The Post-Mortem Interval:

A study of the Body Cooling Rate and Steroid Degradation After Death.

A thesis submitted in part fulfilment of the requirements for admission to the Degree of

Doctor of Philosophy

by

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DEDICATED to my parents who taught me to love science and search for the truth and to my wife who encouraged me to do so

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A C K N O W L E D G E M E N T

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Some of the work described in Part I of this thesis has been published:-

1. Al-Alousi, L.M. and Anderson, R.A. Post-mortem interval by microwave thermography. Proceedings of the meeting on the Uses of Forensic Sciences. Strathclyde University: Glasgow, 1986. (in press)
2. Al-Alousi, L.M. and Anderson, R.A. Microwave thermography in forensic medicine. Police Surgeon, No.30 1986; 30:30-42.
3. Al-Alousi, L.M. and Anderson, R.A. Post-mortem interval by microwave thermography. Forens.Sci. Int. (in press)

SUMMARY

In Part I, the most useful methods for the estimation of the time of death are reviewed, with special emphasis being placed on the post-mortem rate of cooling because this method is commonly used for estimating the interval after death. Theories and models of the post-mortem loss of heat from the human body are summarised and discussed.

The Microwave Thermography System, a new device which is applied to this field for the first time, is described and its mode of operation is discussed. Using this device, it is possible to measure temperatures of internal organs of the body by placing the sensory elements on the skin. The reliability of the system and factors affecting the accuracy of temperature measurements made with the device are assessed and discussed.

Results of a study of the cooling rate of 117 fatalities are given. All cases were studied under controlled conditions and two groups were collected in which the bodies were monitored either naked or covered with blankets. In each case, the environmental temperature as well as the temperatures at three body sites were continuously monitored over a period beginning shortly after death and ending up to 60 hours post-mortem or more. Rectal and environmental temperatures were measured with thermocouples while the temperatures of the

brain and liver were measured using microwave probes, therefore by non-invasive and ethically acceptable methods.

The data were recorded on tape following Analogue to Digital (AD) conversion using a BBC Microcomputer. These data were processed and temperatures at the moment of death for the three body sites were estimated by extrapolation backwards. Processed data were tranferred to a mainframe computer where sophisticated curve-fitting procedures were performed. These indicated that the cooling curves were adequately represented by three-term exponential equations containing six empirically derived parameters. Statistical analysis of the parameters yielded average formulae and the use of these formulae to improve the ease and accuracy of the estimation of the time of death is discussed. Lastly, suggestions for future work are given.

In Part II, biochemical methods of estimating the time of death are reviewed and limitations of their use are discussed. Steroids were selected as potential indicators of the post-mortem interval by virtue of their metabolism and degradation after death. Aspects of steroid biochemistry are summarised. Various methods of steroid analysis were assessed using radioassays and thin layer chromatography. Three reversed phase chromatography systems were evaluated for separation and recovery of steroids extracted from blood, tissues and faeces. The use of different numbers of Sep-Pak C_{18} cartridges for

the purification of steroid extracts was examined and steroid recoveries were measured and compared. The results indicated that recoveries were best when 4-6 cartridges were used. Rapid and slow procedures of enzymatic hydrolysis and acidic solvolysis of steroid conjugates were compared. A new and relatively rapid method for analysis of steroid profiles in biological samples was developed. Assessment of this method showed that steroid recoveries were improved compared to existing methods. A pilot study of the post-mortem changes in the steroid profiles of blood, tissues and faeces was carried out using the rat as a suitable and convenient animal model. Liver and adrenal tissues, faeces and blood samples collected from 30 rats either at the moment of death or at 24 hours after death were analysed and their steroids were studied qualitatively and quantitatively using selective ion monitoring GC-MS techniques. Thus chromatographic peaks were identified by comparision of their retention times and mass spectrometric characteristics with those of standards and quantitative analysis was performed. The occurence of significant steroid changes was difficult to ascertain but some changes in the steroid profiles of the biological samples were shown to have occurred. Lastly, the practicability of this method for the estimation of time of death is discussed.

PART ONE

ESTIMATION OF THE TIME OF DEATH BY CONTINUOUS MONITORING OF POST-MORTEM TEMPERATURES OF THE BRAIN, LIVER, RECTUM AND THE ENVIRONMENT USING THE MICROWAVE THERMOGRAPHY SYSTEM

CHAPTER 1: INTRODUCTION

1.1 Medico-legal Importance of Estimating the Time of Death:

The estimation of the time of death is one of the most important and fundamental problems in forensic medicine. Since the early nineteenth century, when scientific forensic medicine was in its developing stages, the question of the time of death has been repeatedly encountered by forensic pathologists and other medico-legal practitioners [11,12,78,380].

The time of death is frequently considered to be of a "vital" or a "decisive" value to the outcome of a case of suspicious death particularly in relation to homicide [249,250,284,286,317,326,380,387,400,437,531].

Estimation of the post-mortem interval may be of use in determination or confirmation of the cause and manner of death [291]. However, this is not always possible. In some civil proceedings also, the issue of the time of death sometimes becomes of great importance. Many authors have given examples of such cases [317,338,461].

Following the application of recent advances in cardio-pulmonary support devices, several medical and legal problems have arisen [193,275,287,338,495]. Most of these are, in some way, related to the time of death or, in other words, to the determination of the so-called "moment of death". However, from a practical point of view this is extremely difficult to establish.

Timing of death may also be of use in cases where the solution of commorientes is required [275,338].

Accordingly, the subject has been repeatedly and often extensively studied. Nevertheless, an accurate, reliable and easily applicable method of estimating the time since death has not yet been found [276,284,286, 318,326,355,387,400]. This is one reason why it is usually accepted amongst forensic pathologists that estimations of the post-mortem interval have an investigational rather than evidential value [12,265, 286,331,332]. The other reason is due to the fact that the time of death does not necessarily represent the time of assault [13,326,331,332,477]. However, there now exist many reliable methods for the estimation of the age of wounds and other injuries using advanced histochemical and biological techniques [160,161,165, 190,323,347,408-415,425,467,521].

The definition of death is closely related to the issue of estimating the post-mortem interval. Death as a medico-legal problem and other related concepts have been extensively discussed by many authors [2,109,110,187,193,275,287,304,338,389,399, 464,473,494,495]. Somatic death is assigned when death is clinically diagnosed, in other words the commonly accepted concept of death [2,109,110,494], while cellular death means that individual cells or tissues cease to function, which usually takes up to several hours after somatic death. Several authors have

discussed the concept of cellular death and its applications [46,313,316,332,395].

1.2 Methods of Estimating the Time of Death:

Apart from entomological estimation of the post-mortem interval [22,382-385,400,420], all methods depend on the physical, chemical and biochemical changes that occur in the body after death.

The physical changes used for this purpose are numerous. Examples of these are: "rigor mortis", "livor mortis", ocular changes, putrefaction, adipocere formation, histological changes and body cooling. Several reviews of these changes and their applications for estimation of the post-mortem interval have been published [23,24,49,62,92,94,126,155-157,169,191,193, 219,272,281,282,284-286,324-327,332,360,377,400,405,420, 434,436,456,462,475,486-488,496-498,524,525]. Rigor mortis, indicating the continuation of certain metabolic processes and muscular activities, is of particular interest, especially in relation to the production of heat after death. Theoretical and chemical explanations of rigor mortis have been discussed by several authors [23,49,126,193,277,484, 486,524]. Apart from methods which depend on post-mortem body cooling, all methods based on physical changes lack objectivity and are, moreover, not suitable for quantitative calculations. Histological and haematological changes that occur in the body after

death have also been studied and found to be widely variable and of little value in the field [115,118,156, 186,234,248,253,269,294,393,402,424,460,530]. Post-mortem responses of muscles and sweat glands to electrical and other stimuli have been examined but the results have not been encouraging [93,128,175,401,406, 407,417,510]. Most recently a method based on velocity of transmission of ultrasound through the body has been reported [536].

Chemical and biochemical methods will be discussed later.

1.3 Post-mortem Body Cooling as an Indicator of the Time of Death:

Temperature measurements have been used in forensic medicine for the following purposes [78,117, 336,400,490]:

(i) to estimate the time of death,

(ii) to differentiate death from suspended animation, and,

(iii) to help find the cause of death in some cases.

The gradual loss of heat from a dead body has, for a long time, been regarded as providing a scientific basis for the solution of the problem of the time of death [87,166,274,387,396,461,490]. It is now a commonly held opinion that methods of estimating the interval after death based on post-mortem cooling are the most reliable in practice, the easiest to implement

and the most likely to be of value, especially during the first 18-24 hours post-mortem [12,292,293,325,326, 332,388,400,457,461,463,465]. Therefore, recourse to temperature measurements in a criminal case is often an obligatory step [168,326,400].

There is perhaps no forensic problem which has attracted more research or wider discussion in the literature than the problem of estimating the time of death[326,387,400]. It is, in fact, difficult to ascertain the first published work related to this subject. However, it is clear that systematic studies appeared as early as the mid-nineteenth century, or even a decade or so before that date. From a historical point of view, the published work may be classified into three categories. These are:

(1) Work of the nineteenth century [79,86,87,117, 129-133,139,188,192,205,241,335,353,416,435,444,449, 474,522],

(2) Studies of the early and mid-twentieth century [166,260,317,358,359,361,435,436,440,441,443,474,500] and,

(3) Modern studies [8-10,78,79,205,206,242,243, 249,250,265,276,291-293,328-331,333,334,336,340,351-353,362,365,379,380,387,419,438,461].

Most of the workers of the nineteenth century were aware of Newton's Law of cooling and, to some extent, its inapplicability to the post-mortem cooling of the human body [133,353]. In fact some of these

workers pointed out a phenomenon which is similar to what is now known as the "initial temperature plateau". They observed that the "temperature was sustained" but despite this, most of them used a linear drop in post-mortem temperature versus time, or what is known as the "Rule of Thumb", rather than exponential cooling.

In the studies of the second category, the sigmoid shape of the cooling curve became more recognized, and the initial temperature lag was called the temperature plateau [281,458]. In general most investigators of this category used a single-exponential model to describe the cooling curve.

In the last category more advanced techniques of temperature recording were used and more complicated formulae were developed or followed to estimate the time of death. Of these studies, those of Marshall have attracted particular attention [328-331,333,334, 336]. Marshall studied rectal, liver and axillary cooling in 90 cases and published results of about 40 of them [336], mostly related to rectal cooling. He and Hoar introduced the use of the double-exponential formula to the problem of estimating the time of death. They described the cooling curve as having three stages; the plateau, the rapid cooling stage and the slow cooling stage. Marshall and Hoar also emphasized that Newton's Law of Cooling does not apply to human body post-mortem cooling. Although the

double-exponential formula is a prominent and important progression towards a better understanding of the post-mortem cooling behaviour, this method has the following shortcomings:-

(1) The formula contains many constants which should, in practice, be derived separately for each case. It requires several temperature measurements over a period of many hours and in most instances for at least 12 hours.

(2) It requires the measurements of some body parameters such as body weight and surface area. It depends on an assumed correlation between the cooling factor and the "cooling size factor" which is an approximate correlation derived from only 27 cases.

(3) The 'Z' constant, which is very important in the method, cannot be determined until 12 hours or more after death. This presupposes a knowledge of the approximate time of death, which is obviously unknown in practice.

(4) Errors in time estimates resulting from the application of this formula have been evaluated sporadically. It has not been tested either practically or statistically on a significant number of cases.

Several modifications to this formula have been devised to get average equations [78,353,379]. The formula has also been converted to a computer-

applicable form [379]. Although formulae which depend on the double-exponential cooling model are more accurate in describing the actual rectal cooling curve than earlier methods, recent studies have shown that significant time errors may still occur when this method is applied [78,205,353,379,391].

There is therefore a continuing need for more accurate and reliable (albeit more complicated) methods for the estimation of the post-mortem interval or, at least, to achieve a better understanding of the problem. An equally important objective is to establish statistically the extent of errors inherent in the method. These goals have become more possible and practicable in recent years by the application to forensic medicine of sophisticated modern equipment and techniques for the analysis of biological systems and for the acquisition and processing of data. Of particular value are recent advances in computing science [12,40]. In this study a triple-exponential formula has been used and developed for the more accurate description of the cooling curve and extensive computer-based methods for data acquisition and processing have been applied.

1.4 Mathematical Models of Body Cooling:

The post-mortem cooling curve has been described in the past by the following mathematical models:

(1) The linear cooling model:

This is the "rule of thumb" procedure which is traditionally used in the field. It is represented by the formula:

$$t = \frac{B_0 - B_t}{R} \quad \text{.....Equation 1.1}$$

where B_0 is the normal body temperature (usually rectal temperature) at death,

B_t is the body temperature at a given time,

R is the rate of cooling, and,

t is the time after death.

For temperate climates R is given the value of 1.5°F/hour for the first twelve hours after death. Many extended forms of the "rule of thumb" have been published [265,286,335,435,436]. This is to compensate for the effects of factors such as cold or warm weather, body build and others.

(2) Single-exponential model (Newtonian cooling):

Newton's Law of Cooling states that "the rate of loss of heat from the surface of a solid body to the surrounding fluid is directly proportional to the difference in temperature between the surface and the fluid [376].

It is worth noting that this law was originally applied to homogeneous and thermally-thin bodies. The latter means that the temperature in all points of the body is uniform. In other words there is no temperature gradient between points of the body.

Molnar and Brown and Marshall have given discussions on the application of this law to the human body [79,349, 350].

This law is represented by the following formula:

$$q = hA(B_s - E) \quad \text{.....Equation 1.2}$$

where q is the heat transfer rate from the surface of the body to the surrounding fluid (environment),

h is the heat transfer coefficient,

A is the surface area,

B_s is the temperature of the body surface

E is the temperature of the fluid or the environment.

For thermally-thin bodies, this equation can be written:

$$M.S.\ dB_t/dt = hA(B_t - E) \quad \text{.....Equation 1.3}$$

where M is the mass of the body,

S is the specific heat of the body,

dB_t/dt is the rate of change of body temperature,

Assuming that the temperature of the surrounding fluid does not change with time Equation 1.3 can be integrated to give:

$$(B_t - E)/(B_0 - E) = e^{-\frac{hA}{MS}t} \quad \text{.....Equation 1.4}$$

Many extended single-exponential formulae have been published to compensate for some factors particularly the initial temperature plateau [166,435,436].

(3) Models based on the Heat Transmission Theory:

According to the heat transmission theory, the cooling curve of any body of a non-uniform temperature distribution (i.e. not thermally-thin), varies with its shape [350]. In practice this theory has been applied directly to the human body as the "Infinite Cylinder Model" (Figure 4.1). This model derived from work originally developed by Fourier which was published in 1822 in "Theorie Analytique de la Chaleur" [172,438], and which was subsequently reconsidered by Carslaw and Jaeger [98]. A slightly modified form has also been used consisting of an infinite sum of exponential terms [249]. The cooling curves and formulae for a solid sphere, the "Infinite Sphere Model" [516], have been used in industry but have never been applied to post-mortem cooling of the human body. The Infinite Cylinder means that all or most of the heat of the cylinder flows radially and not axially, except to a very small extent. The Infinite Cylinder and the Infinite Sphere models and their formulae simply mean that the temperature gradient along a radius from the centre to the surface at any moment of cooling can be described by the sum of an infinite number of terms each of which is the product of a constant and an exponential term which diminishes with time.

The validity of this formula for the human body has been shown by many authors [166,249,250,276,331]. Also, although Marshall in 1969 stated that "the more

exponential terms in the series the more accurate is the formula" [331], a more recent conclusion was that only the first few terms of the formula were important for the cooling of the human body [79]. The double-exponential formula of Marshall and Hoare, and also its amendments, and the triple-exponential formulae which are developed and used in this study are all, in fact, modified forms of the Infinite Cylinder Model.

For an infinite cylinder where there is no heat input, with uniform thermal properties in all directions (isotropy), and uniform temperature distribution initially, the formula is:

$$\frac{(T_r - T_e)t}{(T_r - T_e)_0} = \sum_{n=1}^{\infty} \frac{2AJ_0(r\beta_n/a)}{(\beta_n^2+A^2)J_0(\beta_n)} \cdot e^{-\beta_n^2 \frac{\alpha}{a^2} \cdot t}$$

.....Equation.1.5

where

T_r = temperature on a radius at a distance r from the axis,

T_e = temperature of the environment or ambient temperature, and,

$(T_e-T_e)t$ = temperature difference between T_r and T_e at any given time.

$(T_r-T_e)_0$ = temperature difference between T_r and T_e at the commencement of cooling (i.e. at the moment of death)

$\sum_{n=1}^{\infty}$ = sum of terms of number n from 1 to infinity

$A = a\frac{h}{K}$, where a is the radius, h is the heat transfer

coefficient and K is the thermal conductivity.

J_0= Bessel function of order zero, first kind. Bessel functions are used in problems concerned with cylinders.

β_n = roots of the equation $\beta_n J_1(\beta_n)=AJ_0(\beta_n)$

where J_1 is a Bessel function of order one, first kind. These roots are obtainable from certain tables [98]

α = thermal diffusivity.

This formula seems formidable but, in fact, it can be simplified and made easily usable as follows:

Let $\frac{(T_r - T_e)t}{(T_r - T_e)_0}$ = temperature difference ratio = R

Assuming that the environmental temperature does not change with time, therefore R can be written as:

$$R = \frac{(T_r - T_e)_t}{T_{r0} - T_{et}} \qquad \text{.....Equation 1.6}$$

Assuming that the body parameters, such as and K, are constant, and that the temperature is measured at the centre of the body where r = 0, therefore the formula can be written as:

$$R = \sum_{n=1}^{\infty} P_1 . e^{-\frac{D}{a^2} t} \qquad \text{.....Equation 1.7}$$

where D is constant.

For the purpose of curve-fitting by a computer where the value of a^2 can be deduced automatically from the data and then implied in the constant, and if

three exponential terms are to be used, as is the case with this study, therefore the final formula can be now written as:

$$R = P_1.e^{-P_2 \cdot t} + P_3.e^{-P_4 \cdot t} + P_5.e^{-P_6 \cdot t}$$

.....Equation 1.8

where P_1 to P_6 are constants deduced from the curve-fitting of the original data.

1.5 Devices used to measure the temperature after death:

The thermometer has commonly been used [79,86, 87,117,129-133,192,241,416,490,522]. This was usually a mercury thermometer [166,265,291,336,419,435,474]. Sometimes the thermometer was specially made for the purpose; for instance Burman used a right angled long thermometer to facilitate axillary temperature measurement [87]. Rectal thermometers are usually long to enable measurements to be taken in situ. Electrical thermocouples, which are sometimes known as electrical thermometers, have been widely used by many authors, particularly when continuous recording is required [12,166,249,276,292,293,317,388,461]. Fiddes and Patten used what they called an "electrical continuous recording apparatus" which is most probably based on a type of electrical thermocouple [166]. The rototherm, which is a version of the domestic meat thermometer, was proposed for the measurement of the temperature of the abdominal cavity in the dead. However, it has subsequently been found to be insufficiently accurate

for the purpose [334]. In this study electrical thermocouples were used as well as the Microwave Thermograph, which enabled the temperature of the interior of the body to be measured by a non-invasive and ethically acceptable method.

1.6 Sites Used for Post-mortem Temperature Measurement:

The rectum was chosen for temperature measurements by all investigators except a few in the 19th Century such as Taylor and Wilks and Burman and Womack [87,490,522]. This was mainly because it is less influenced by the environment than the skin surface of the body and also because it is easily accessed with a thermometer compared with other internal organs like the liver or brain. Usually the thermometer is inserted about 3-4 inches into the rectum. Difficulties of temperature measurement in the rectum and the unreliability of this site in representing the actual changes in the 'core' temperature, have been mentioned by some authors [342] and will be discussed later (Paragraph 4.4). Also, the site of the rectum has been found not to be in the centre of the body [250,276,336]. Other sites have been used like the forehead surface [317], axilla [87,133,336,461], surface of the chest [317], surface of the abdomen [490,522], umbilicus [416], surface of the thigh [317], crural muscle [133,461], quadriceps

muscle of the thigh [129,133,317] and internally, the brain [133,317,388, 461], oesophagus [61,206], liver [133,317,336,436,461], heart [133] and abdominal and chest cavities [133]. Many authors have preferred the measurement to be made in internal sites like the brain and the liver [276,387,388,436, 461]. The main obstacle is that these organs cannot be accessed unless a stab or perforating wound is made, for example in the abdomen, to access to the liver or in one of the eyes to access the brain [336,461]. This may not only be ethically unacceptable but also may affect the actual cooling process. Using the microwave thermography technique it is now possible to take the temperature of these and other organs by a non-invasive and ethically acceptable method as explained later.

CHAPTER 2 : EXPERIMENTAL

2.1 Microwave Thermograph

The device used for temperature measurements in this study was the Microwave Thermography System which was recently developed in the Department of Natural Philosophy at Glasgow University. The System consisted of the following parts (Figures 2.1 and 2.2):

1. Two microwave radio aerials (microwave probes) which were held in place by two anglepoise lamp arms. The aerials were insulated by thick layers of cotton and plastic tape. Each microwave probe was cylindrical in shape, 2.8 cm. in diameter and 6.5 cm. in length, and connected to the receiver unit through a 1.5 m flexible microwave cable assembly. The construction of the probes consisted of a solid core of low-loss dielectric material designed to simulate the microwave refractive index of body tissues.
2. A microwave radio receiver unit designed to amplify the microwave signals collected by the aerials. The intensity of microwave signals was ordinarily small, therefore one hundred millivolts corresponded to one Celsius degree.
3. An electronic signal processing system which operated on the signal from the receiver so that the temperature of the two body sites in question would be alternately measured and temperature

Figure 2.1: A photograph of the Microwave Thermography System.

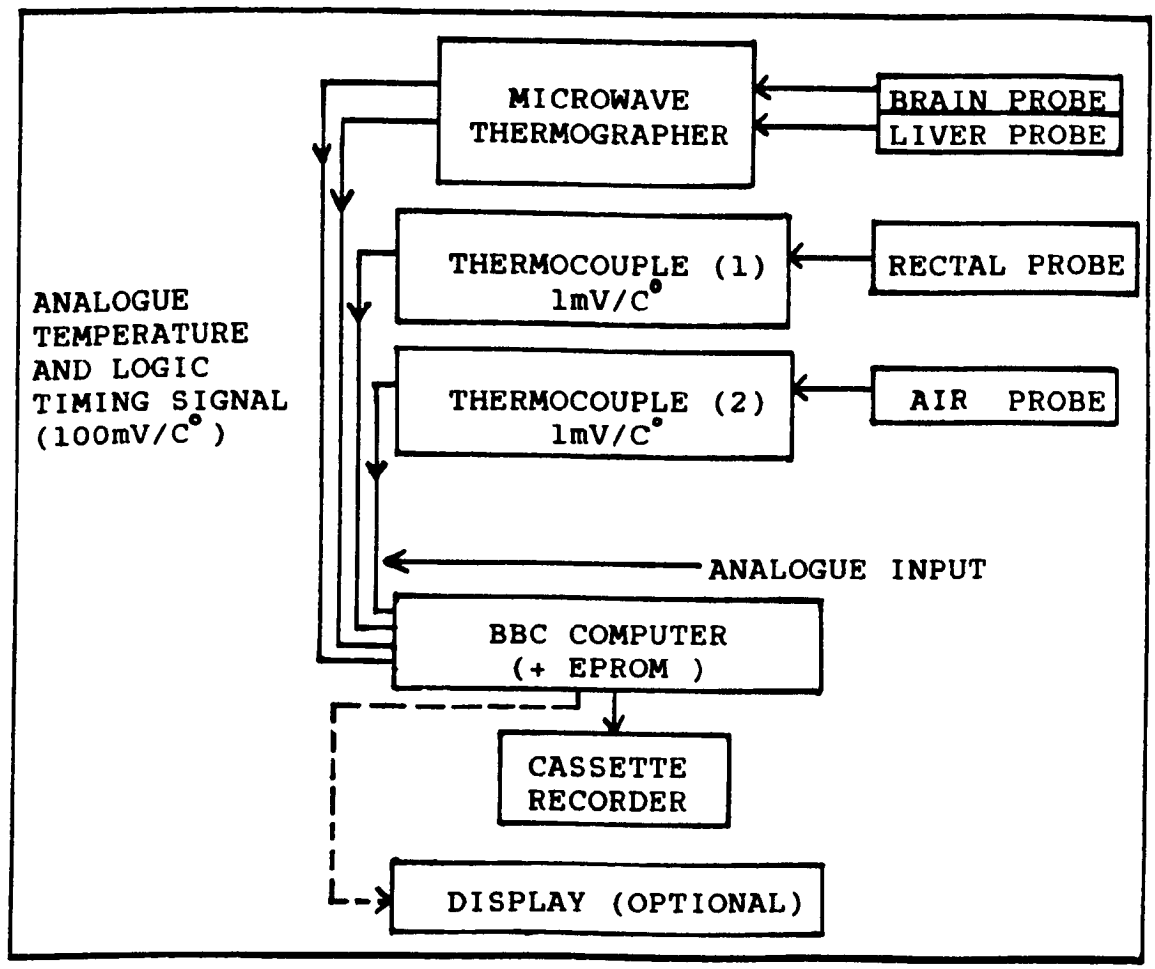


Figure 2.2: Data Recording Sytem

readings displayed immediately on a small screen. The output was also compatible with a chart-recorder and microcomputer.

4. Two digital pyrometers fitted with rigid thermocouples. The latter were used to monitor the rectum and the environment, as the temperature of the experimental room was not regulated. The rectal thermocouple was specially made in the Department of Natural Philosophy, having a length of 30 cm and a diameter of 0.5 cm, to allow it to be inserted for 9-10 cm into the rectum and left in situ throughout all the monitoring period. The environmental thermocouple was wall-mounted adjacent to the body at a height of 2 m.

The temperature resolution of the system was 0.1°C and the response time was 2 seconds. The absolute accuracy of temperature measurements by the microwave probe and the theromcouples was ±0.6°C. The equipment used in this research operated at radiation frequencies from 3.0 to 3.5 GHz corresponding to wavelengths, in air, from 10 to 8.6 cm. As a result, the penetration distances obtained (the effective depth at which temperatures were recorded) were 1.6 cm and 10 cm in high and low water content tissues, respectively.

2.2 Calibration of the Instrument

The microwave system was calibrated, with the

assistance of the Natural Philosphy Department, with liquid and solid media at different temperatures, using a mercury thermometer or a thermocouple as a control. In this respect, a solution of glycerol in water (1:3 v/v) was used to simulate the microwave refraction of body tissues. The end of each microwave probe was placed in contact with the surface of an equilibrated bath of the solution and allowed to stabilise for 5 minutes. The temperature readout of the thermograph was noted as was the temperature of the bath. This was repeated at several other temperatures over the range 10-37°C. Similar procedures were carried out using water alone and solid media, i.e. meat (beef). Following these procedures, the instrument was tested on normal living subjects to show any discrepancies between observed and actual temperatures. The calibration curves are given in Figure 3.1 and temperatures of some body organs measured by the microwave aerials in a clinical case are given in Table 3.1.

2.3 Assessment of Microwave Attenuation

Absorption of microwave radiation by tissues was assessed via 2 sets of experiments:-

1. Temperatures of the liver and the brain were measured in normal healthy subjects using the microwave probes as described above. The mean values were then compared with the mean body

temperature in health.

2. In a group of 5 fatalities both microwave probes were used to measure the temperature of the liver in the usual way. At the same time, two small intradermal thermocouples (type KC4 and KC3, Kane-May Ltd., Welwyn Garden City) alternately connected to a pyrometer, were used to measure the temperatures of the tissues in the right hypochondrial region including the skin, subcutaneous fat and muscle, and the liver. Thus temperatures in this region were monitored at different depths from the body surface. Also brain, environmental and rectal temperatures were simultaneously measured as usual. These measurements were recorded intermittently, with the first reading being taken soon after death and then once or twice later, several hours post-mortem. The readings from the microwave probes and the thermocouples were then compared.

2.4 Assessment of the Effects of Environmental Temperature on the Microwave Probes

To test whether the microwave probes were affected by cooling or heating of the environment, another group of experiments was carried out. The body temperature of a fatal case was measured in the normal manner decribed below (Paragraph 2.5.2). After the temperature values were taken, the shafts of the probes

were successively warmed and cooled using a hair dryer. Any changes in the temperature readings were then noted.

For the same purpose another experiment was performed in which a healthy adult subject lay naked in a supine position. His liver temperature was measured with a microwave probe. Then his body and the probe were covered with a blanket and the temperature was monitored for several hours.

2.5 Temperature Monitoring

2.5.1 Selection of Cases

The work described here was carried out in the Glasgow City Mortuary during the years 1983-1986. The experiments were performed in a room, approximately 5m by 4m by 3.5m, which had one window, about 3m by 2m, with no blinds. The window was kept closed at all times. The room was usually illuminated by daylight or fluorescent light but not by direct sunlight. It had one door which was closed except to permit occasional entry and exit. There was no significant air movement in the room.

The body was conveyed to the mortuary by ambulance and was divested in the reception room. The experiment was started immediately. The cases were selected according to the following criteria:

1. The exact time of death should be known to within 15 minutes.

2. The elapsed time between death and the arrival of the body in the mortuary should be as short as possible, preferably less than 45 minutes, so that the body should feel quite warm to the touch.
3. The circumstances of the death should allow the body to be kept, undisturbed, at the mortuary for a period of at least 20-36 hours, preferably up to 60 hours or more, to allow many hours of monitoring. In practice, this criterion meant that cases were restricted to those for which the cause of death was either natural or non-suspicious.

On arrival of a case satisfying the above criteria, the body was accurately weighed using a weighing trolley, its crown-sole length (height) was measured with an autopsy ruler and the circumference of the head and the width of the hip were then obtained with a tape measure. Information concerning the name, sex, body measurements, the exact time of death and the time of beginnning and end of monitoring were recorded. The causes of death were obtained from the death certificates or the medico-legal necropsy reports.

2.5.2 Operating Procedure

The body was laid in a supine position, with legs together and arms by the side, on a metal-topped hospital trolley which was covered by a plastic sheet. The body was either left naked or covered with two cotton blankets (Figure 2.3). In the latter case, a third blanket was put underneath the body to cover the

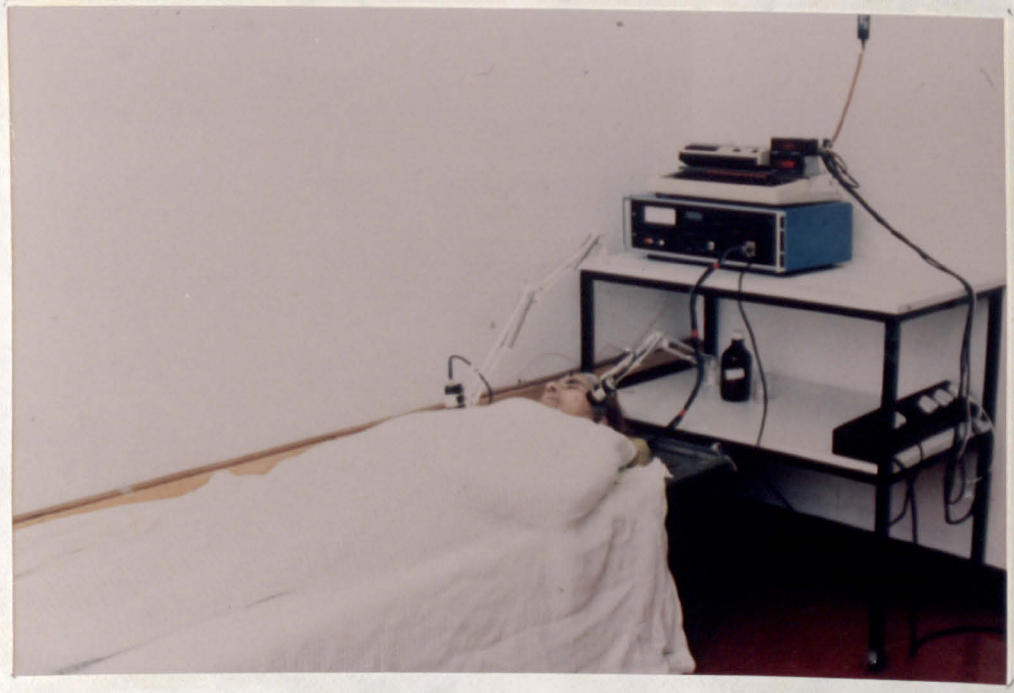


Figure 2.3: A photograph illustrating a body covered by two blankets, being monitored by the Microwave Thermography System.

trolley. In this group the liver probe was left uncovered and was placed in contact with the body through small holes in the blankets.

The temperatures of three body sites, namely, the brain, liver and the rectum, as well as the temperature of the environment, were monitored. During monitoring, the microwave probes were placed in contact with the skin of one of the temporal regions and of the right hypochondrium, to monitor the temperature of the brain and the liver, respectively (Figures 2.4, 2.5). Measurements were started as soon as possible after death (normally less than 45 minutes) and continued for 24-36 hours or longer, up to 60 hours or more if possible.

Occasionally, a pathologist, policeman or family doctor required to inspect or examine the body for the purpose of identification prior to issuing a death certificate. This was mostly carried out without interruption of the temperature monitoring and with as little disturbance as possible. If, for any reason, the monitoring was interrupted for any length of time, the case would be excluded from further analysis.

2.6 Data Acquisition and Processing

2.6.1 Analogue to Digital Conversion

For most cases, analogue to digital (AD) conversion of the signals from the microwave receiver and pyrometers was carried out using a BBC Model B

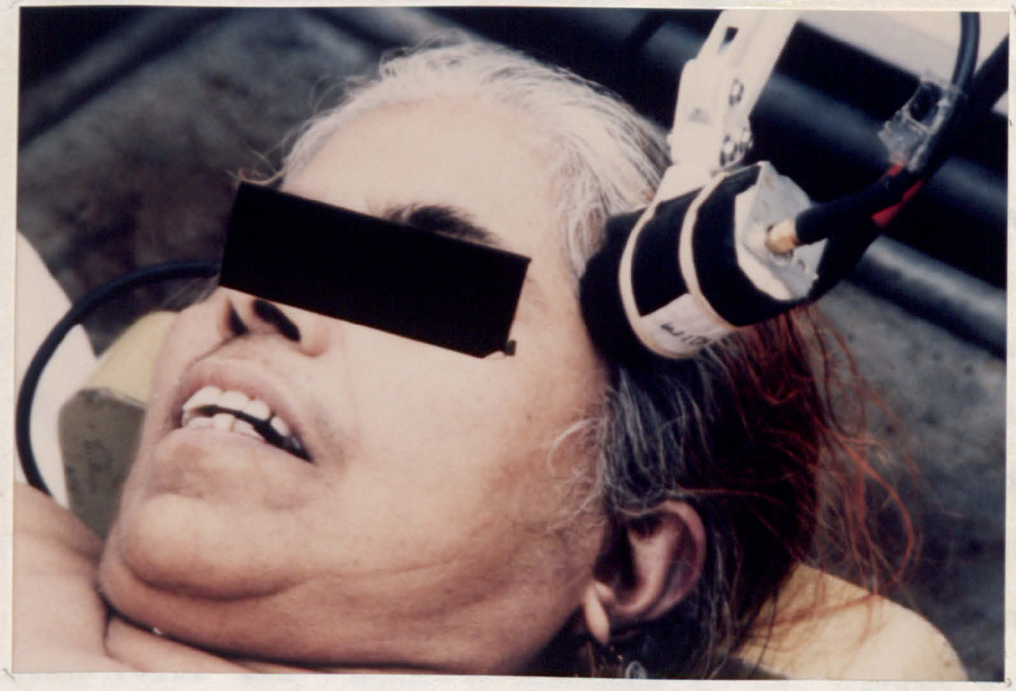


Figure 2.4: The microwave probe was placed in contact with the skin of the temporal region to monitor the brain temperature after death.

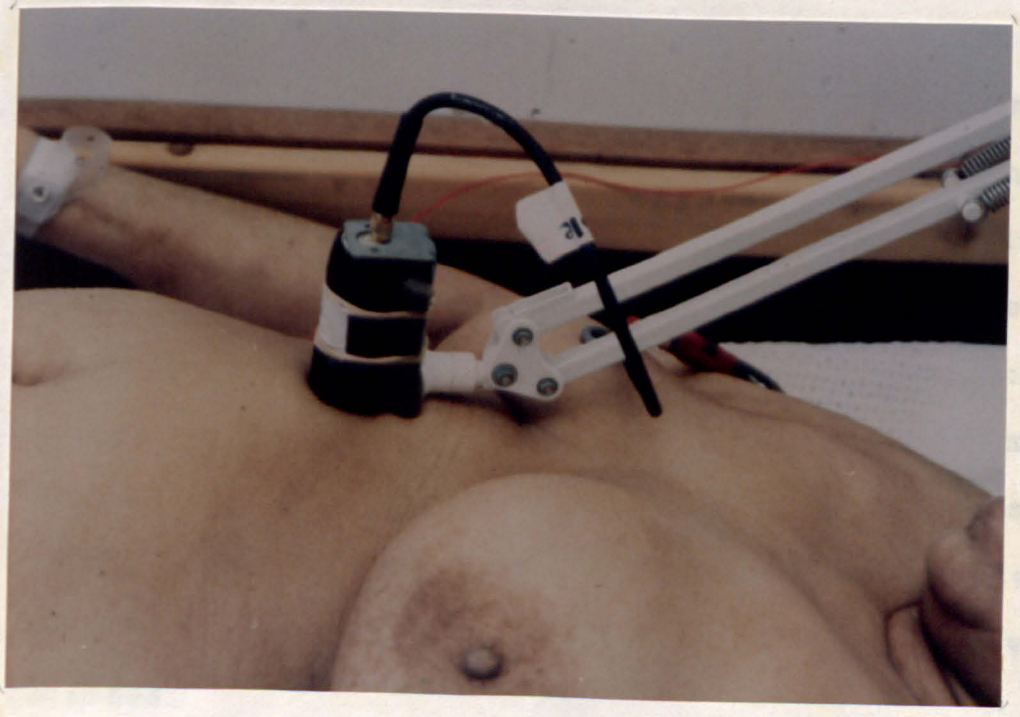


Figure 2.5: The microwave probe was in contact with the skin of the right hypochondrium to monitor the temperature of the liver after death.

microcomputer which has an inbuilt 12-bit analogue-to-digital converter (ADC). The function ADVAL, part of the computer operating system, returned values from the ADC in the range 0 (zero) to 65520. This corresponded to voltages in the range 0 (zero) to the reference voltage V_{ref} (maximum voltage which can be converted). V_{ref} was measured with a digital voltmeter and found to be 2.454 volts. However, the last four bits of ADVAL values were not significant and each value was therefore divided by 16, giving a range of 0 to 4095. This meant that there was a digital value of 1669 per volt, or in other words the value returned by the ADVAL function was equal to the input voltage multiplied by (16 x 1669). Similarly 0.6 millivolts corresponded to one digital unit which represented the precision of the voltage measurement.

Each input channel took only 10 milliseconds to convert an input voltage to a digital value. It was therefore possible to average a large number of converted values before storing the readings: an average of 300 values was taken in approximately 12 seconds. The reproducibility of the AD conversion was examined by applying a constant voltage to one channel and comparing the average digital values returned.

The linearity and accuracy of the ADC were tested by applying calibrated voltages and plotting these versus the digital values returned. This is

shown in Figure 2.6 which also shows the value of V_{ref} measured with a digital voltmeter.

2.6.2 Analogue Recording and Digitisation

In the first 25 cases, before the microcomputer was used for AD conversion, data was recorded on a four-pen chart recorder. The temperature traces in those charts were digitized using a PAD digitizer connected to a BBC microcomputer, and copied to disks.

An example of these charts is given in Figure 2.7 and the programme used for digitization is shown in Appendix I: Programme 2.

2.6.3 Data Logging and Processing

Data acquisition steps carried out in the mortuary are shown systematically in Figure 2.2. Other steps of data processing and curve fitting were performed in the Forensic Medicine and Computing Services Departments, Glasgow University. These steps are shown in a schematic diagram in Figure 2.8. The data logging control programme (Appendix I: Programme 1) was implemented in an Erasable Programmable Read Only Memory (EPROM) and had an auto-start capacity. This meant that it could be run very simply by pressing the 'BREAK' key and even by switching on the equipment. Temperature data from four channels were recorded on magnetic tapes and a logical timing scale was also provided by the computer. Tapes from the mortuary were taken in batches to the Forensic Medicine Department, Glasgow University, where a second BBC

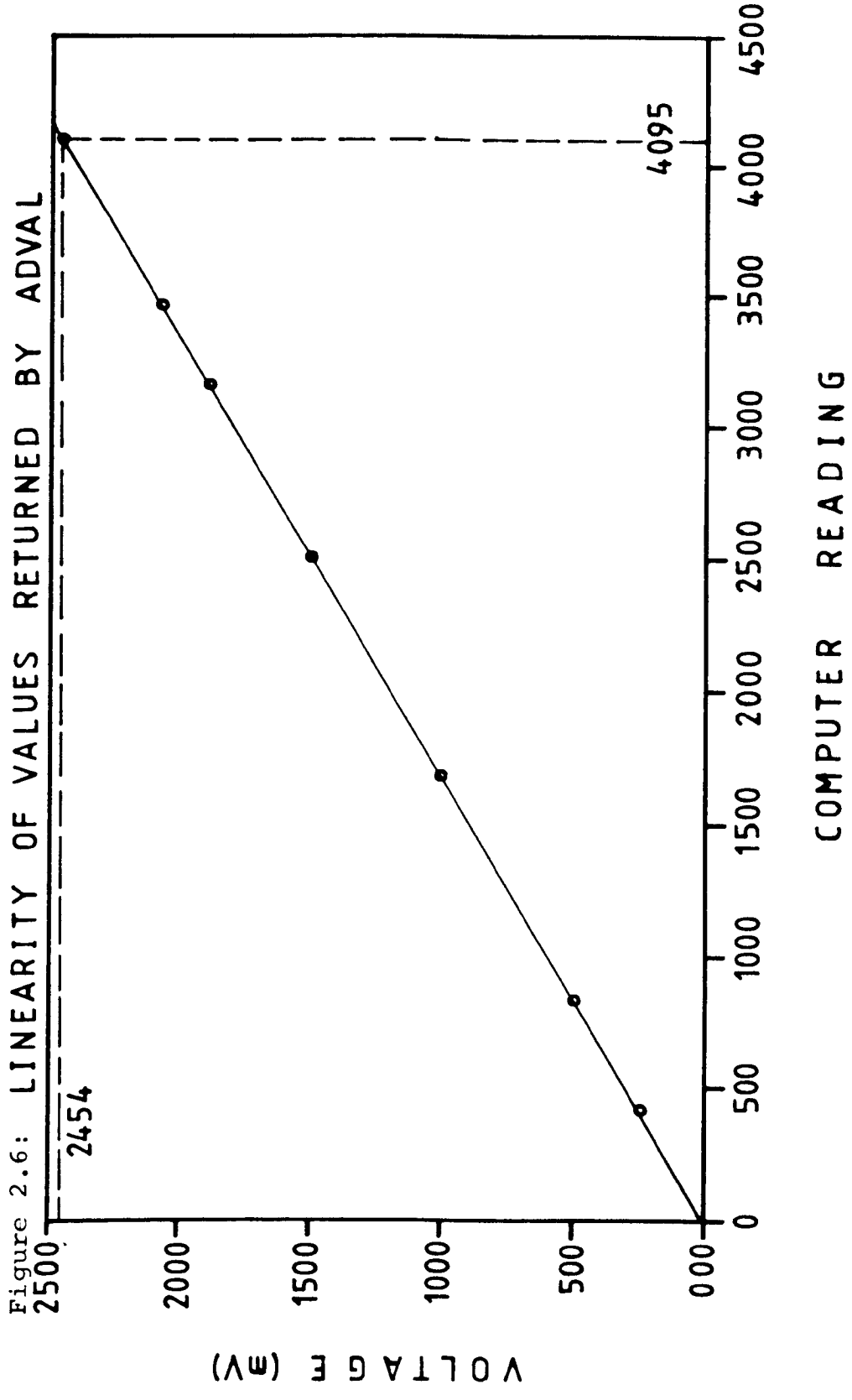

LINEARITY OF VALUES RETURNED BY ADVAL
2454
4095
2500
2000
1500
1000
500
000
0
500
1000
1500
2000
2500
3000
3500
4000
4500
VOLTAGE (mV)
COMPUTER READING

Figure 2.6:

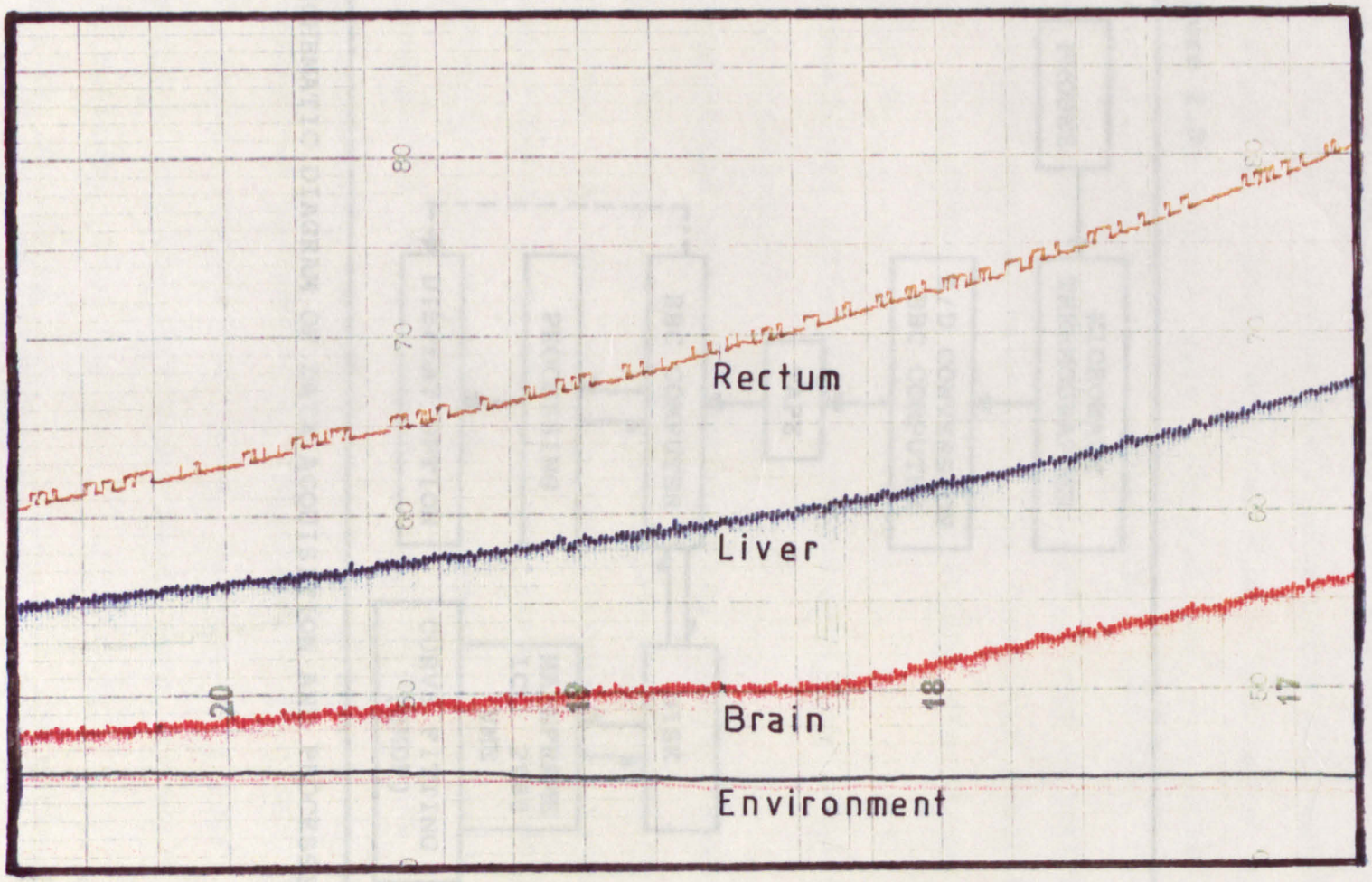
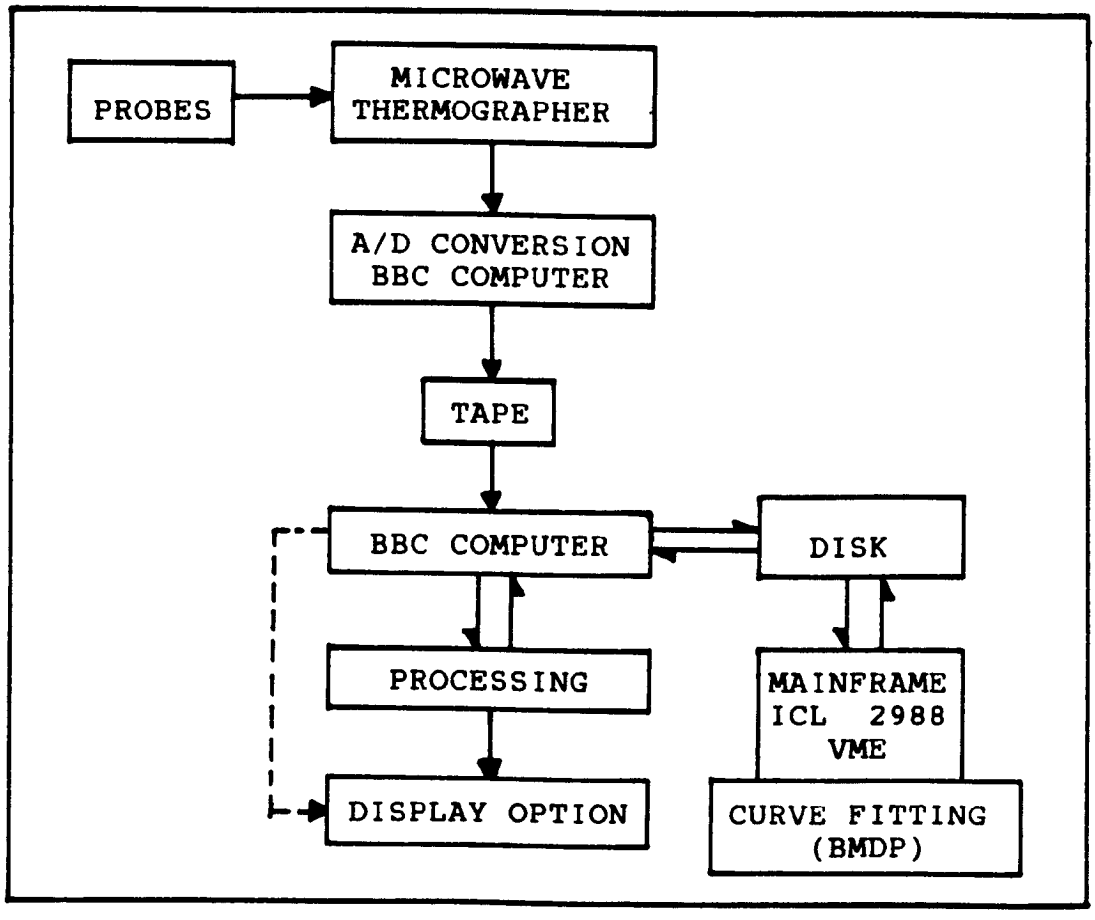


Figure 2.7: An example of temperature traces recorded on four-pen chart recorder.

Figure 2.8:



SCHEMATIC DIAGRAM OF DATA ACQUISITION AND PROCESSING

Model B microcomputer was used to transfer and store all data to disks. Plots of the raw temperature data versus time were performed and visualised on a monitor screen, to assess their uniformity and validity for further processing and analysis. Artifacts such as electrical spikes were revealed and corrected by editing the file to remove the aberrant data. Irremediably bad traces were excluded. Data processing was carried out to calculate the functions described below: programmes for these operations are given in Appendix I.

1. Temperature differences between each body site and the environment.
2. Temperature ratios between body sites, namely, rectum/brain, rectum/liver and liver/brain ratios.
3. Changes in the temperature difference between measurements made at a given site in successive intervals. These changes represent the fall in temperature of a given site relative to the environmental temperature. For instance, if the rectal and environmental temperatures at 1 hour and 2 hours post-mortem were r_1, e_1, r_2 and e_2 respectively the difference meant here (D) would be as follows:

   $D = (r_1-e_1)-(r_2-e_2)$ .....Equation 2.1
4. The temperature of each body site at the moment of death (T_{b0}) which was established as described below (Paragraph 2.6.4)

5. Temperature difference ratios which were computed according to this equation:-

$$R = \frac{T_{bt} - T_{et}}{T_{bO} - T_{et}} \qquad \text{.....Equation 2.2}$$

where

T_{bt} = temperature at any body site measured at any given time t.

T_{bO} = temperature of any body site at the moment of death as established above.

T_{et} = temperature of environment measured at time t.

and R = temperature difference ratio.

Temperature difference ratios were transferred to a main frame computer (ICL 2988 VME) for regression analysis and statistics. A listing of the programme used to transfer the data from the BBC microcomputer to the mainframe computer is given in Appendix I: Programme 7.

2.6.4 <u>Body Temperature at the Moment of Death</u>

This was obtained for each body site by the following procedures:-

1. Curve-fitting of mathematical equations of different types to the data for the first three hours of monitoring. To find the best equation which properly described that data. For this purpose, 2nd, 3rd and 4th order polynomial as well as single-exponential equations were tried for 30 curves (10 cases). The latter equation was found to be the best for this aim.

2. Single-exponential equations were obtained in this way for each body site in each case and were extrapolated backwards over the post-mortem interval prior to the start of monitoring, t_0. The intercept on the Y-axis (temperature axis) was the calculated temperature at the moment of death of the body organ to which the curve belonged at the moment of death. The programme used for this calculation is listed in the Appendix I: Programme 5.

2.7 Data Analysis

2.7.1 Curve-fitting procedures.

Two methods of curve-fitting were performed. The first, for a small number of cases, was conducted on the BBC microcomputer using single-exponential equations. In subsequent discussion it will be referred to as curve-fiting Class 1. The second method, the major one, was carried out on the mainframe computer using programme P3R of the Biomedical Programme package (BMDP), a collection of statistical software routines [125]. Detailed operating instructions for this programme are given in Reference 125: an example of a job control file is given and discussed in Chapter 3, section 3.3.4. and Chapter 4, section 4.8.3. This method of curve-fitting will be referred to below as curve-fitting Class 2.

Three types of data were fitted for each case,

corresponding to the three body sites, namely, brain, liver and rectum.

2.7.2 Derivative Curves and Extended Analysis

Other analytical computations were made such as calculations of slopes of curves (i.e. the rate of cooling) for the three body sites in individual cases and for average curves. These were calculated using the first derivative of the Class 2 equations of the curves. The derivative curves had equations of the form given in Equation 3.3 below.

In addition, slope ratios (SR) were calculated using Equation 3.5 (below) for individual cases and for the average formulae.

Finally, in five cases, the method of Marshall and Hoare[336] for estimating the time of death was tested. Also, the triple-exponential formulae developed in this study were compared with methods and formulae used by other investigators.

2.7.3 Subdivision of Cases into Groups

Cases were subdivided initially into either naked or covered groups. These, in turn, were split into two categories, fat and thin bodies. In this respect, use was made of the body surface area and the Cooling Size Factor [336,435]. These were calculated using the formulae given in Equations 2.3 and 2.4 respectively.

$A = 71.84 \cdot (W^{0.425}) \cdot (H^{0.725})$ .....Equation 2.3

where A is the surface area in cm^2,

W is the weight of the body in kg,

and H is the height in cm.

$Z = A/W$ .....Equation 2.4

i.e. $Z = 71.84 \cdot (W^{-0.575}) \cdot (H^{0.725})$ ...Equation 2.5

where Z is the Cooling Size Factor.

A body was considered to be thin if Z was greater than 0.028 or fat if Z was below this threshold (Table 3.18).

2.7.4 Average equations

Parameters (i.e. constants) derived from the curve-fitting processes were then classified according to the above groups. Averaging of these parameters for each body site and in each group of related cases was carried out as follows:-

1. A simple numerical average was taken of the parameters.
2. Temperature difference ratios were calculated initially using the equations obtained in curve-fitting Class 2. The ratios for groups of related cases at selected intervals after death were averaged and the average data thus obtained were refitted using the BMDP P3R programme.

In this way, two types of average formulae were obtained for each body site and for each group of related cases.

2.7.5 Assessment of the Formulae

The validity of the average formulae for predicting the post-mortem interval was tested by comparing the average cooling curve with curves for individual cases. Using the average formulae, predicted times were calculated for each value of the temperature difference ratios (R) in a case. The actual times corresponding to these values of R had been recorded experimentally. Differences between the actual times and the predicted times were calculated for each case for the entire monitoring period. For selected times after death, the mean and standard deviation of the differences were then calculated and plotted versus time. Also, reference graphs and a simple computer programme were developed to enable practising forensic investigators to use the triple-exponential formulae of this study for more accurate estimation of the time since death.

CHAPTER 3 : RESULTS

3.1 General Assessment of Microwave Thermograph

3.1.1 Performance of the Instrument

The Microwave Thermography System was found to be reliable and easy to use in practice. Very few equipment faults occurred and these related primarily to the connectors used to couple the microwave cable to the probes and the main unit. Poor connection in this respect gave rise to background electrical noise ("spikes") which could often be corrected by editing the data records unless the noise problem was irremediable, when the case was abandoned. The other equipment fault, which occurred rarely, was a failure of the EPROM system auto-start facility in the data-logging computer in very cold weather: this problem was easily avoided by using a keyboard command (RUN) to begin acquisition.

During the initial period of use of the instrument, calibration procedures were carried out and it was found that a linear relationship existed between the response of the microwave thermograph and the temperature of the calibration medium. This relationship was found to be unaffected by the room temperature and also to be true for different calibrating media including water, water/glycerol mixtures and meat (beef). The calibration curves for two different room temperatures are shown in

Figure 3.1. This figure also shows the temperatures of several body organs recorded by microwave thermography in one clinical case using the Clinical Model of the thermograph. In this case the actual temperature of the patient was known from clinical measurements and it agreed with the temperatures measured by the device. Further measurements of this type were carried out in the laboratory with the Forensic Medicine instrument and are described below. In addition, the two microwave probes were compared and found to give similar temperature readings, varying by less than 1°C, and these are summarised in Table 3.1. In all subsequent monitoring experiments, the same probes were used for liver or brain respectively i.e. Probe 1 was used for the measurement of the liver temperature while Probe 2 was used to monitor the brain temperature.

3.1.2 Effect of Heat on Microwave Probes

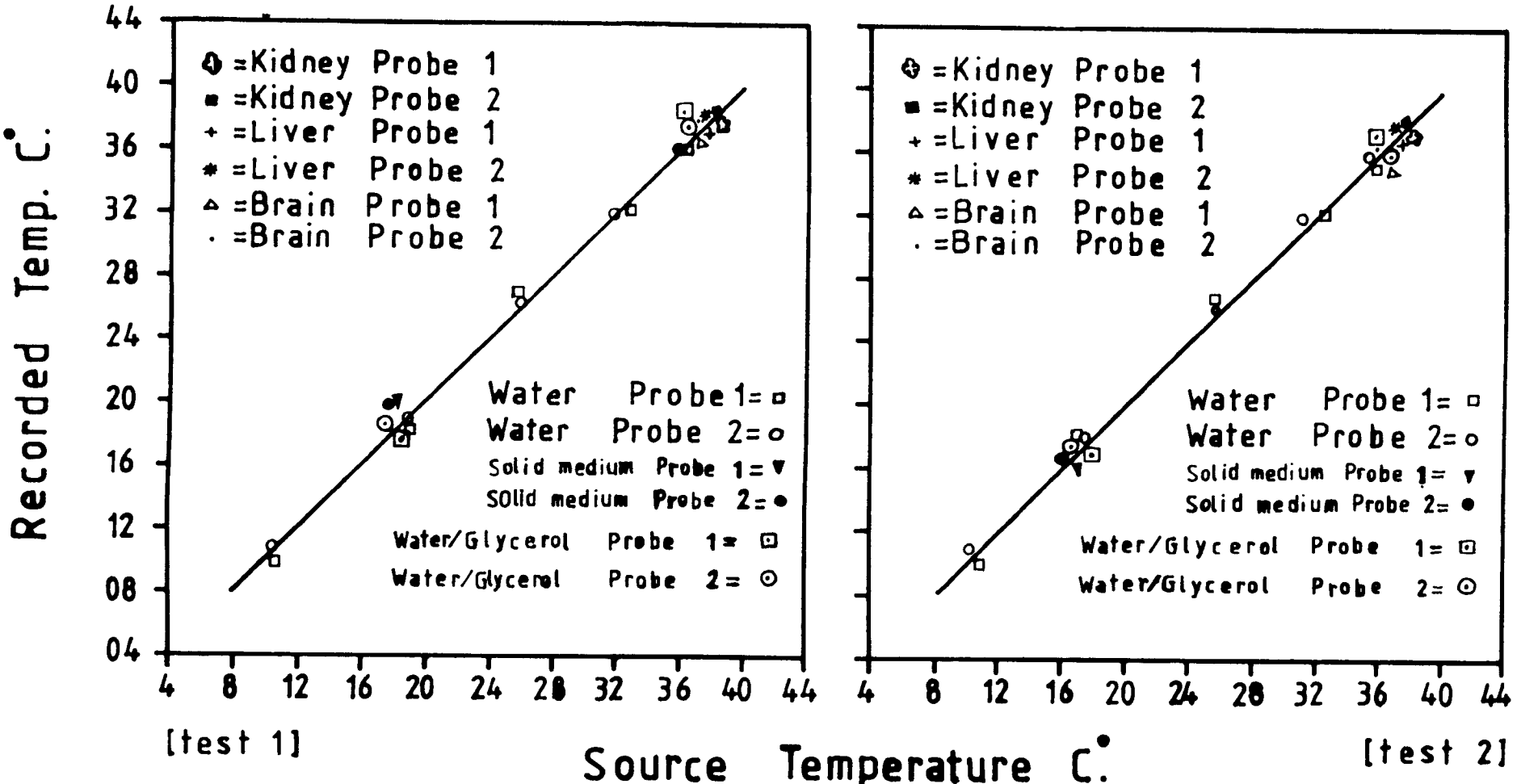
During monitoring of the first few fatalities in the "covered" group, both the body and the liver probe were draped with blankets. In these cases it was observed that the initial temperature lag for the liver was very pronounced or even that the temperature increased significantly soon after monitoring began. This was thought to be due to heating of the probe by body heat when insulated by the blankets. The effect of heat on the temperature readout from the probes was examined by two methods and found to cause a significant increase in the temperature reading. The

Table 3.1 Temperature readings in (^{o}C) of some body organs and source media obtained by microwave probes. Experiments were conducted in warm and cold environments.

I T E M	Source Temperature (^{o}C)	Recorded Temperature (^{o}C) by Probe 1		Recorded Temperature (^{o}C) by Probe 2	
		Test (1)*	Test (2)**	Test (1)*	Test (2)**
Brain	-	36.4	35.8	37.3	36.4
Kidney	-	36.8	36.6	37.4	37.1
Liver	-	36.6	36.4	37.1	36.9
Water	18.5	18.4	18.6	18.4	18.3
	26.0	26.6	26.2	26.4	26.0
	30 - 33	32.8	31.4	32.7	30.5
Water/Glycerol 3/1 v/v	36 - 37	37.2	36.7	36.8	36
	16 - 17	17	16.8	17.0	16.2
Solid Medium	16	-	-	16	16.2
	18	19.9	19.9	-	-

* Warm Environment (23^{o}C)
** Cold Environment (10^{o}C)

Figure 3.1: Calibration Curves for Microwave Probes



Test 1 = Room temperature was 10°C
Test 2 = Room temperature was 23°C

results of these tests are summarised in Table 3.2. In all subsequent monitoring experiments, the liver probe was left uncovered.

3.1.3 Microwave Attenuation in Living and Dead Bodies

The attenuation of microwaves by body tissues was examined in two ways. Firstly, 14 normal healthy adult subjects, with moderate body build (8 male and 6 female), were studied. The mean age was 31.9 ±8.3 years. The average brain temperature, as measured by the microwave probes at the temporal region, was found to be 36.4 ±0.9°C, while when the measurements were made at the mid-frontal region (forehead) the average brain temperature was 35.8 ±0.8°C. Similarly the average liver temperature was 34.12 ±1.8°C. These results are summarized in Table 3.3. Assuming the normal body temperature is 37°C, the conclusion drawn from this experiment was that attenuation of microwaves in living subjects resulted in a lowering of the temperature reading by 0.6 ±0.9°C in the temporal region and 2.9 ±1.8°C in the liver region. It should be mentioned that in all subsequent cases the brain temperature was monitored by placing the microwave probe on the temporal region and not on the mid-frontal area.

Secondly, 5 fatalities were studied as described earlier using both microwave probes and intradermal thermocouples to measure the tissue temperatures of the

Table 3.2: Effect of heat on microwave probes.

METHOD	Probe 1		Probe 2	
	Cold	Warm	Cold	Warm
1*	32.5 ± 0.4	32.2 ± 0.3	33.7 ± 0.4	34.6 ± 0.4
	Uncovered	Covered	Uncovered	Covered
2**	33.8 ± 0.4	35.4 ± 0.4	34.5 ± 0.5	36.9 ± 0.4

* Probes heated with a hairdryer.

** Probes tested on a healthy volunteer (for both probes 1 and 2 uncovered or covered, $z = 8.9$, $P < 0.2\%$ and $Z = 10.7$, $P < 0.2\%$, respectively).

Table 3.3: Temperature of different body sites in ^{o}C taken in living subjects by the microwave probes. (N = 14)

Item	Mean Temperature (^{o}C)	Standard Deviation
Brain (Temple)	36.4	0.84
Brain (Mid-front)	35.8	0.72
Liver (Right Hypochondrium)	34.12	1.73
Age of Cases	31.9	8.26

right hypochondrium in successive layers. Body characteristics such as sex, age, weight, height and the thickness of the abdominal wall of these fatalities are summarized in Table 3.4, which also shows details of the thicknesses of skin, muscle and fat layers in two cases. The latter cases represented the thickest and the thinnest abdominal walls amongst the 5 fatalities studied. The relevance of these details is discussed later (Paragraph 4.2.4).

The results of this work are given in Tables 3.5-3.7 and Figures 3.2-3.5. Temperature values of successive layers of the right hypochondrium, measured at different post-mortem intervals for individual cases of the fatalities studied, are given in Table 3.5. This table also shows brain, environmental and rectal temperatures which were simultaneously taken. Results of each of these fatalities are illustrated in Figure 3.2.

The temperature of the liver centre was compared with the temperature of the body surface, brain, rectum and the liver. These readings were taken from individual cases at different post-mortem intervals: only one set of measurements was made with each cadaver. Temperatures of liver centre and body surface were measured by intradermal thermocouples, brain and rectal temperatures were measured as usual and the temperature of the liver was measured using both Probe 1 and probe 2. Results of this comparision are given

Table 3.4: Characteristics of 5 fatalities studied by microwave probes and intradermal thermocouples for the assessment of microwave attenuation by post-mortem tissues.

CASE NUMBER	SEX	AGE (Years)	WEIGHT (kg)	HEIGHT (m)	ABDOMINAL WALL THICKNESS (cm)
1	Male	61	67	1.7	2.3
2	Female	77	65	1.65	1.8
3	Male	63	70	1.8	2.5
4	Female	75	55	1.5	Skin = 0.2 cm Muscle = 1.0 cm Fat = 0.4 cm Total = 1.6 cm
5	Male	65	90	1.72	Skin = 0.3 cm Muscle = 1.5 cm Fat = 1.7 cm Total = 3.5 cm

Table 3.5: Temperatures measured at successive layers of the right hypochondrium in 5 fatalities studied for the assessment of the microwave attenuation using microwave probes and intradermal thermocouples; brain, environmental and rectal temperatures (°C), which were measured as usual, are also shown; measurements were made at different post-mortem intervals (hours)

CASE NUMBER	POST-MORTEM INTERVAL (hours)	TEMPERATURES IN °C													
		AT BODY SURFACE	at indicated depths from the body surface (centimetres)									measured by Probe (1)	measured by Probe (2)	Rectal	Brain	Environ-mental
			1	2	3	4	5	6	7	8	9-10					
1	4.75	21.4	25	26	27.2	27.7	30.2	–	–	–	32.0	25.5	28.4	32.2	23.1	10.3
	10.45	16.6	17.7	19.3	19.7	–	21.2	22.0	–	22.9	24.6	21.1	22.4	24.4	18.8	10.8
2	3.5	21.9	24.8	25.5	26.6	29.4	29.7	29.7	29.7	29.7	29.7	27.0	29.0	30.8	20.9	13.0
	8.5	18.9	19.3	20.1	20.7	21.5	23.8	–	23.6	–	23.8	22.2	23.2	–	15.5	14.6
	23.0	14.6	15.5	15.7	16.0	16.1	16.5	16.7	16.6	–	16.7	16.0	16.0	16.3	14.8	14.6
3	7.0	21.4	24.7	26.3	28.5	29.5	31.6	31.8	–	32.4	32.9	28.5	29.0	32.5	19.4	11.0
	23.0	12.6	13.9	15.0	–	15.7	18.1	18.1	18.3	18.7	19.2	18.4	18.2	18.4	15.5	9.9
4	2.25	25.9	30.7	32.4	32.9	–	33.6	33.7	–	33.8	33.8	31.7	32.7	32.0	27.6	14.0
	16.4	16.0	16.2	16.5	16.7	–	17.2	18.3	19.9	21.5	24.1	22.0	22.2	26.1	20.5	14.0
5	4.5	19.5	23.4	24.9	25.7	–	29.6	29.5	29.4	30.8	32.2	27.0	27.5	30.2	31.3	12.2
	19.2	12.1	13.4	14.3	14.8	15.2	17.1	17.4	17.2	18.0	20.1	18.7	18.8	22.3	20.9	10.0

Table 3.6: Temperature differences (°C) between the centre of the liver(C) and the body surface(S), brain(B), rectum(R) and readings obtained by Probe 1 and Probe 2 at different post-mortem intervals in hours for individual cases.

POST-MORTEM INTERVAL (hours)	C-S(°C)	C-B(°C)	C-1(°C)	C-2(°C)	C-R(°C)
2.25	7.9	6.2	2.1	1.1	1.8
3.5	7.8	8.8	1.7	0.7	-1.1
4.5	12.7	0.9	5.2	4.7	2.0
4.75	10.6	8.9	6.5	3.6	-0.2
7.0	11.5	13.5	4.4	3.9	0.4
8.5	4.9	8.3	1.6	0.6	-
10.45	8.0	5.8	3.5	2.2	0.2
16.4	8.1	3.6	2.1	1.9	-2.0
19.2	8.0	-0.8	1.4	1.3	-2.2
23.0	4.4	2.9	0.8	0.9	0.7

Table 3.7a: Temperature measurements in ^{o}C taken at different depths from the body surface using the intradermal thermocouples, in addition to the measurements taken by the microwave probes. Measurements were made at different post-mortem intervals.

Site	Mean	S.D.	Number of Measurements
Probe 1	24.1	4.15	11
Probe 2	24.97	4.6	11
Body Surface	18.3	4.3	11
1 cm. deep	20.5	5.6	11
2 cm. deep	21.5	5.9	11
3 cm. deep	23.6	6.0	9
5 cm. deep	24.8	6.3	11
- 10 cm. deep	26.6	5.15	11

Table 3.7b: z and P values of microwave probe readings (^{o}C) as compared with temperature measurements (^{o}C) made at the indicated sites; (N = 11).

SITE FOR COMPARISON	PROBE 1		PROBE 2	
	z	P%	z	P
Body Surface	5.6	<0.2	4.8	<0.2
1cm Deep	2.9	<1	3.2	<0.2
2cm Deep	2.1	<5	2.5	~1
3cm Deep	0.4	>10	1	>10

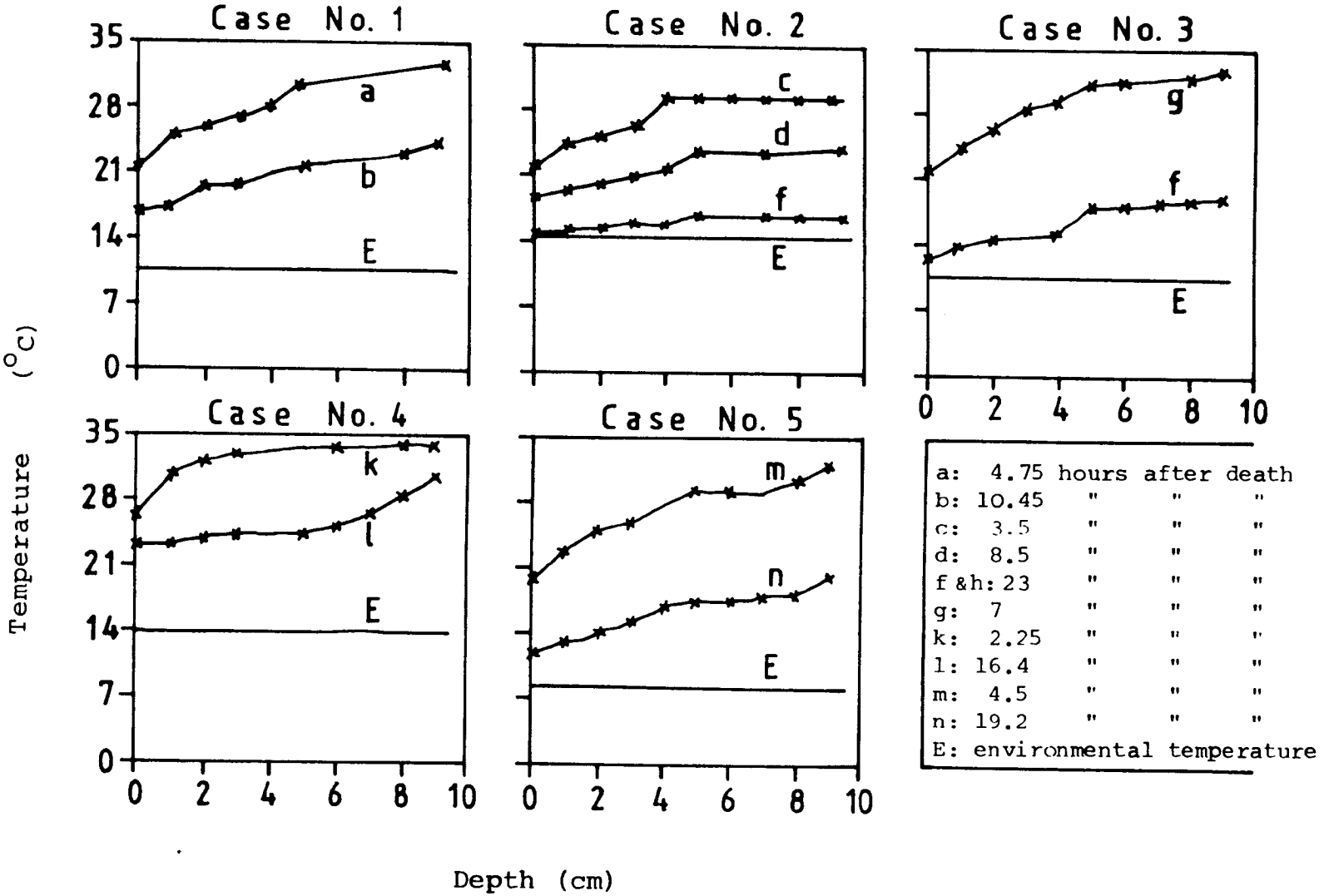
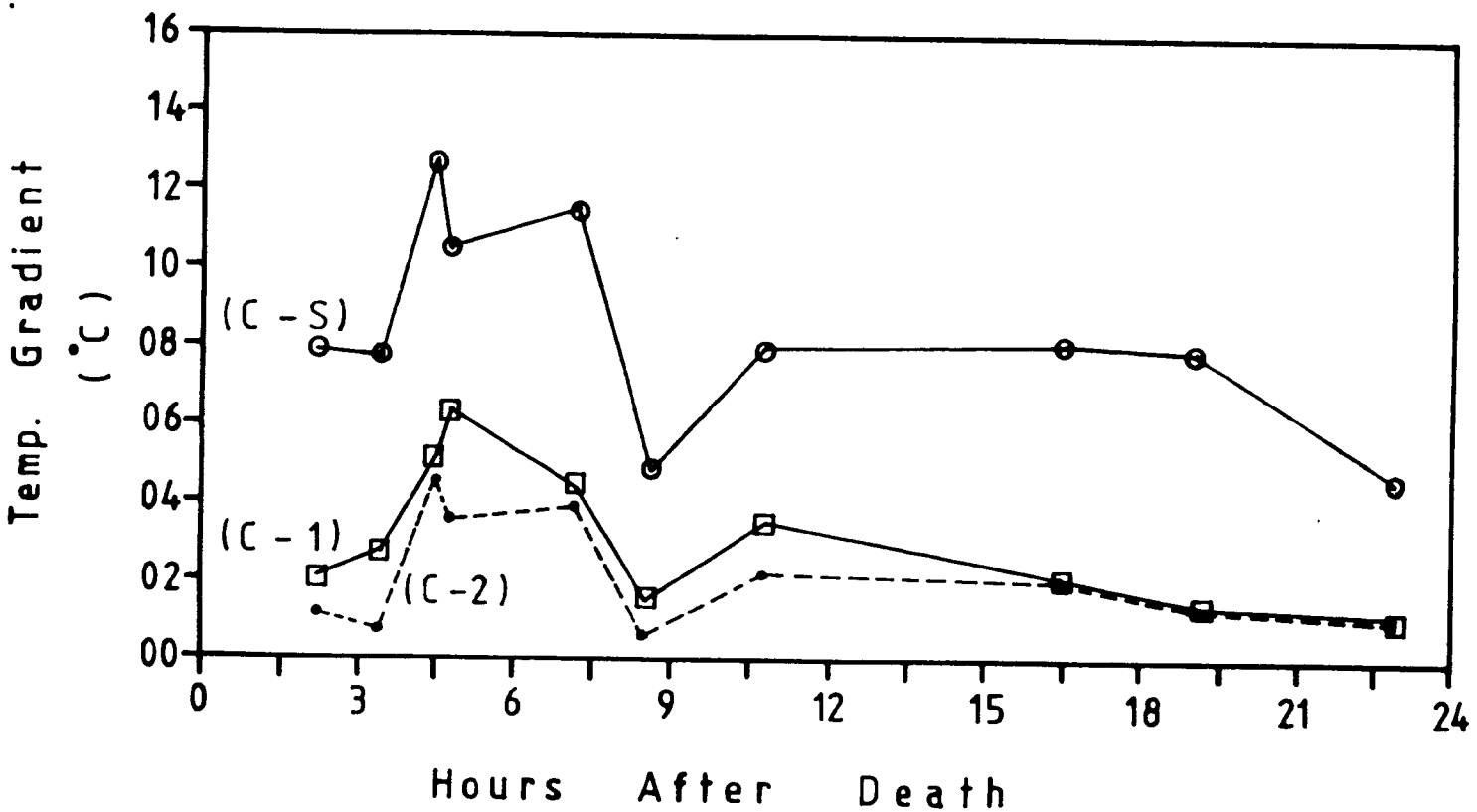


Figure 3.2: Temperature (°C) versus body depth (cm): Measurements made at right hypochondrium using Intradermal Thermocouples for five fatalities.

Figure 3.3 : Temperature differences(°C), between the Body Surface, Microwave Probes Readings and the Centre of the Liver, versus post-mortem interval (hour): (C-S) = Centre - Surface, (C-1) = Centre - Probe 1, (C-2) = Centre - Probe 2.



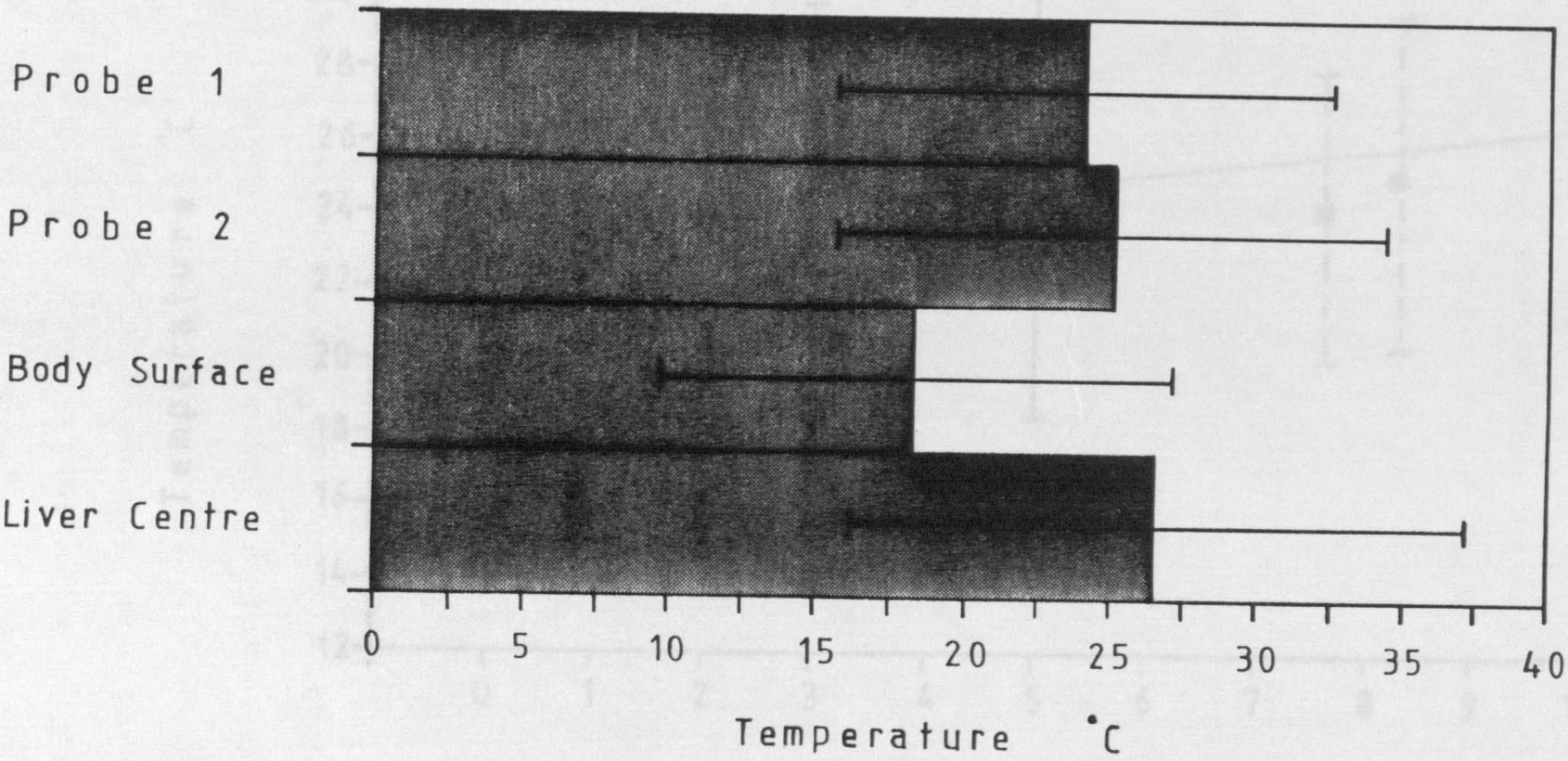
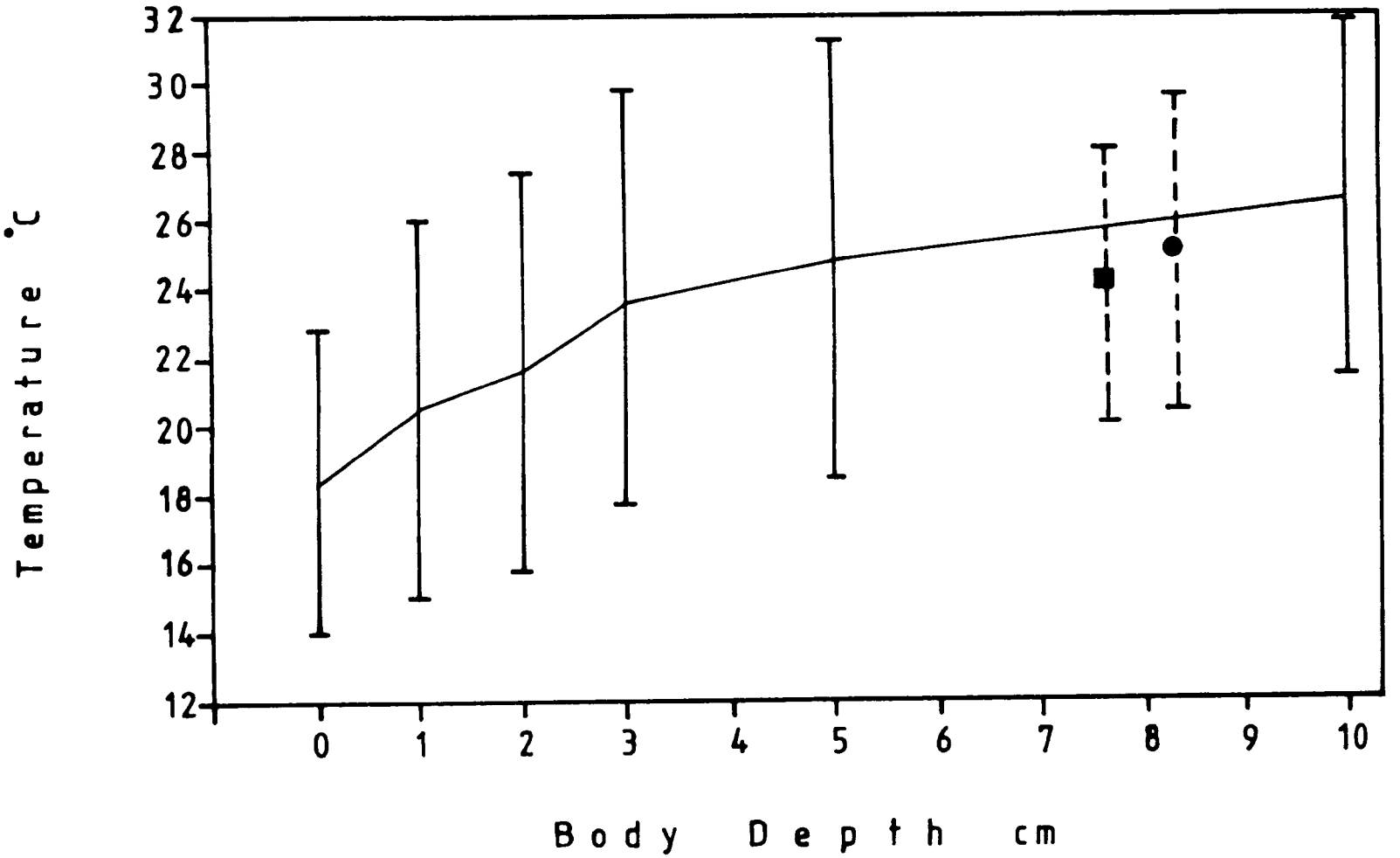


Figure 3.4 : Average values ± two standard deviations of temperature measured at the right hypochondrium using Microwave Probes and Intradermal Thermocouples at different depths from the body surface.

Figure 3.5 : Average temperature(°C) ± one standard deviation versus body depth (Cm); Measurements made at right hypochondrium using Intradermal Thermocouples.



and = Average ± one standard deviation values of measurements made by Microwave probes 1 and 2 respectively.

in Table 3.6. Mean and standard deviations obtained from 11 temperature measurements, made at successive post-mortem intervals for each of Probe 1, Probe 2, body surface and at 1, 2, 3, 5 and 10cm deep from the surface, are given in Table 3.7a. Values of z and P for Probe 1 and 2 compared to the body surface and to measurements made at 1, 2 and 3cm from the surface are shown in Table 3.7b. Values of the body surface temperature and the reading of the microwave probes were subtracted from those of the liver centre temperature. The differences thus obtained were plotted against post-mortem intervals and shown in Figure 3.3. Thus 3 curves were obtained for 2.25-23 hours after death, representing:

Liver centre - body surface temperature differences (C-S)

Liver centre temperatures - temperature readings from Probe 1 (C-1), and,

Liver centre temperatures - readings from Probe 2 (C-2).

Average values of temperature measurements made at 10 post-mortem intervals for each of the liver centre, the body surface and microwave Probes 1 and 2, ±2 standard deviations, are presented in histograms in Figure 3.4. Lastly, average temperature measurements obtained at 10 post-mortem intervals for the body surface, selected distances from the liver centre and also readings from Probes 1 and 2, ±1 standard deviation, are given in Figure 3.5.

The following inferences were drawn from the

study of these 5 fatalities:-

1. Temperature readings obtained by microwave probes were, from a statistical point of view, significantly different from temperature measurements obtained by intradermal thermocouples at the body surface and at 1 and 2cm deep from the surface (Tables 3.5 and 3.7 and Figures 3.2 and 3.3). However, this was not significant for depths of 3cm or more from the surface. In fact, in most cases, temperature readings of the microwave probes represented temperatures of deep body structures, perhaps between 5 and 9cm deep (Figure 3.5). This is better understood if the problem of microwave attenuation is considered.

2. Attenuation of the microwaves by the tissues after death continued over a long post-mortem interval (23 hours in these experiments) and would be expected to continue for longer periods. Data presented in Table 3.6 and Figure 3.3 indicated that the effect of the microwave attenuation was greater at 4.5-7.0 hours after death than at earlier or later intervals. The average values of (C-1) and (C-2) (See Table 3.6) throughout all post-mortem intervals were 2.9 ±1.8°C and 2.0 ±1.4°C respectively. As both probes in these experiments were used to measure the temperature of the liver, both average values were compared with the average attenuation effect on temperature

readings in the liver region obtained from experiments conducted on living subjects (2.97 ±1.8°C). Values of z for both comparisons were found to be 0.17 and 1.79 and hence P values were greater than 10% and 5% respectively. Accordingly it was concluded that on average, the effect of microwave attenuation by body tissues on temperature readings obtained by microwave probes was similar before and after death.

3. The average values for (C-1) and C-2) were also compared with the average attenuation effect on temperature readings in the temporal region obtained in the experiments on living subjects (0.6 ±0.9°C). Values of z this time were 8.4 and 5.16 respectively. Hence P values were less than 0.2% in both situations. This meant that the effects of microwave attenuation by the tissues in the head and those in the right hypochondrium were significantly different. The attenuation effect was also found to be slightly variable from case to case when measurements made at the same post-mortem interval were compared (Table 3.5, Cases 2 and 3, interval 23 hours). However, these inferences should not be generalized as the number of measurements concerned were few.

4. It was found that temperature differences between various regions in the body existed not only immediately after death, but also that these

differences continued for a long post-mortem interval (23 hours in the above experiments and probably longer) (Tables 3.5 and 3.6 and Figures 3.2, 3.3 and 3.5). The greatest temperature differences existed between the liver centre and the surface of the body at the right hypochondrium. The differences between the liver centre and the brain temperatures were also high. Only in one measurement, made at post-mortem interval 19.2 hours, was the brain temperature found to be higher than that of the centre of the liver. In that particular case, the head and neck were markedly congested due to manipulation of the body after death and perhaps the cause or mode of death; congestive heart failure.

3.2 Statistical Analysis of the Cases

3.2.1 Numbers and Characteristics of the Cases

During the period of the research, 128 cases were collected. After the initial data processing 11 cases were found not to be useful for further analysis and were excluded due to faults in the recording, interruption of monitoring or irregularities in the traces as a result of artifacts or electrical noise (spikes). The results described here represent the study of 117 cases, including 74 cases (63%) monitored as naked bodies and 43 cases (37%) monitored as covered bodies.

The range, the mean and the standared deviation values for each of the body parameters of the cases are shown in Table 3.8. These parameters include the age, weight, height, surface area, cooling size factor and the circumferences of the head and the hip.

The age and sex distributions of the cases are summarized in Table 3.9. A histogram of age distribution according to age groups used by the Office of Population Censuses and Surveys is shown in Figure 3.6 which also shows the male/female (M/F) ratio in each age group. The age of the cases ranged from 30-90 years with a mean value of 63.4 ±13.4 years. Most of the cases (69%) were between 55-79 years. The average M/F ratio of the cases was 3.

The weights, heights and the surface areas of the cases are given in Table 3.10, and histograms of the distributions of these parameters are demonstrated in Figure 3.7. Body weights were not measured in 10 cases. In the remainder (i.e. 107 cases) body weights ranged from 42-117 kg with an average weight of 70.5 ±16.3 kg. In respect of the crown-sole lengths (heights) of the examined bodies, the height was not taken in only 4 cases. Minimal and maximal values were 1.52 and 1.98m respectively. The mean was 1.74 ±0.1m. The surface area, in 94 cases, ranged from 1.39 to 2.37m^2. The average was 1.84 ±0.23m^2. The cooling size factor was found to range from 0.019 to 0.037m^2/kg. The mean was

Table 3.8: Characteristics of Cases Studied
(N = 117)

Item	Age (Years)	Hip Circumference (m)	Head Circumference (m)	Weight (kg)	Height (m)	Surface Area (m^2)	Cooling Size Factor (m^2/kg)
Range	30-85	0.76-1.19	0.46-0.71	42-117	1.52-1.98	1.39-2.37	0.019-0.037
Mean	63.4	0.997	0.57	70.5	1.74	1.84	0.027
S.D.	13.4	0.095	0.057	16.3	0.098	0.23	0.0032

Table 3.9: Age and sex distribution of the cases studied.

Age Group (year)	Number of Cases						
	Female	Percent of all cases	Male	Percent of all cases	Total	Percent of all cases	Male/Female Ratio
30 -34	3	2.6	0	0	3	2.6	*
35 -39	0	0	3	2.6	3	2.6	*
40 -44	0	0	1	0.9	1	0.9	*
45 -49	2	1.7	10	8.6	12	10.3	5
50 -54	0	0	8	6.8	8	6.8	*
55 -59	3	2.6	8	6.8	11	9.4	2.7
60 -64	5	4.3	17	14.5	22	18.8	3.4
65 -69	3	2.6	12	10.3	15	12.8	4
70 -74	6	5.1	13	11.1	19	16.2	2.2
75 -79	2	1.7	12	10.3	14	12.0	6
80 -84	3	2.6	4	3.4	7	6.0	1.3
85 -89	2	1.7	0	0	2	1.7	-*
30 -89 (total)	29	24.8	88	75.2	117	100	3

* Either No. of females or No. of males = 0

Table 3.10: Distributions of height, weight and surface area amongst the cases studied. (N = 117)

Height (Number of Cases = 113)			Weight (Number of Cases = 107)			Surface Area (Number of Cases = 94)		
Group(m)	Number	Percent	Group(kg)	Number	Percent	Group(m^2)	Number	Percent
1.52 -1.57	6	5.3	40 -49	8	7.5	1.35 -1.47	5	5.3
1.58 -1.63	9	8.0	50 -59	13	12.2	1.48 -1.6	9	9.6
1.64 -1.69	24	21.2	60 -69	30	28.0	1.61 -1.73	16	17.0
1.7 - 1.75	31	27.4	70 -79	26	24.3	1.74 -1.86	27	28.7
1.76 -1.81	16	14.2	80 -89	16	15.0	1.87 -1.99	13	13.8
1.82 -1.87	16	14.2	90 -99	4	3.7	2.0 - 2.12	12	12.8
1.88 -1.93	9	8.0	100 -109	6	5.6	2.13 -2.25	7	7.5
1.94 -2.00	2	1.7	110 -119	4	3.7	2.26 -2.38	5	5.3
Total 1.52 -2.00	113	100	40 -119	107	100	Total 1.35 -2.38	94	100

Figure 3.6:

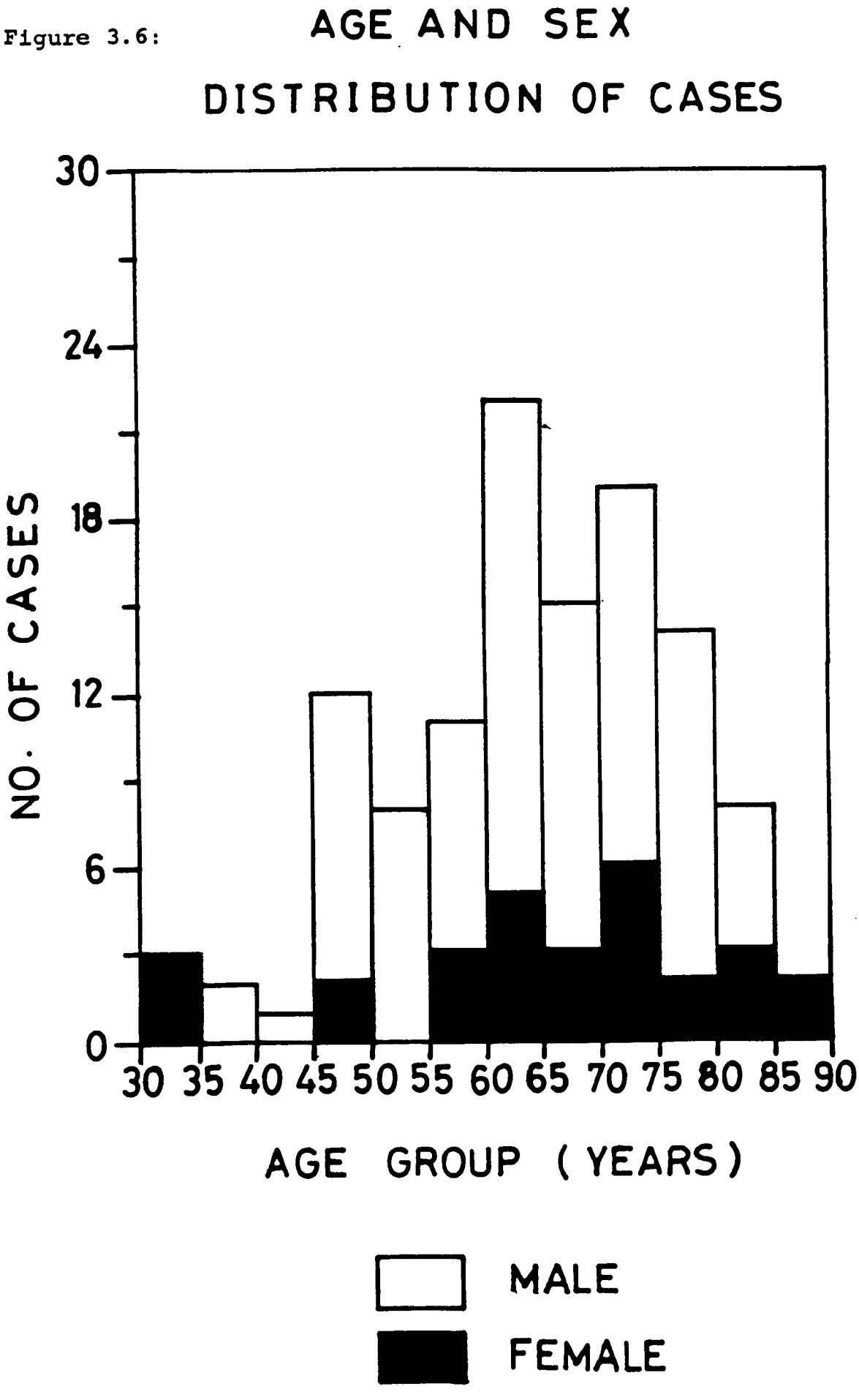
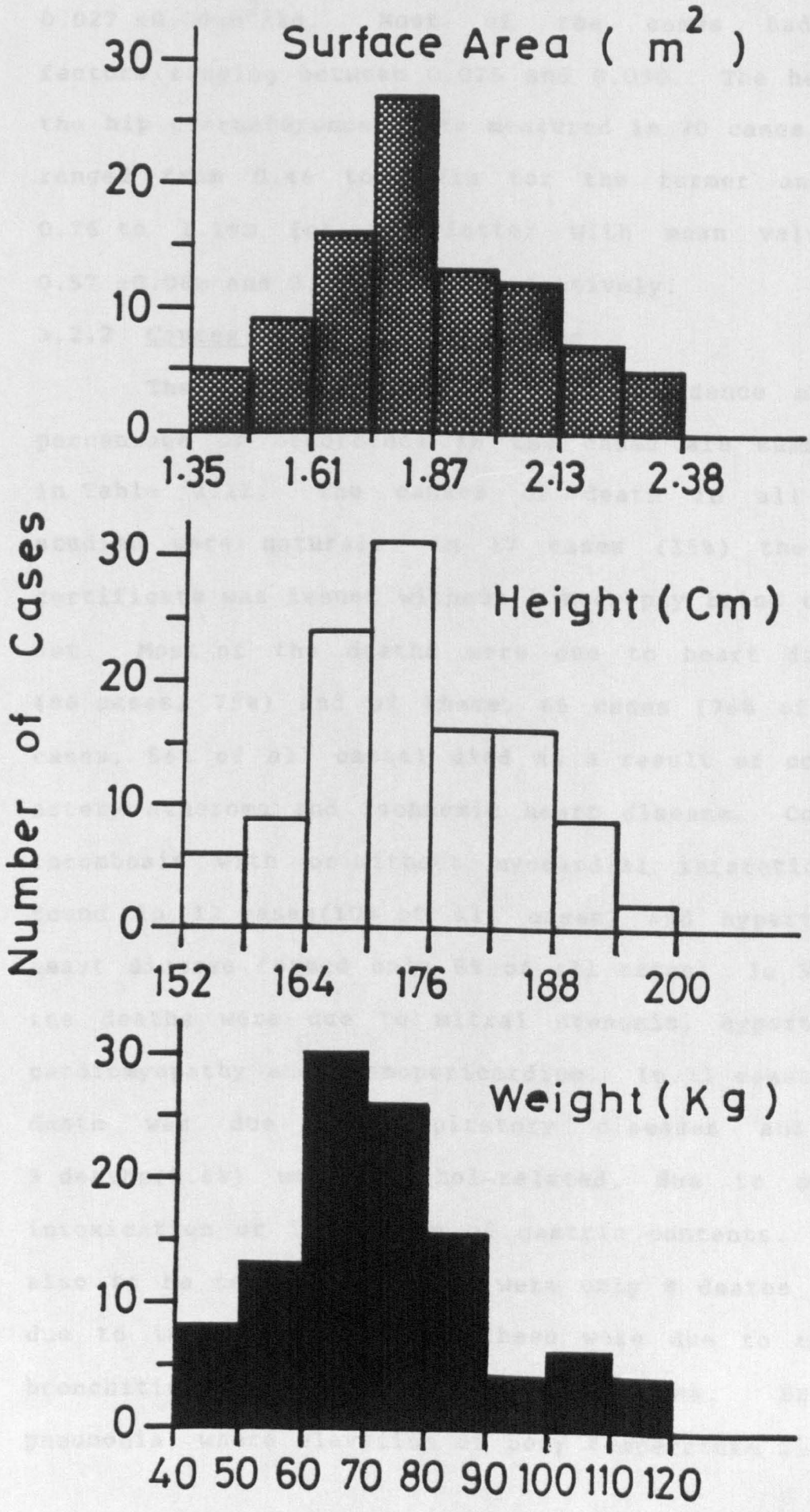

AGE AND SEX
DISTRIBUTION OF CASES
30
24
18
12
6
0
NO. OF CASES
30 35 40 45 50 55 60 65 70 75 80 85 90
AGE GROUP (YEARS)
MALE
FEMALE

Figure 3.7: Size Factors Distribution



0.027 ±0.003m^2/kg. Most of the cases had size factors ranging between 0.025 and 0.030. The head and the hip circumferences were measured in 70 cases. They ranged from 0.46 to 0.71m for the former and from 0.76 to 1.19m for the latter with mean values of 0.57 ±0.06m and 0.89 ±0.09m respectively.

3.2.2 Causes of Death In the Cases

The causes of death, their incidence and the percentage of occurrence in the cases are summarized in Table 3.11. The causes of death in all cases studied were natural. In 17 cases (15%) the death certificate was issued without a necropsy being carried out. Most of the deaths were due to heart diseases (86 cases, 75%) and of these, 65 cases (76% of heart cases, 56% of all cases) died as a result of coronary artery atheroma and ischaemic heart disease. Coronary thrombosis with or without myocardial infarction was found in 12 cases(10% of all cases) and hypertensive heart disease formed only 5% of all cases. In 3 cases the deaths were due to mitral stenosis, hypertrophic cardiomyopathy and haemopericardium. In 11 cases(9.4%) death was due to respiratory diseases and only 3 deaths(2.6%) were alcohol-related, due to alcohol intoxication or inhalation of gastric contents. It is also to be noted that there were only 8 deaths (6.8%) due to infection and 6 of these were due to chronic bronchitis with or without emphysema. Broncho-pneumonia, where elevation of body temperature might be

Table 3.11: Causes of death in cases studied

Cause of death	Number of Cases	Percent of all %
Ischaemic Heart Disease	36	30.8
Coronary Artery Atheroma and Ischaemic Heart Disease	29	24.8
Coronary Thrombosis	6	5.1
Myocardial Infarction	6	5.1
Hypertensive Heart Disease	6	5.1
Other Heart Diseases*	3	2.6
All Heart Causes	86	73.5
Chronic Bronchitis with or without Emphysema	6	5.1
Bronchopneumonia	2	1.7
Asthma and Chronic Obstructive Airways Diseases	2	1.7
Bronchial Carcinoma	1	0.85
All Respiratory Causes	11	9.4
Alcohol Related Deaths**	3	2.6
Unknown (Natural Causes without Necropsy)	17	14.5
TOTAL	117	100

* such as: mitral stenosis, hypertrophic cardiomyopathy and haemopericardium.

** such as: alcoholism, alcohol intoxication and inhalation of vomit.

expected, occurred in only 2 cases (1.7% of all cases). The remaining 3 cases involved asthma, bronchial carcinoma and chronic obstructive airways disease.

3.2.3 Monitoring Periods

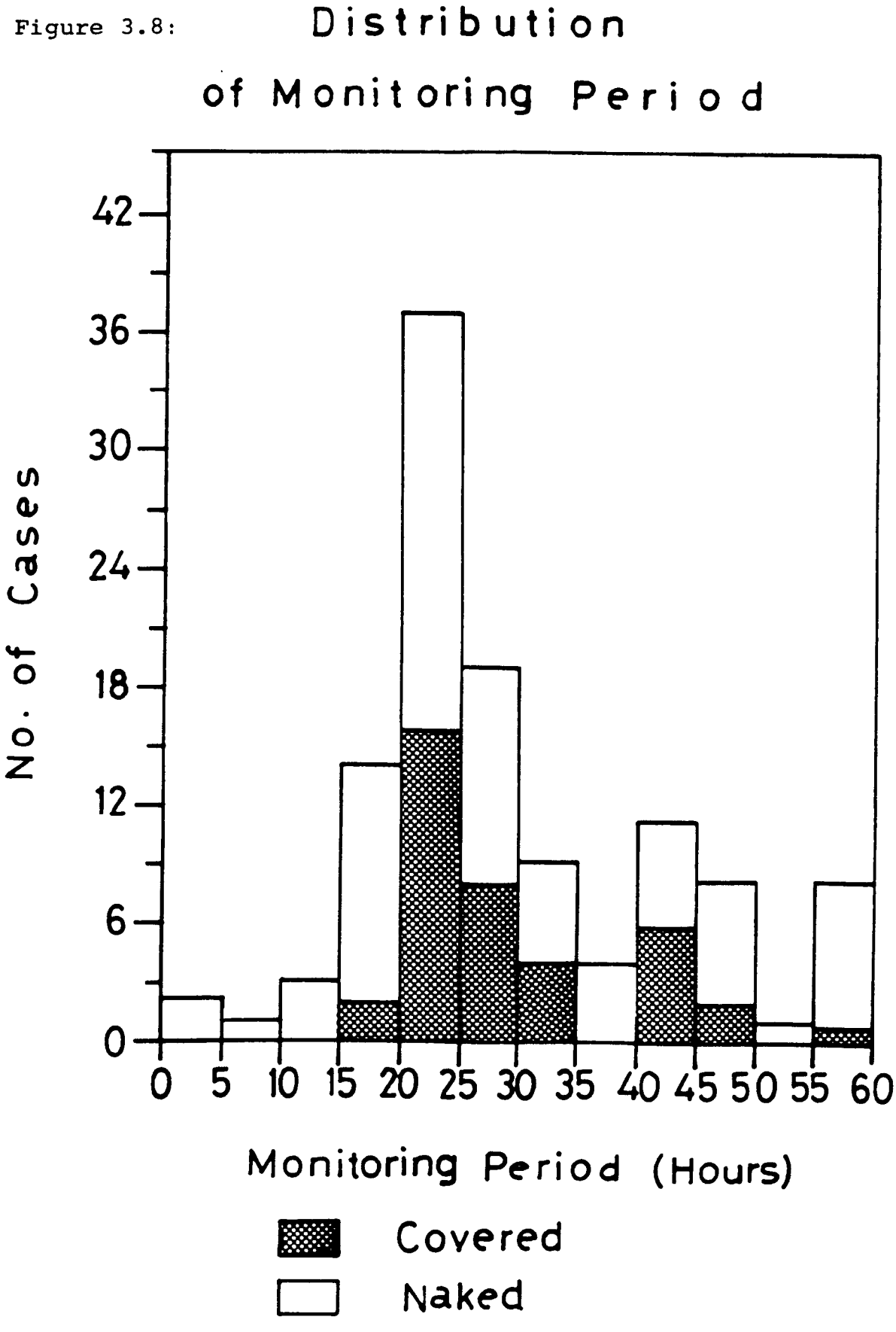
Distributions of the monitoring periods and the post-mortem intervals which had elapsed before the start of the monitoring are shown in Table 3.12. In the same table are given the number and percentage of cases in each group for naked and covered bodies as well as values for all cases. An illustrative histogram of the distribution of the monitoring periods among the cases is shown in Figure 3.8 which also shows the number of naked and covered bodies studied. The distribution of the post-mortem intervals before the beginning of monitoring is shown in a histogram in Figure 3.9.

The monitoring periods ranged from 2.5-60 hours with an average value of 30.25 hours. It is clear from Figure 3.8 that, apart from three cases which were monitored for less than 10 hours, all cases were monitored successfully for long periods. Most of the cases (79 cases, 68%) were monitored for 15-36 hours after death. In fact there were 3 cases which were originally monitored for 72 hours. Although the data of the whole 72 hours were recorded with a four-pen recorder on a chart as described earlier (Paragraph 2.6.2), only the data for the first 60 hours

Table 3.12: Distributions of monitoring periods (hours) and time intervals elapsed between death and the beginning of monitoring in the cases studied.

	Monitoring Period (hours) Mean = 30.25, S.D. = 12.7						Post-mortem Interval Before Monitoring (minutes) Mean = 32, S.D. = 15		
Range Group	N a k e d		C o v e r e d		T o t a l		Range Group	Number	Percent
(hours)	Number	Percent	Number	Percent	Number	Percent	(minutes)		
0-5	2	1.7	0	0	2	1.7	0-14	0	0
6-10	1	0.9	0	0	1	0.9	15-29	35	29.9
11-15	3	2.6	0	0	3	2.6	30-44	39	33.3
16-20	11	9.4	3	2.6	14	12.0	45-59	16	13.7
21-25	20	17.1	17	14.5	37	31.6	60-74	14	12.0
26-30	10	8.5	9	7.7	19	16.2	75-89	4	3.4
31-35	5	4.3	4	3.4	9	7.7	90-104	3	2.6
36-40	4	3.4	0	0	4	3.4	105-120	2	1.7
41-45	4	3.4	7	6	11	9.4	121-135	1	0.9
46-50	6	5.1	2	1.7	8	6.8	136-150	1	0.9
51-55	1	0.9	0	0	1	0.9	151-165	0	0
56-60	7	6	1	0.9	8	6.8	166-180	2	1.7
(Total) 6-60	74	63.3	43	36.7	117	100	(Total) 15-18	117	100

Figure 3.8:


Distribution
of Monitoring Period
No. of Cases
42
36
30
24
18
12
6
0
0 5 10 15 20 25 30 35 40 45 50 55 60
Monitoring Period (Hours)
Covered
Naked

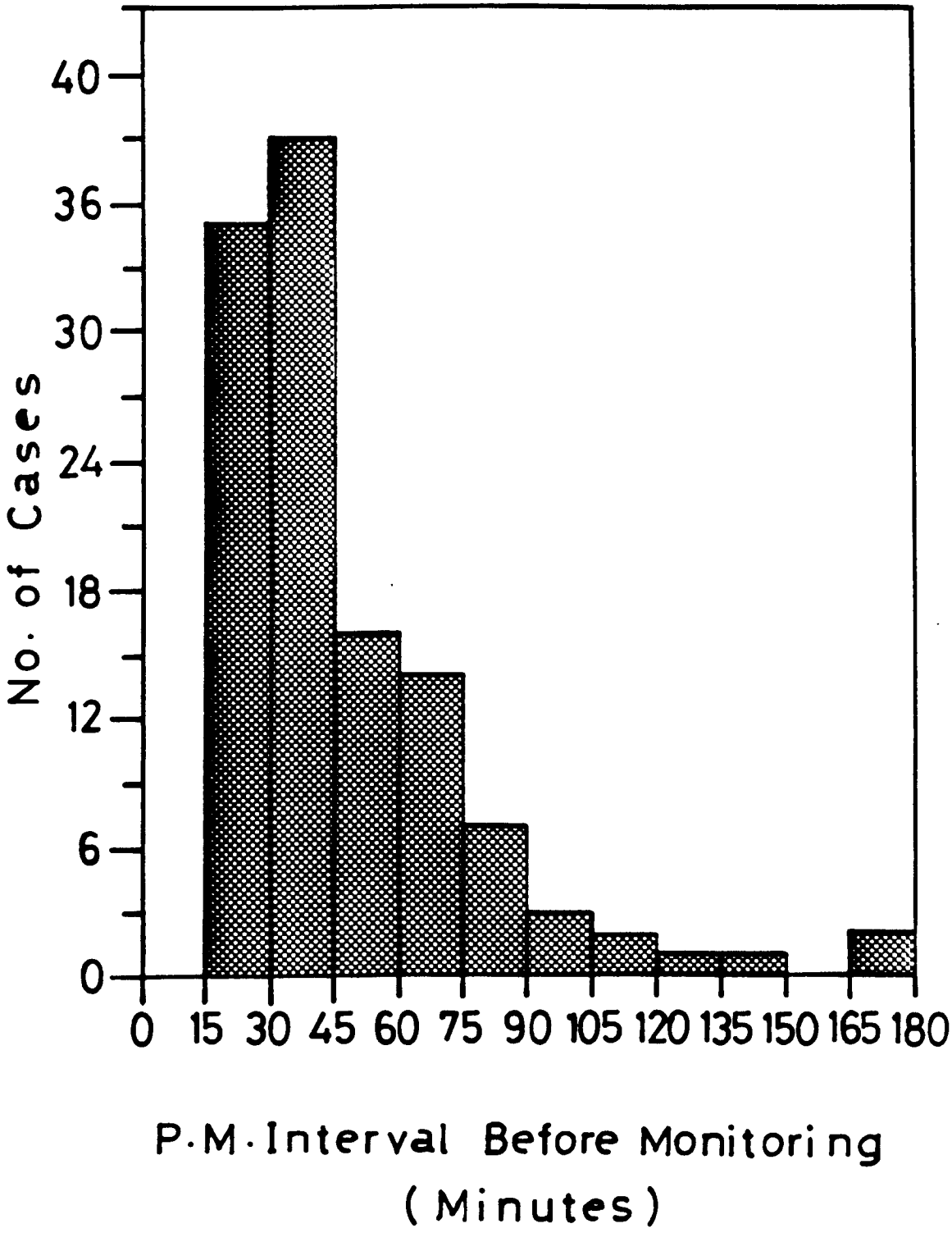


Figure 3.9: Distribution of the elapsed time between the moment of death and initiation of monitoring (all cases).

were digitized and kept for further processing and analysis. It is also worth noting that the distributions of monitoring periods in naked and covered bodies were almost identical.

The mean lapsed time between death and the start of monitoring was 32.3 ±15 minutes. Again the distribution shown in Figure 3.9 is asymmetrical because most cases (74 cases or 63.3%) are aggregated in the region from 15-45 minutes. In fact no body was monitored within 15 minutes post-mortem. As efforts were made to begin monitoring soon after death, there were only two cases in which monitoring started after an elapsed period of 3 hours.

Environmental temperatures under which monitoring was carried out ranged from 8.38°C to 22.76°C. The mean value was 15.2 ±3.2°C.

3.3 Data Recording and Processing

3.3.1 Recording of the Data

For each case, the temperatures of the brain, liver, rectum and the environment were measured every 5 or 10 minutes throughout the monitoring period. In the cases where temperatures were originally recorded by a four-pen recorder on charts, data were stored on disks following manual digitization as described earlier (Paragraph 2.6.2). For most of the cases the microcomputer was used for the AD conversion (Paragraph 2.6.1). In this method temperature data were recorded

on magnetic tapes which were easily conveyed or stored. Although the manual digitization procedure was accurate and relatively easy to perform, the AD conversion was found to be far more accurate and easier in practice as well as being more rapid. The values returned by the ADVAL function were found to have a good linear relationship to the converted voltages as measured by a voltmeter (Figure 2.6). Accordingly, the ADC was used in all subsequent data acquisition.

3.3.2 Primary Processing of the Data

Initially, raw temperature data for the brain, liver, rectum and the environment in every case were plotted versus post-mortem time. These plots were then visualized on a monitor screen and electrical spikes were located as described earlier (Paragraph 2.6.3). Correction of these spikes was successfully carried out by editing the file and removing the aberrant data. An in-built word processor programme (Wordwise) was used for this purpose. Examples of uncorrected and corrected traces are shown in Figure 3.10. It is important to notice that the correction procedure has not changed the shape of the traces or altered the remaining correct data. This procedure was not difficult but was time consuming. Other primary data reduction was satisfactorily carried out to produce, for example, plots of temperature versus time, temperature difference (body site minus environment) versus time, site temperature ratio (ratio of temperature at two

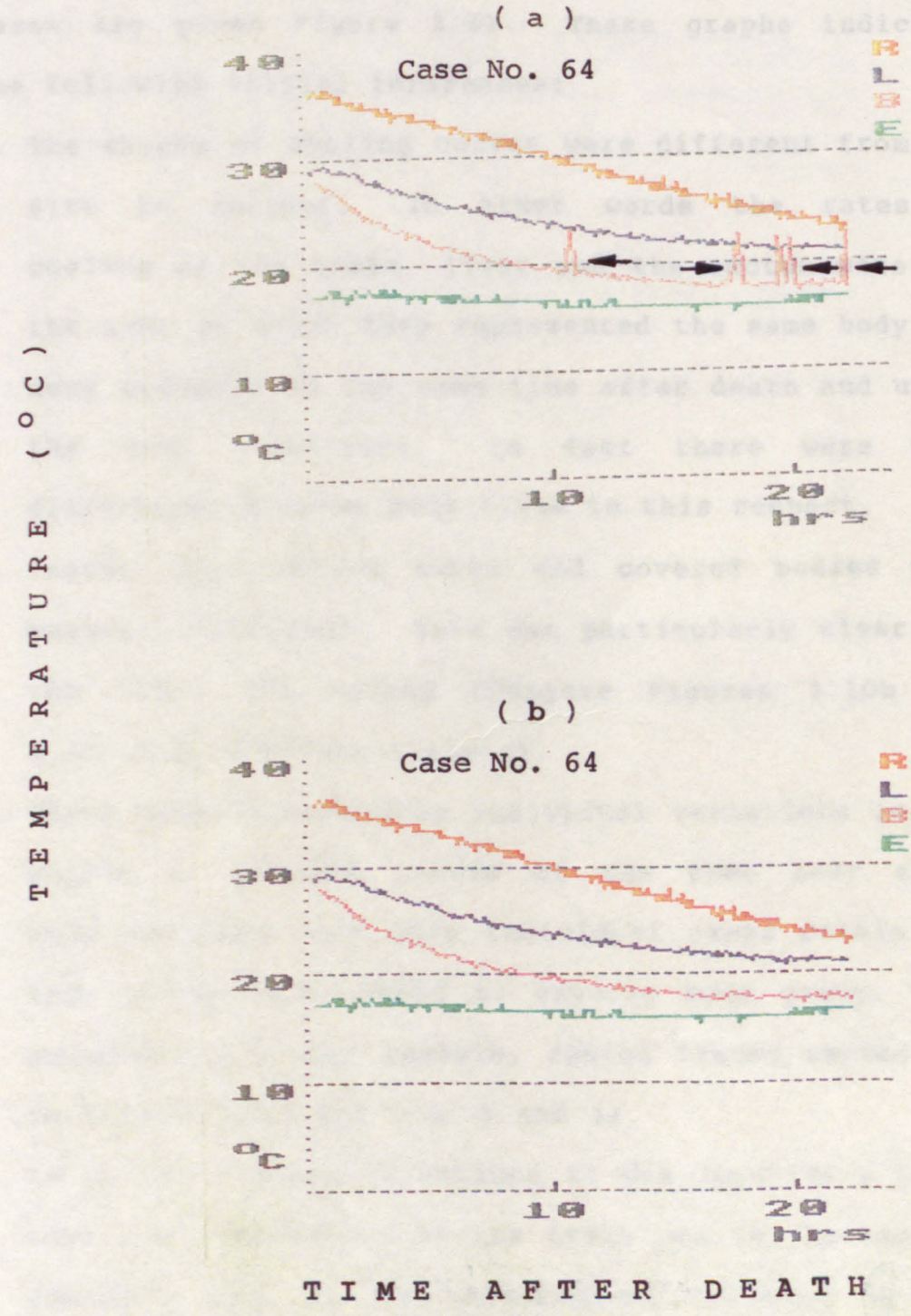


Figure 3.10: Plots of temperatures (^{o}C) of three body sites and environment versus post-mortem interval (hours): (a) An example of uncorrected trace of brain temperature where "spikes"(arrows) are shown. (b) The same case after the correction of the spikes. R = rectal (red), L = liver (blue), B = brain (magenta) and E = environment (green).

body sites) versus time and temperature difference ratio (Equation 2.2) versus time. Plots of typical cases are given Figure 3.11. These graphs indicated the following initial inferences:

1. The shapes of cooling curves were different from one site to another. In other words the rates of cooling of the brain, liver and the rectum were not the same although they represented the same body and were measured at the same time after death and under the same conditions. In fact there were wide differences between body sites in this respect.
2. Traces representing naked and covered bodies were markedly different. This was particularly clear for the liver and rectum (Compare Figures 3.10b and 3.11a-f with Figure 3.11g-j).
3. There were considerable individual variations in the shapes of cooling curves of the same body site. This was also true when individual cases within the same group, i.e. naked or covered body group, were compared (See, for example, rectal traces marked (R) in Figure 3.11a and b or h and j).
4. In spite of these variations it was repeatedly noted that the temperature of the brain was the fastest to approach that of the environment followed by the liver then the rectum. This order was changed in the covered body group where the liver became the slowest to cool. Also in the covered group, it was observed that the initial temperature lag for the

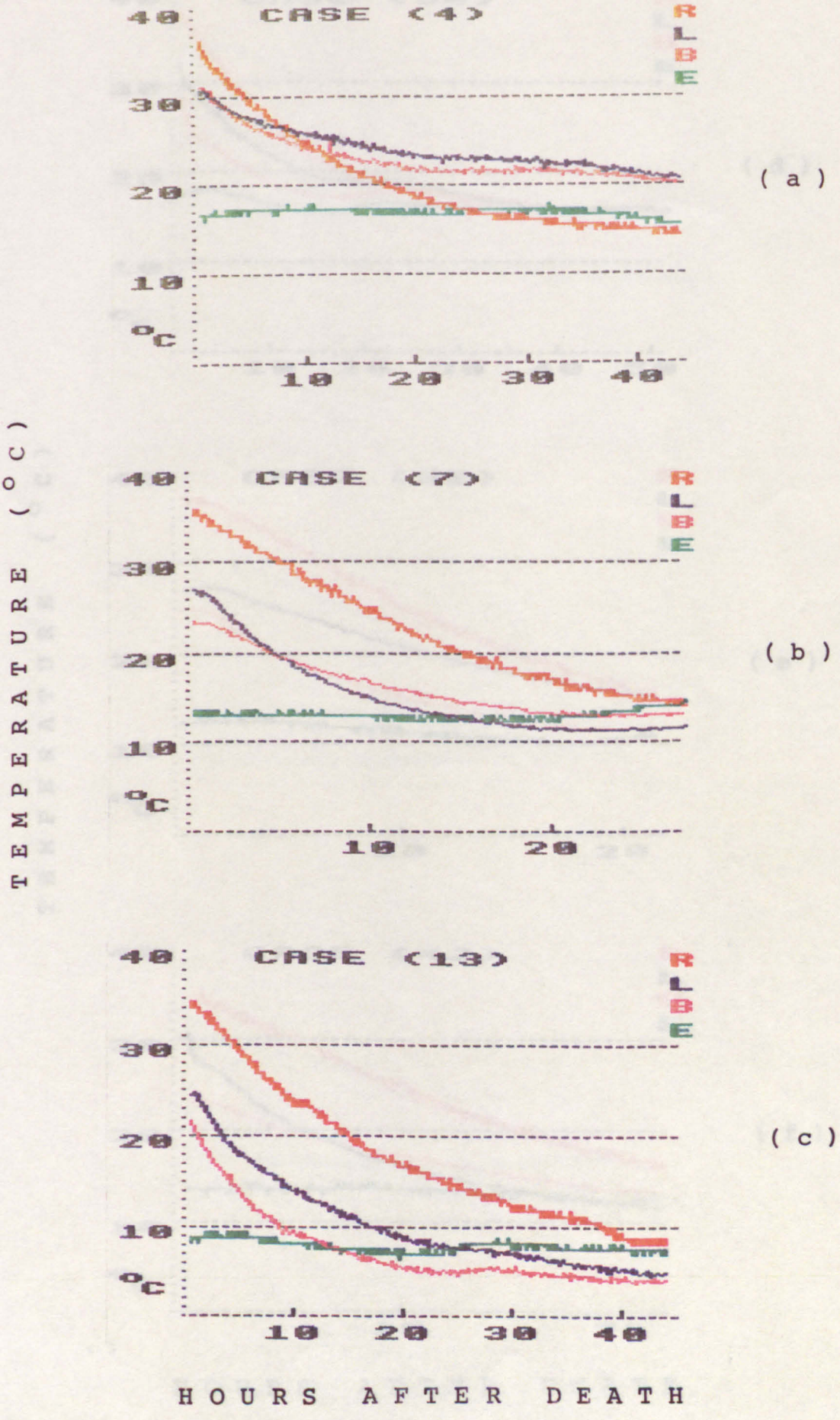


Figure 3.11: Graphs of temperature versus time for selected cases (continued next page).

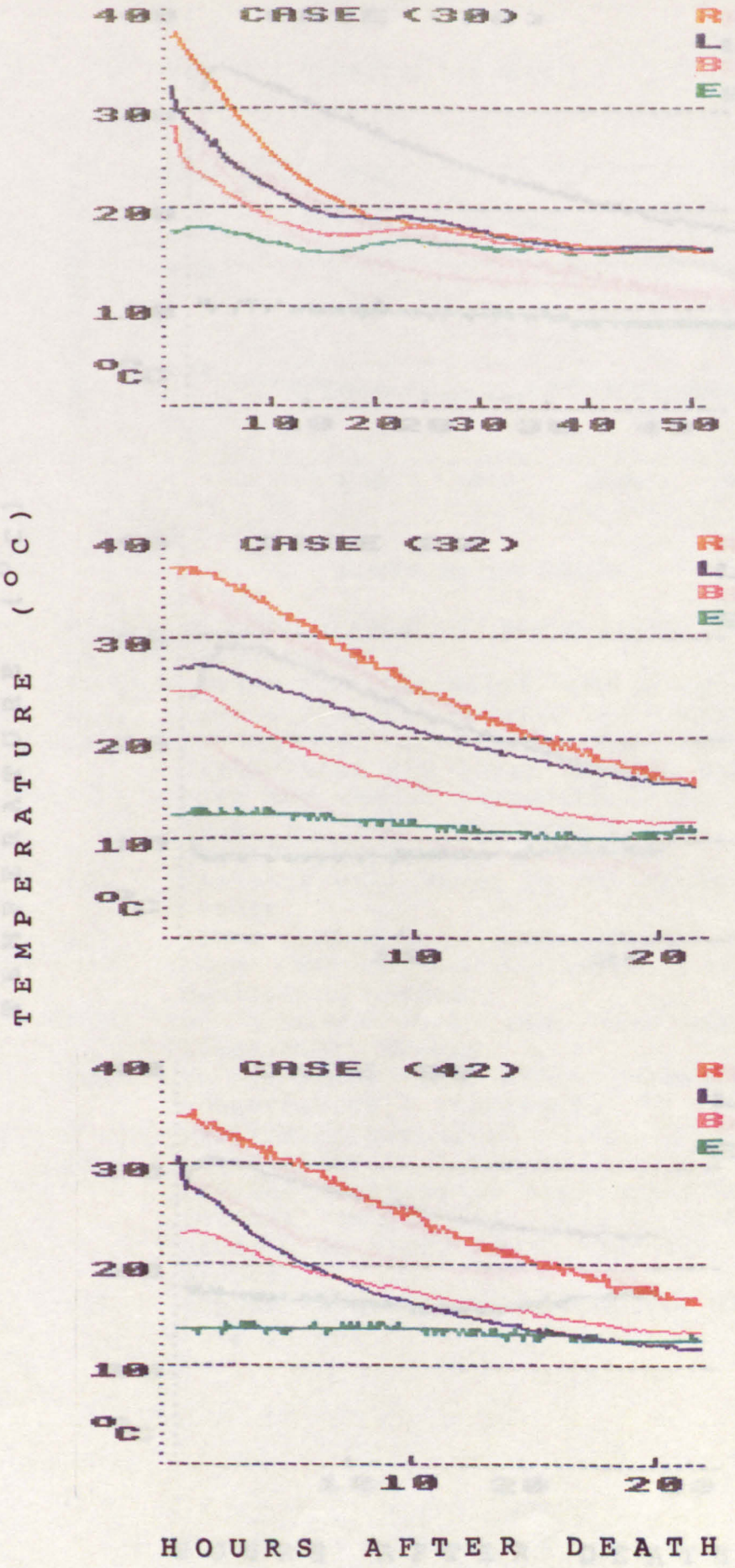


Figure 3.11: Graphs of temperature versus time for selected cases (continued next page).

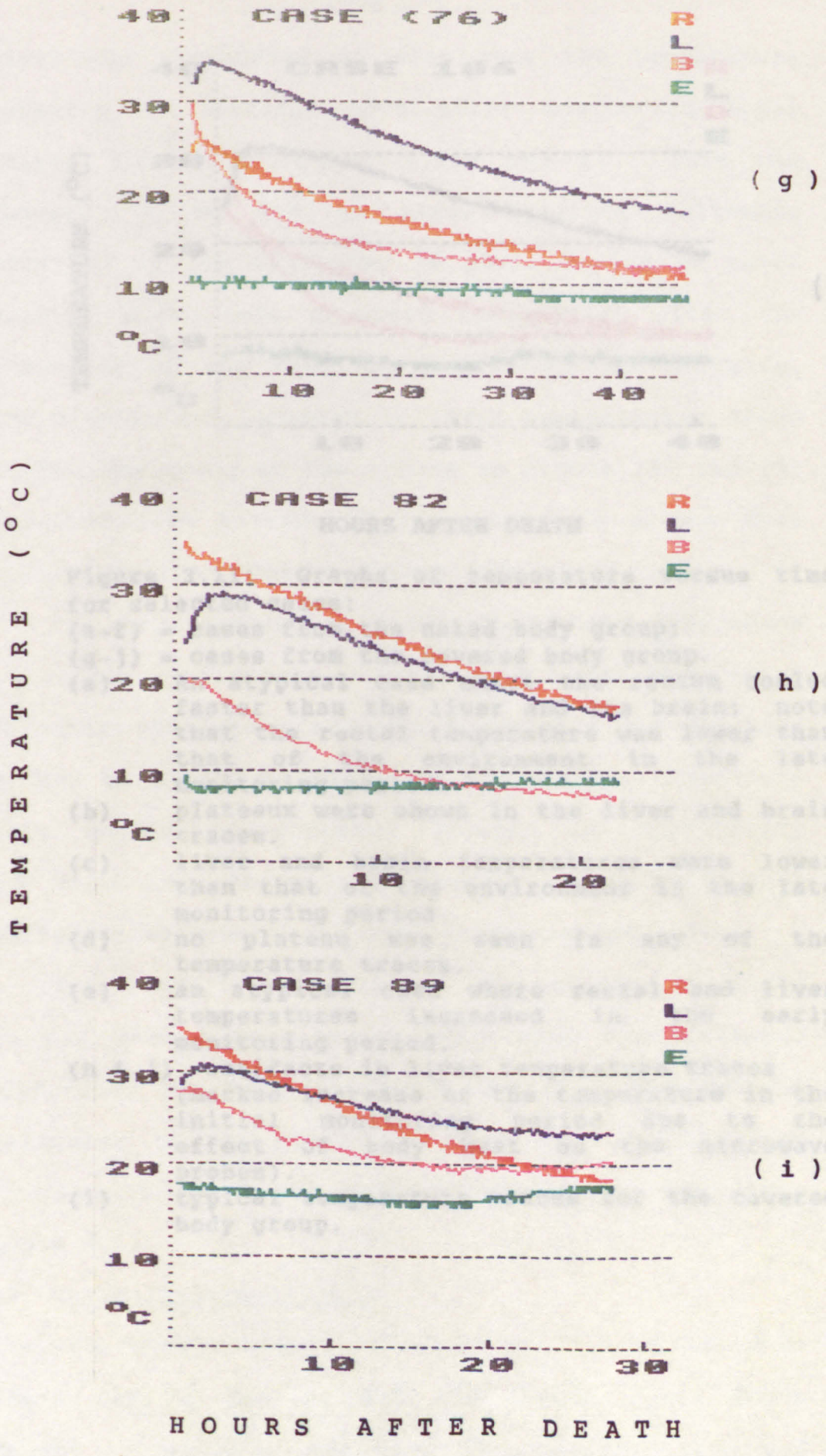
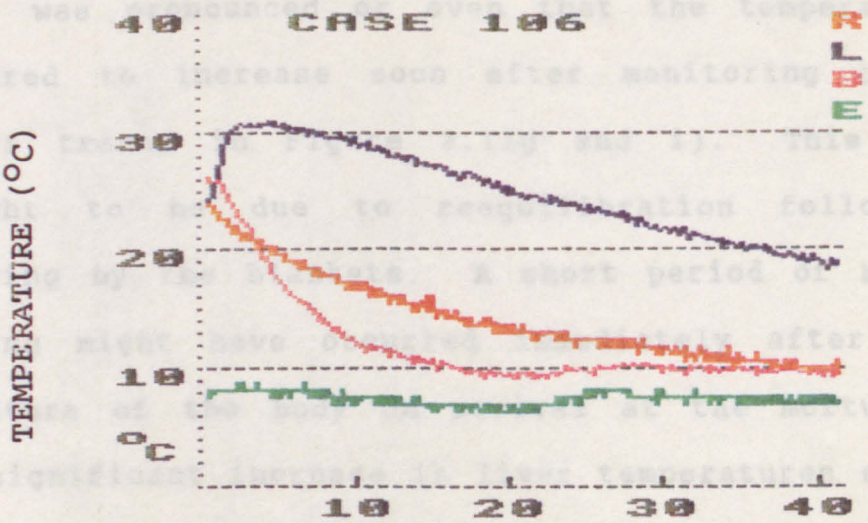


Figure 3.11: Graphs of temperature versus time for selected cases (continued next page).



(j)

Figure 3.11: Graphs of temperature versus time for selected cases:
(a-f) = cases from the naked body group;
(g-j) = cases from the covered body group.

(a) An atypical case where the rectum cooled faster than the liver and the brain; note that the rectal temperature was lower than that of the environment in the late monitoring period.

(b) plateaux were shown in the liver and brain traces.

(c) liver and brain temperatures were lower than that of the environment in the late monitoring period.

(d) no plateau was seen in any of the temperature traces.

(e) an atypical case where rectal and liver temperatures increased in the early monitoring period.

(h & j) artifacts in liver temperature traces (marked increase of the temperature in the initial monitoring period due to the effect of body heat on the microwave probes).

(i) typical temperature traces for the covered body group.

liver was pronounced or even that the temperature appeared to increase soon after monitoring began (liver traces in Figure 3.11g and i). This was thought to be due to reequilibration following covering by the blankets. A short period of rapid cooling might have occurred immediately after the divesture of the body on arrival at the mortuary. The significant increase in liver temperatures shown at the beginning of the traces in Figure (h) and (j) resulted from heating of the microwave probes when covered by the blankets (Paragraph 3.1.2).

The main conclusion drawn from these inferences was that the post-mortem cooling of a human body was a complicated phenomenon and would probably not be well described by simple mathematical procedures. Therefore efforts should be directed towards an appropriate method which was chosen to be curve-fitting of the data to different mathematical formulae (Paragraph 3.3.3).

The body temperature at the moment of death (T_{b0}) for each body site in every case was estimated as described earlier (Paragraph 2.6.4). The average temperatures at the moment of death for brain, liver and rectum in naked and covered body groups are given in Table 3.13.

3.3.3 Curve-fitting Class 1

Following the initial treatment, the validity of Newton's Law of Cooling and the linear model (Rule of Thumb) to describe the data was tested. This was

Table 3.13: Average values for body site temperatures at the moment of death in naked and covered bodies (N = 74 and 43 respectively)

BODY SITE	Average Temperature	Standard Deviation
Brain (naked)	26.5	3.1
Brain (covered)	27.7	3.7
Liver (naked)	27.5	3.1
Liver (covered)	32.7	2.9
Rectum (naked)	36.6	2.0
Rectum (covered)	32.2	4.8

carried out for thirty curves (i.e. 10 cases, each with a curve for each of the three body sites). The test was conducted as follows:-

1. The whole course of each cooling curve of this group was fitted to a single-exponential formula (Newtonian formula) and also to a first order polynomial (straight line) equation. Actual and calculated data were plotted versus time and compared. Examples of these plots are shown in Figures 3.12 and 3.13. These figures indicated that both models gave an unsatisfactory description of the data. It was also found that Newton's Law was unable to describe the curve accurately in 8 cases (24 curves, 87%). This was particularly true at the beginning and at the end stages (Figure 3.12). However, there were 4 curves, i.e. 13% of all tested curves, where the Law and its formula were sufficient to produce good fits (RMS = 0.8 ±0.4). An example of these cases and their Newtonian fit is given in Figure 3.14. The curve-fitting was said to be good when the Residual Mean Square (RMS) resulting from it was small and the curves of the fitted and the actual data were close to each other throughout most or all of the monitoring period (compare Figures 3.12 and 3.13 with Figure 3.14). The average value of the RMS for curve-fitting to polynomial equations was 1.9 ±0.3, and the value for Newtonian curve-fitting was 1.3 ±0.5.

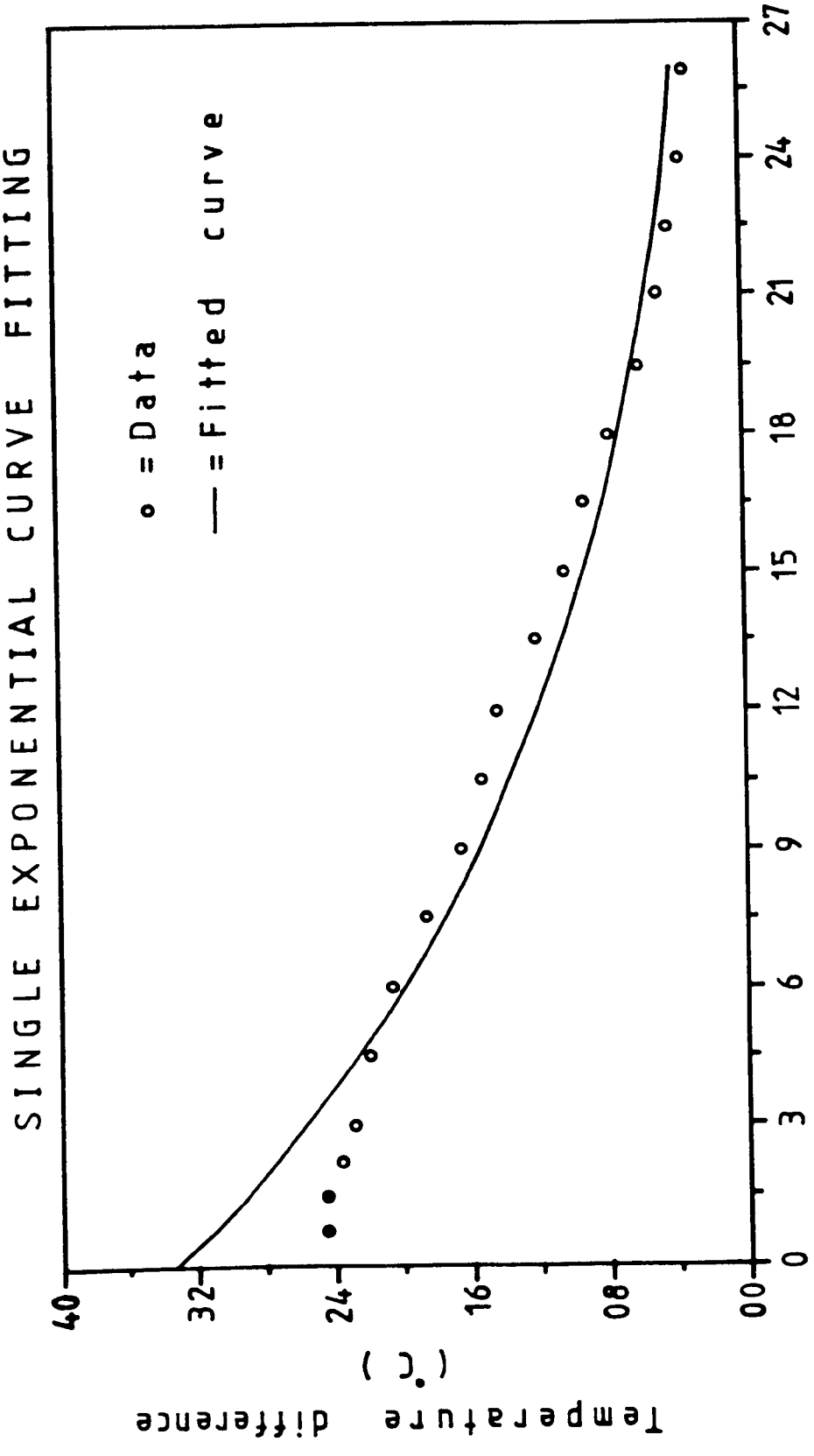

SINGLE EXPONENTIAL CURVE FITTING
o = Data
— = Fitted curve
Temperature difference (°C)
40
32
24
16
08
00
0
3
6
9
12
15
18
21
24
27
Hours after death

Figure 3.12:

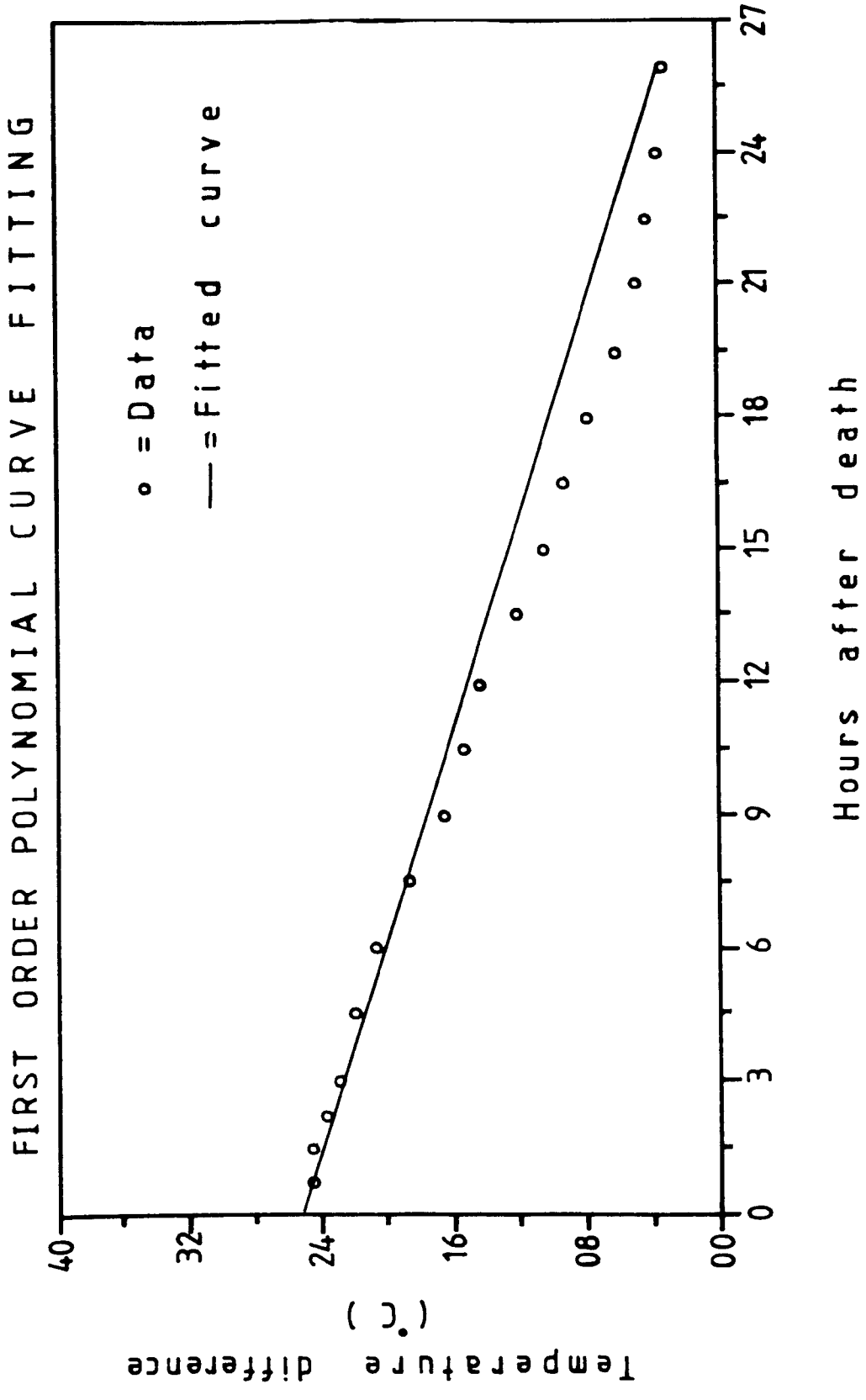
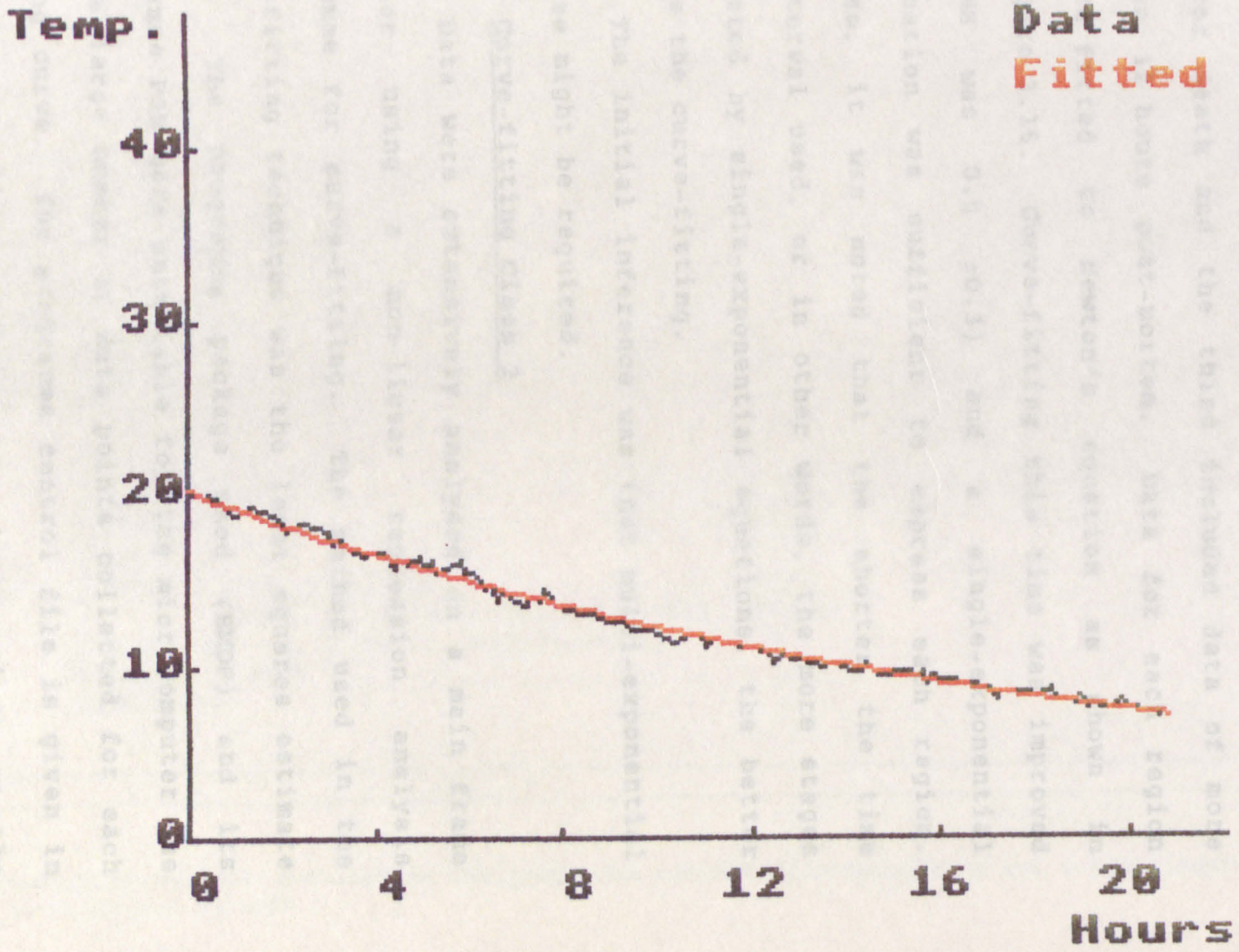

FIRST ORDER POLYNOMIAL CURVE FITTING
o = Data
—— = Fitted curve
Temperature difference (°C)
40
32
24
16
08
00
0
3
6
9
12
15
18
21
24
27
Hours after death

Figure 3.13:

Figure 3.14: Good fit by Newtonian Law.



2. The data of each curve were classified into three intervals or regions; the first comprised the period from the moment of death to 5 hours post-mortem, the second was from 5 to 12 hours after death and the third included data of more than 12 hours post-mortem. Data for each region were fitted to Newton's equation as shown in Figure 3.15. Curve-fitting this time was improved (RMS was 0.5 ±0.3) and a single-exponential equation was sufficient to express each region. Also, it was noted that the shorter the time interval used, or in other words, the more stages fitted by single-exponential equations, the better was the curve-fitting.

The initial inference was that multi-exponential formulae might be required.

3.3.4 Curve-fitting Class 2

Data were extensively analysed on a main frame computer using a non-linear regression analysis programme for curve-fitting. The method used in the curve-fitting technique was the least squares estimate (LSE). The programme package used (BMDP) and its programme P3R were unsuitable for the microcomputer due to the large number of data points collected for each cooling curve. The programme control file is given in Table 3.14a. This contains the instructions or the commands necessary to run the P3R programme. These commands are self-explanatory but some of them may

Table 3.14a: Programme Control File for P3R of BMDP

LINES		EXPLANATION
/ PROBLEM	TITLE IS 'POST MORTEM RATE OF COOLING'.	Two variables are considered, time and ratio. Each is a real number with 2 decimal places. Unit 11 is a specific input file for BMDP.
/ INPUT	VARIABLES ARE 2.	
	FORMAT IS '(2F8.2)'.	
	UNIT IS 11.	
/ VARIABLES	NAMES ARE TIME, RATIO.	
/ TRANSFORM	TIME=TIME/10	
	RATIO=RATIO*10	
/ REGRESS	DEPENDENT IS RATIO.	Regression is on Ratio (y) versus Time (x) Number 1 indicates that exponential functions are required to be used for curve-fitting.
	INDEPENDENT IS TIME.	
	NUMBER IS 1.	
	PARAMETERS ARE 6.	
	CONSTRAINT=1.	
	ITER=900.	
	HALVE=120.	
/ PARAMETER	CONSTRAINT=(1)1,(3)1,(5)1. K=10.	
	[(2)-1.(1)1,(4)-1.(3)1,(6)-1.(5)1. K=0.]*	
	INITIAL ARE -60,-0.036,51,-0.02,19,-0.05.	
/ PLOT	VARIABLE IS TIME.	
	SIZE=40,25	
/ END		

* This constraint was not included in the programme control file because it was a non-linear constraint and was not accepted by BMDP.

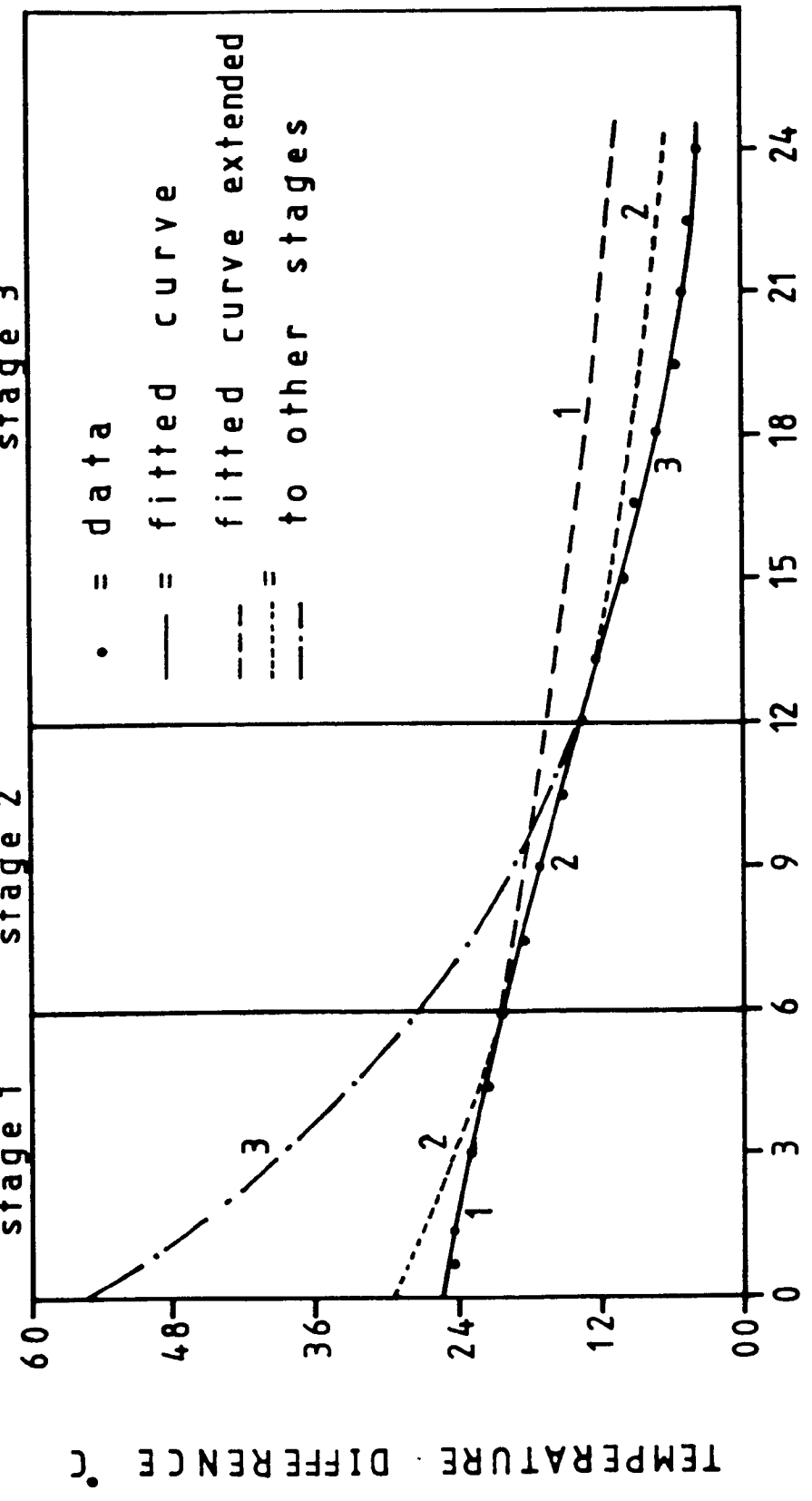


Figure 3.15: THREE STAGES OF SINGLE EXPONENTIAL CURVE FITTING

require further explanation; for example, multiplication or division of the variables by 10, in the TRANSFORM paragraph, was carried out to reduce the differences between the scales of the X and Y axes to make the data usable by the programme. If the data were used without transformation the programme did not manage to process them and an error message was returned. Also, in the REGRESSION paragraph the code number (1) meant the use of an exponential equation whose number of terms was indicated by the number given in the PARAMETER paragraph; each two parameters (coefficients) meant one exponential term, hence when six parameters were instructed this meant a three exponential equation was required. The least number of iterations (trials at curve-fitting) was 50 by default, but in most cases up to 900 iterations were required. The constraint used meant that the temperature ratio at the moment of death for any body site was equal to 1 (or 10 after transformation). Another constraint (shown in brackets in the table) was attempted but was not accepted by the programme. The latter constraint meant that there was no temperature loss from the body in life or at the moment of death and therefore the rate of cooling at this moment was equal to 0 (zero). These constraints are based on hypothetical premises which will be discussed later (Paragraph 4.8.3).

The programme required to know the initial values of the parameters from which to start the

iterations. These initials should be estimated by the user and put in the INITIAL paragraph. If the initials were not suitable the programme could not process the data and an error message would appear, or the programme would be terminated. This procedure was tedious as it might require many trials before the correct initials had been found. Also it happened that many sets of acceptable initials were found and the task was to discover the best amongst them to describe the curve under concern. The PLOT paragraph instructed the programme to plot both observed and predicted data and also the residuals versus time. The size of these plots was indicated by the numbers given in the same paragraph, for example 25 and 40 characters on X and Y axes respectively (as in the table). Examples of these plots are shown in Figure 3.16a and b. Although the data could be weighted by further instructions given in the WEIGHT paragraph, this was not required as the error variance of the data was already homogeneous.

If the initials were accepted by the programme the processing would continue, and at the end the programme listed useful information (shown in Table 3.14b). The most important items were the parameters (coefficients) which were found by the programme to give the best fit. In other words, these indicated the best formula to represent the curve under consideration. Also listed were the observed (input) data, the predicted (calculated) data, their

Table 3.14b Output from BMDP programme following curve-fitting
F = Predicted temperature difference ratio

PROBLEM TITLE IS
POST MORTEM RATE OF COOLING

NUMBER OF VARIABLES TO READ IN	2
NUMBER OF VARIABLES ADDED BY TRANSFORMATIONS	0
TOTAL NUMBER OF VARIABLES	2
NUMBER OF CASES TO READ IN	TO END
CASE LABELING VARIABLES	
MISSING VALUES CHECKED BEFORE OR AFTER TRANS.	NEITHER
BLANKS ARE	MISSING
INPUT FILE . . . UNIT 11	
REWIND INPUT UNIT PRIOR TO READING . . DATA	YES
NUMBER OF WORDS OF DYNAMIC STORAGE	14998
NUMBER OF CASES DESCRIBED BY INPUT FORMAT	1

VARIABLES TO BE USED
1 TIME 2 RATIO
INPUT FORMAT IS
(2F8.2)
MAXIMUM LENGTH DATA RECORD IS 16 CHARACTERS.
INPUT VARIABLES

VARIABLE INDEX	NAME	RECORD NO	COLUMNS BEGIN	COLUMNS END	FIELD WIDTH	TYPE
1	TIME	1	1	8	8.2	F
2	RATIO	1	9	16	8.2	F

VARIABLES TO BE PLOTTED
1 TIME

PLOT OF PREDICTED VALUES VERSUS RESIDUALS	NO
NORMAL PROBABILITY PLOT	NO
DETRENDED NORMAL PROBABILITY PLOT	NO

Table 3.14b (Continuation 1.)

```
1 PAGE 2 BMDP3R POST MORTEM RATE OF COOLING
REGRESSION TITLE
POST MORTEM RATE OF COOLING
REGRESSION NUMBER . . . . . .  1
INDEPENDENT VARIABLE (FOR BUILT-IN FUNCTION) . . . . TIME
DEPENDENT VARIABLE . . . . . . . RATIO
WEIGHTING VARIABLE . . . . . .
NUMBER OF PARAMETERS . . . . . .  6
NUMBER OF CONSTRAINTS . . . . . .  1
TOLERANCE FOR PIVOTING . . . . . .  0.00000001000
TOLERANCE FOR CONVERGENCE . . . . . .  0.00001000000
MAXIMUM NUMBER OF ITERATIONS . . . . . 900
MAXIMUM NUMBER OF INCREMENT HALVINGS . . . . . 120
NUMBER OF DATA PASSES PER CASE . . . . . .  1
COMPUTE LOSS FUNCTION . . . . . .  NO

CONSTRAINTS
      1.000000
      0.000000
      1.000000
      0.000000
      1.000000
      0.000000

CONSTANTS
     10.000000
```

Table 3.14b (Continuation 2.)

```
USING THE ABOVE SPECIFICATIONS THIS PROGRAM COULD PROCESS 1575 CASES (i.e. sets of two data on Y and X axes)
BASED ON INPUT FORMAT SUPPLIED    1 RECORDS READ PER CASE.
NUMBER OF CASES READ     .     .     .     .     .     .     .  242
       CASES WITH DATE MISSING OR BEYOND LIMITS  .     .     .     .    1
             REMAINING NUMBER OF CASES     .     .     .     .     .  241

VARIABLE          MEAN            STANDARD          MINIMUM         MAXIMUM
NO.    NAME                       DEVIATION

1       TIME    64.000000       34.857437           4.000000       124.000000
2       RATIO    4.401197        2.689733           0.600000         9.599999

PARAMETER MAXIMA  0.2126765E+38  0.2126765E+38  0.2126765E+38  0.2126765E+38  0.2126765E+38   0.2126765E+38
PARAMETER MINIMA -0.2126765E+38  0.2126765E+38 -0.2126765E+38 -0.2126765E+38 -0.21226765E+38 -0.2126765E+38
ITERATION   INCREMENT   RESIDUAL SUM      P1           P2           P3           P4           P5           P6
NUMBER      HALVINGS     OF SQUARES
   0           0       4330.32      -60.000000   -0.036000    51.000000    -0.020000    19.000000    -0.0500000
   1           0        158.048     -60.000000   -0.038134    51.000000    -0.025830    19.000000    -0.0589800
   2           0          6.07792   -60.000000   -0.044541    51.000000    -0.030406    19.000000    -0.0682730
   3           0          4.57076   -60.000000   -0.046493    51.000000    -0.031395    19.000000    -0.0722970
   4           0          4.56620   -60.000000   -0.046323    51.000000    -0.031325    19.000000    -0.0718250
   5           0          4.56615   -60.000000   -0.046342    51.000000    -0.031332    19.000000    -0.0718880
   6           0          4.56595   -60.023363   -0.046342    51.000000    -0.031328    19.023363    -0.0718880
   7           0          4.56575   -60.023363   -0.046345    51.000000    -0.031331    19.023363    -0.0718630
   8           0          4.56555   -60.046798   -0.046345    51.000000    -0.031327    19.046798    -0.0718630
   9           0          4.56536   -60.046798   -0.046351    51.000000    -0.031332    19.046798    -0.0718470
  10           0          4.56516   -60.070212   -0.046351    51.000000    -0.031328    19.070212    -0.0718470
```

Table 3.14b (Continuation 3.)

ITERATION 10 HAS THE SMALLEST RESIDUAL SUM OF SQUARES (SUBJECT TO CONSTRAINTS, IF ANY).
REMAINING CALCULATIONS ARE BASED ON THE RESULTS OF THIS ITERATION.
STANDARD FUNCTION FORM USED WITH IND=1
F =P(1) EXP(P(2) X(IND)) + P(3) EXP(P(4) X(IND)) + ...
1 PAGE 3 BMDP3R POST MORTEM RATE OF COOLING

ASYMPTOTIC CORRELATION MATRIX OF THE PARAMETERS

		P1	P2	P3	P4	P5	P6
		1	2	3	4	5	6
P1	1	0.0000					
P2	2	0.0000	1.0000				
P3	3	0.0000	0.0000	0.0000			
P4	4	0.0000	0.9686	0.0000			
P5	5	0.0000	0.0000	0.0000	0.0000		
P6	6	0.0000	0.0582	0.0000	0.8723	0.0000	1.0000

RESIDUAL MEAN SQUARE 0.191813E-01
DEGREES OF FREEDOM 238

PARAMETER	ESTIMATE	ASYMPTOTIC STANDARD DEVIATION	TOLERANCE
P1	-60.070202	0.000000	0.0000000000
P2	-0.046351	0.000363	0.0080799716
P3	51.000000	0.000000	0.0000000000
P4	-0.031328	0.000140	0.0236136090
P5	19.070212	0.000000	0.0000004058
P6	-0.071847	0.001128	0.0312827774

Table 3.14b (Continuation 4)

IF LINEAR DEPENDENCE IS FOUND OR IF PARAMETER IS ON THE BOUNDARY IT IS ASSIGNED A STANDARD DEVIATION OF ZERO

1 PAGE 4 BMDP3R POST MORTEM RATE OF COOLING

CASE NO	LABEL.	PREDICTED RATIO	S.D. OF PRED VALUE	OBSERVED RATIO	RESIDUAL	COOK DISTANCE	TIME
1		9.395658	0.019701	5.599999	0.204341	0.007647	4.000000
2		9.334799	0.020998	9.499999	0.165200	0.005711	4.500000
3		9.276431	0.022100	9.299999	0.023568	0.000129	5.000000
4		9.220356	0.023021	9.299999	0.079643	0.001611	5.500000
5		9.166389	0.023778	9.199999	0.033610	0.000307	6.000000
6.		9.114352	0.024384	9.199999	9.985647	0.002104	7.000000
7		9.06081	0.024852	9.099999	0.035918	0.000073	7.500000
8		9.015419	0.025197	8.999999	-0.015421	0.000073	7.500000
9		8.968220	0.025428	9.099999	0.131779	0.005447	8.000000
10		8.922344	0.025558	8.900000	-0.022345	0.000158	8.500000

etc. etc.....

Serial correlation = 0.856

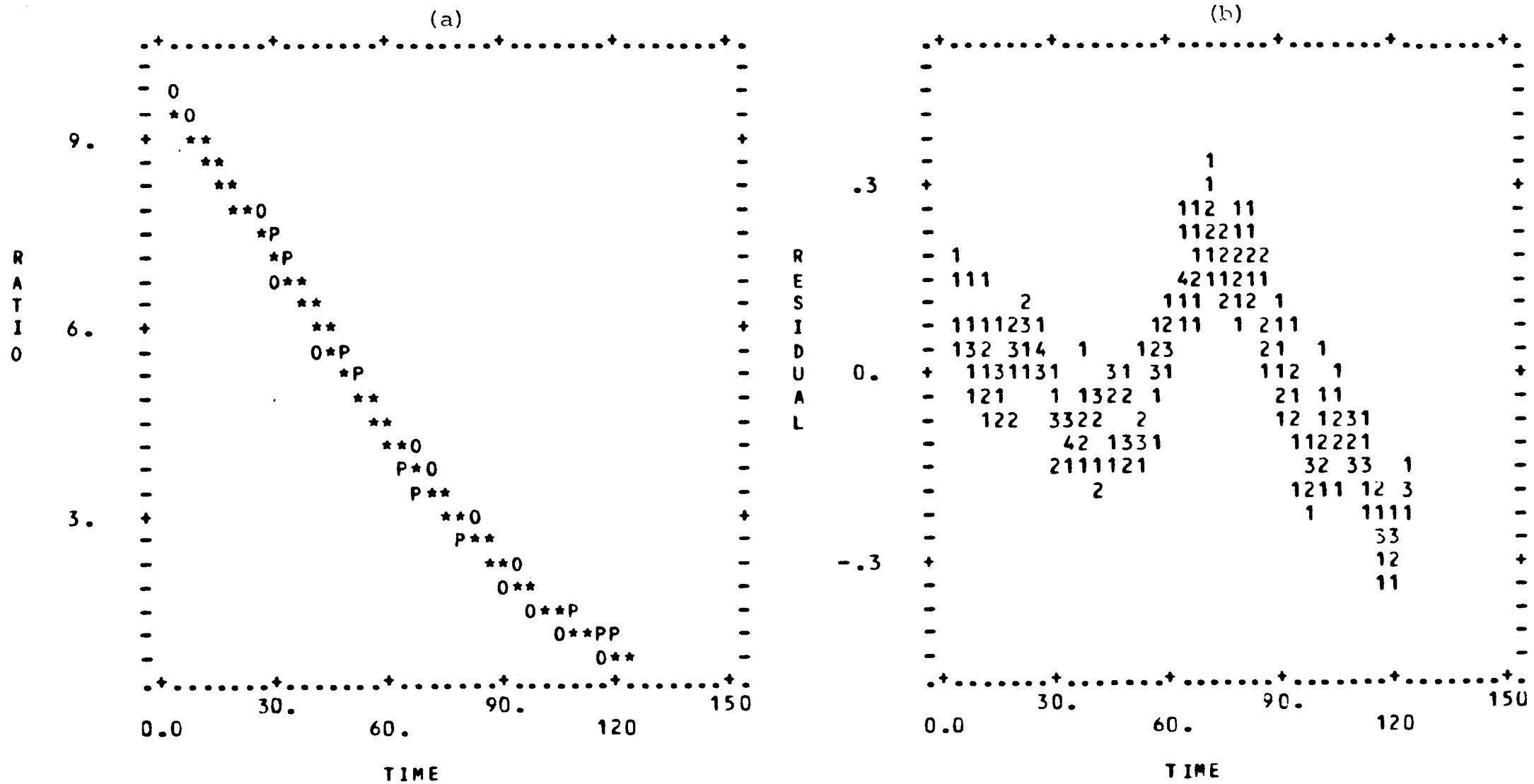


Figure 3.16: Examples of plots printed out during Curve-fitting Class 2; (a) temperature difference ratios were plotted versus time after death in minutes divided by 10. (b) residuals (differences between observed and predicted values of temperature difference ratios) were plotted versus time after death in minutes divided by 10.

corresponding residuals (differences), standard deviations and the post-mortem intervals (the X-axis data).

To obtain the optimum fit for each curve, equations involving two, three and four exponential functions were compared. In this way, the following curve-fitting operations were performed:-

1. Approximately 200 curves were fitted to a double-exponential equation. These included most of the naked body cases.
2. About 350 curves were fitted to a triple-exponential equation in each of the naked and covered body groups.
3. Four exponential terms were evaluated in 15 curves.
4. More than half of the cases were fitted twice for each of the operations mentioned above. This was to obtain the best formula to describe the data as will be explained below (Paragraph 3.3.5).

3.3.5 Criteria of best fit

The best fit was considered to be the one having the least residual mean square of the deviations between the observed (actual) data and those calculated by the function. Also, both sets of data were plotted versus time and a fit was considered to be good if both curves were close to each other throughout the whole monitoring period. In addition to this the fitted (predicted) curve should behave logically throughout a 60 hour period and beyond. Therefore before an

equation resulting from curve-fitting was accepted and the process was ended the ability of that equation to describe the input data was assessed over a period of at least 60 hours. Sometimes, particularly when two or more parameters resulting from the curve-fitting were positive, the fitted curve was in close agreement with the input curve during the monitoring period of the case but it behaved aberrantly outside this period: for example, it increased or alternatively decreased rapidly as shown in Figures 3.17a and b respectively. In this case the observed data were refitted using other initials until the correct formula was found. Accordingly more than half of the cases were fitted twice.

3.4 Triple-Exponential Formula

3.4.1 Best Formula to fit the Data

Statistical analysis of the residual mean squares resulting from curve-fitting Class 2 using different exponential formulae and for different body sites is shown in Tables 3.15 a and b. According to this analysis the use of the triple-exponential equation in all or most body sites resulted in significantly lower RMS than those resulting from the 2- or 4-term equations. Also the comparative ability of the double and the triple-exponential equations to fit the data is shown in Figures 3.18a-b and 3.19a-b. The first two figures, typical of most cases,

Table 3.15a: Residual mean square (RMS) of curve-fitting Class 2, for different body sites using two, three and four exponential equations.

I T E M	Curve-fitting based on two-exponential equations			Curve-fitting based on three-exponential equations			Curve-fitting to four-exponential equations
	Rectum	Liver	Brain	Rectum	Liver	Brain	R e c t u m
Range	0.0041 to 0.398	0.011 to 0.63	0.019 to 0.67	0.00864 to 0.233	0.00813 to 0.979	0.01239 to 0.552	0.0341 to 0.244
Mean	0.076	0.106	0.153	0.052	0.157	0.104	0.095
S.D.	0.075	0.139	0.105	0.038	0.219	0.1	0.069
Number of Curves	70	49	50	56	66	56	15

Table 3.15b: Comparison of triple-exponential formula with two- and four- term formulae using Student's t-test and the zM test.

FUNCTION	Comparison of curve-fitting of 2 exponential equations with curve-fittings of 3 exponential equations			Comparison of curve-fitting of 4 exponential equations with curve-fittings of 3 exponential equations
	Rectum	Liver	Brain	Rectum
t	2.68	2.57	3.3	2.4
P	<1%	~1%	<0.2%	<5% and >1%
z	5.28	1.63	3.47	4.38
P	<0.2%	>10%	<0.2%	<0.2%

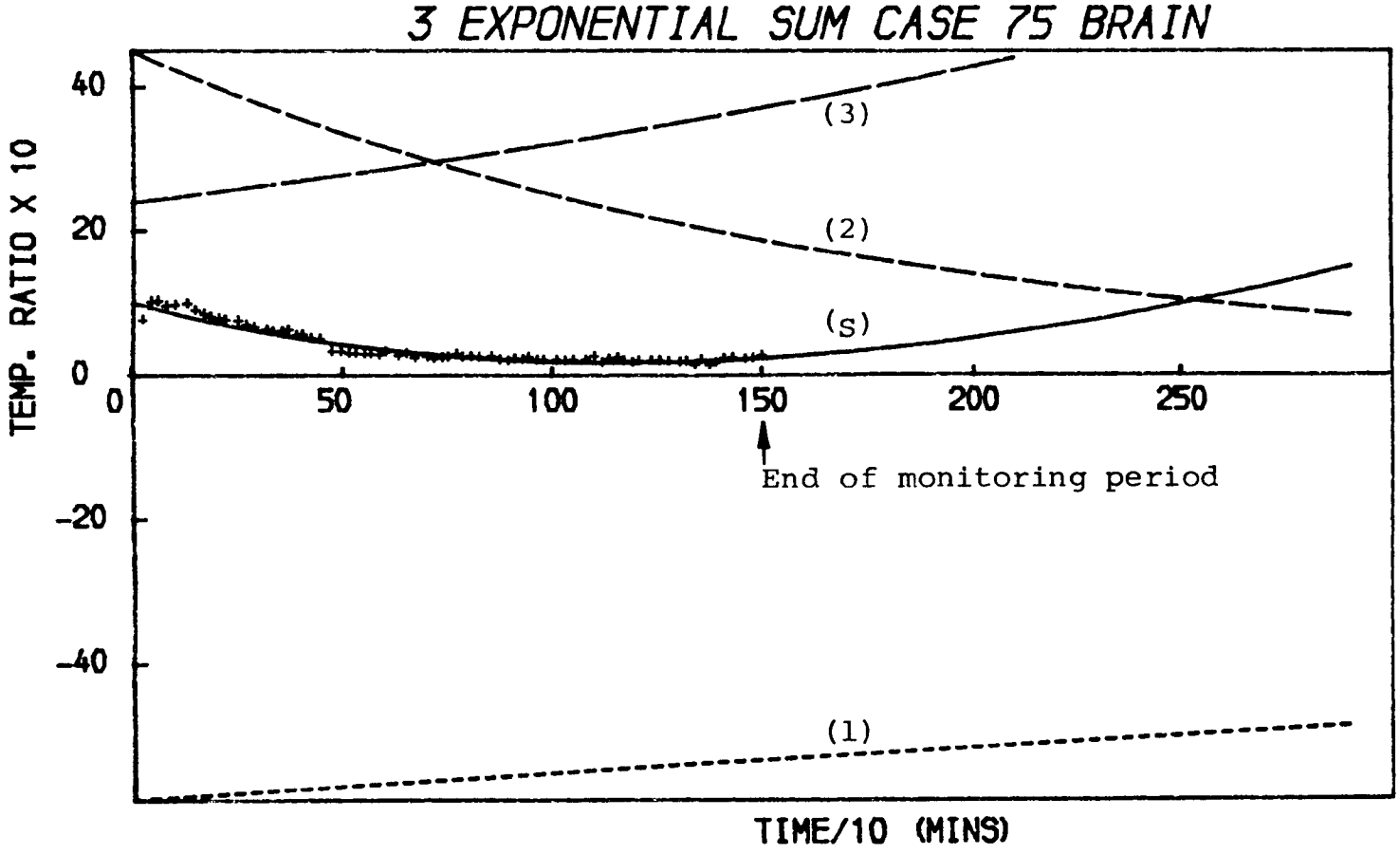


Figure 3.17a: Example of fitted curve (S) which deviates in a positive direction from the logically expected pattern outside the monitoring period. Curve S is the sum of curves 1-3, each of which represents one exponential term.

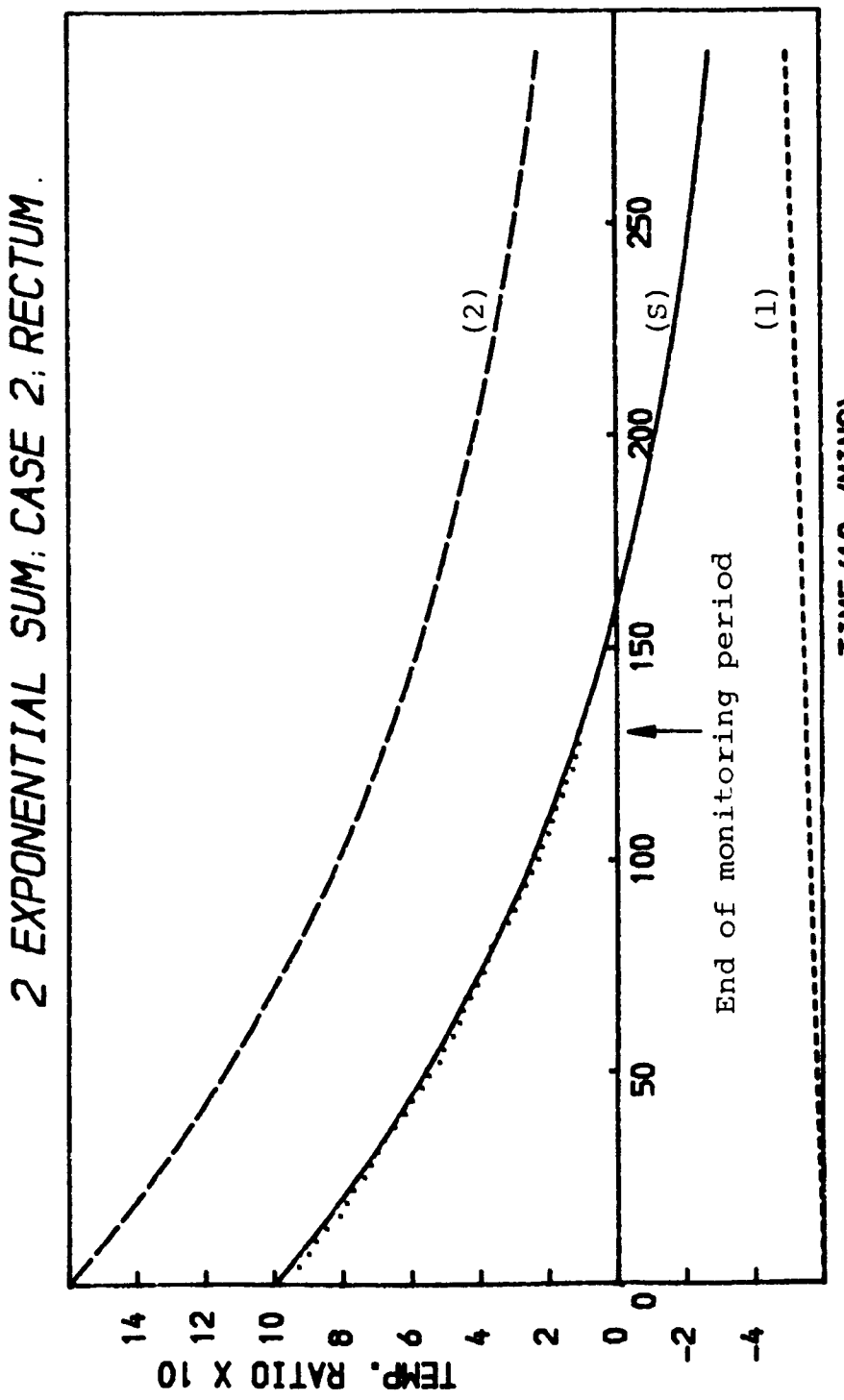


Figure 3.17b: Example of fitted curve (S) showing negative deviation from expected pattern outside the monitoring period.

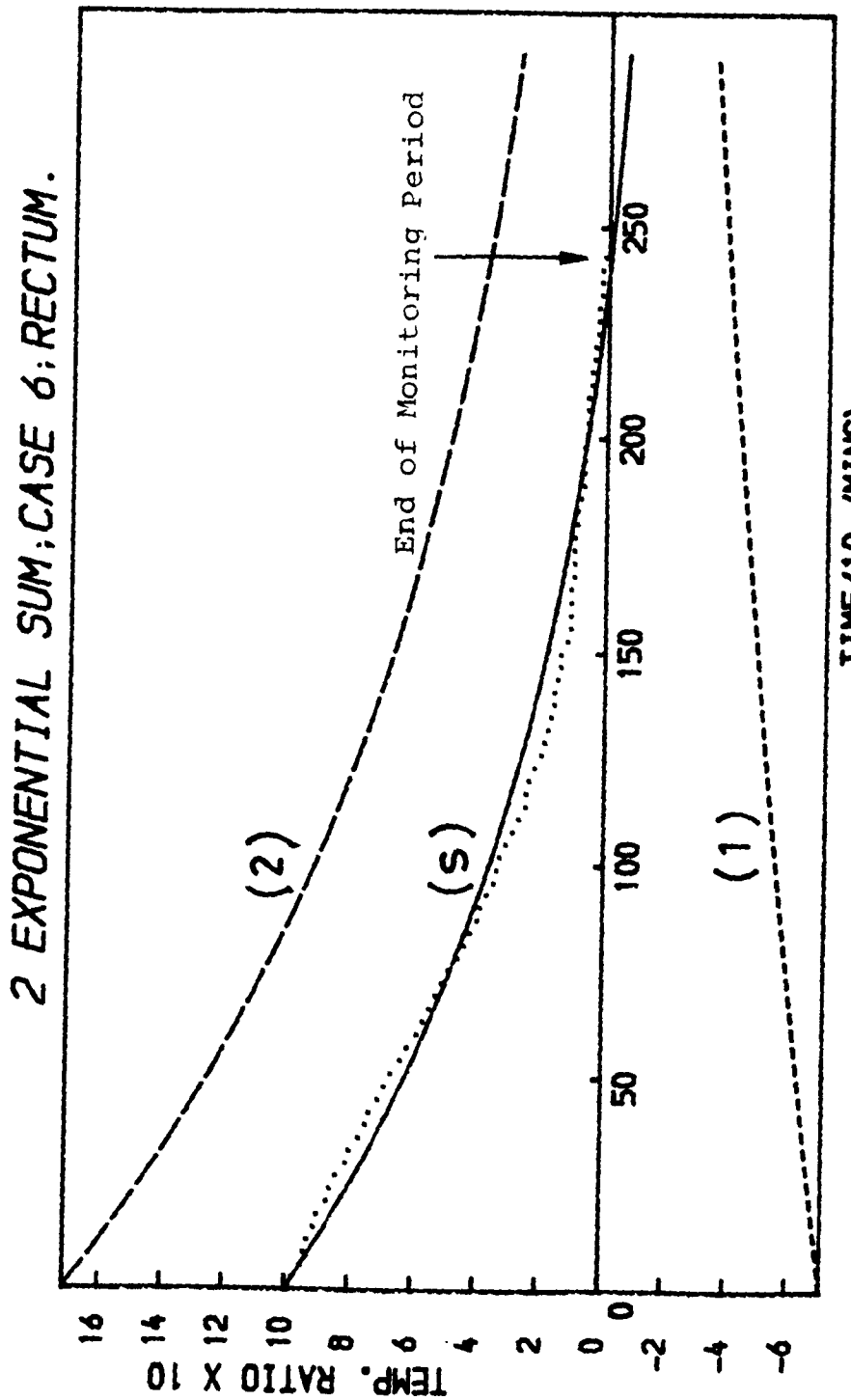


Figure 3.18a: Typical example of curve-fitting using a 2-exponential equation in which curve (S) inadequately describes the input date (RMS = 0.088).

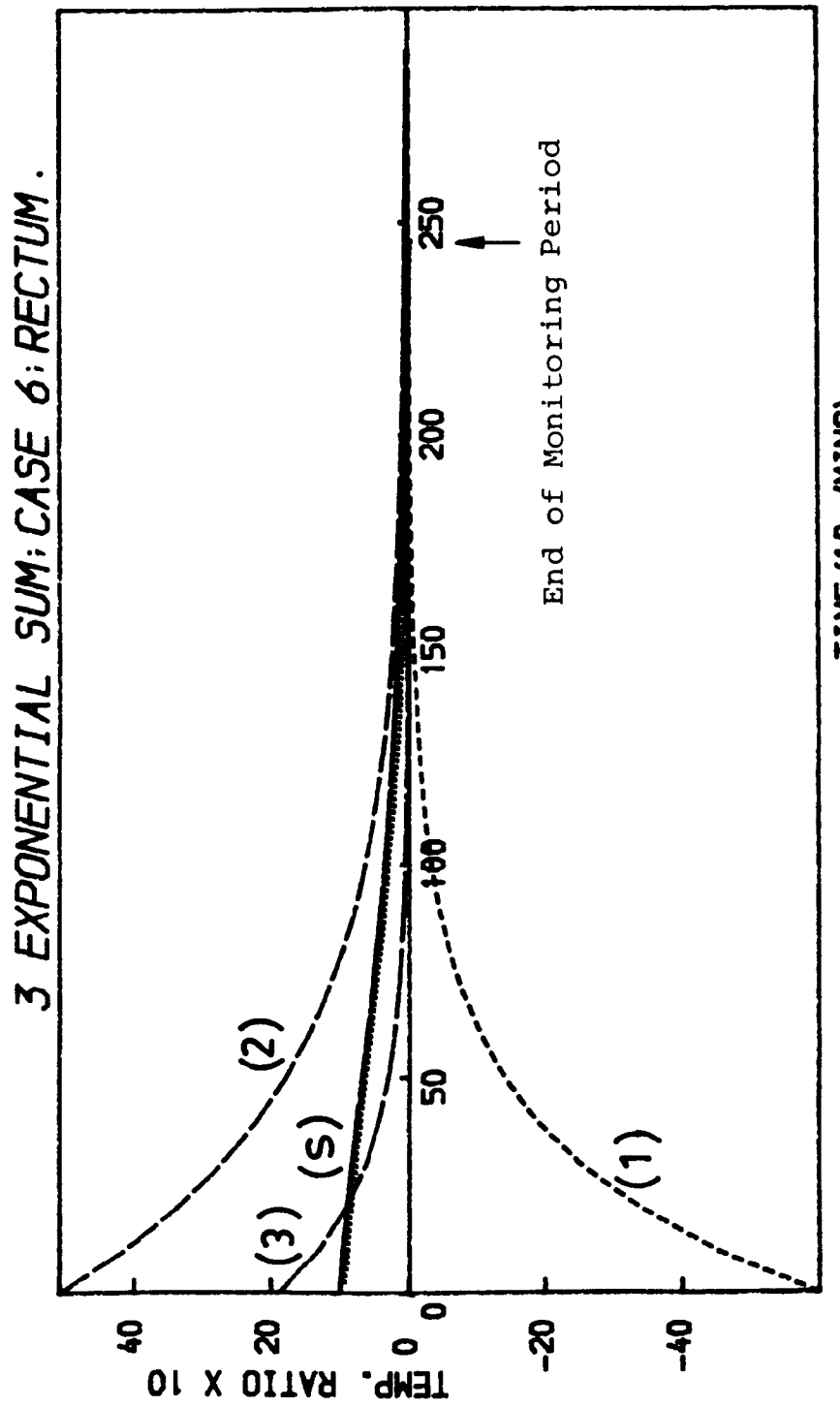


Figure 3.18b: Typical example of good curve-fitting using a 3-exponential equation (RMS = 0.005).

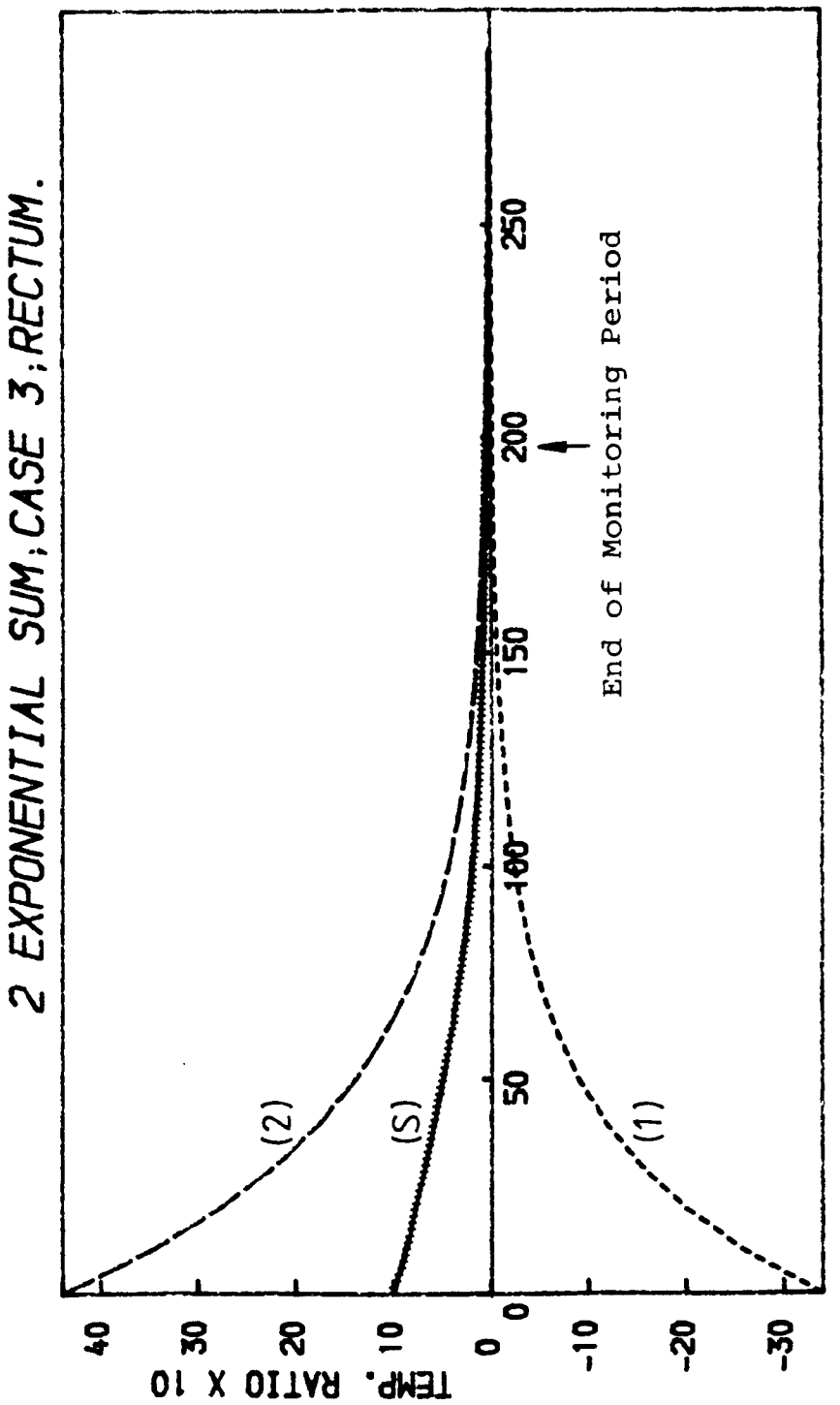


Figure 3.19a: Example of a case in which the input curve is adequately described by a 2-exponential equation (RMS = 0.0048).

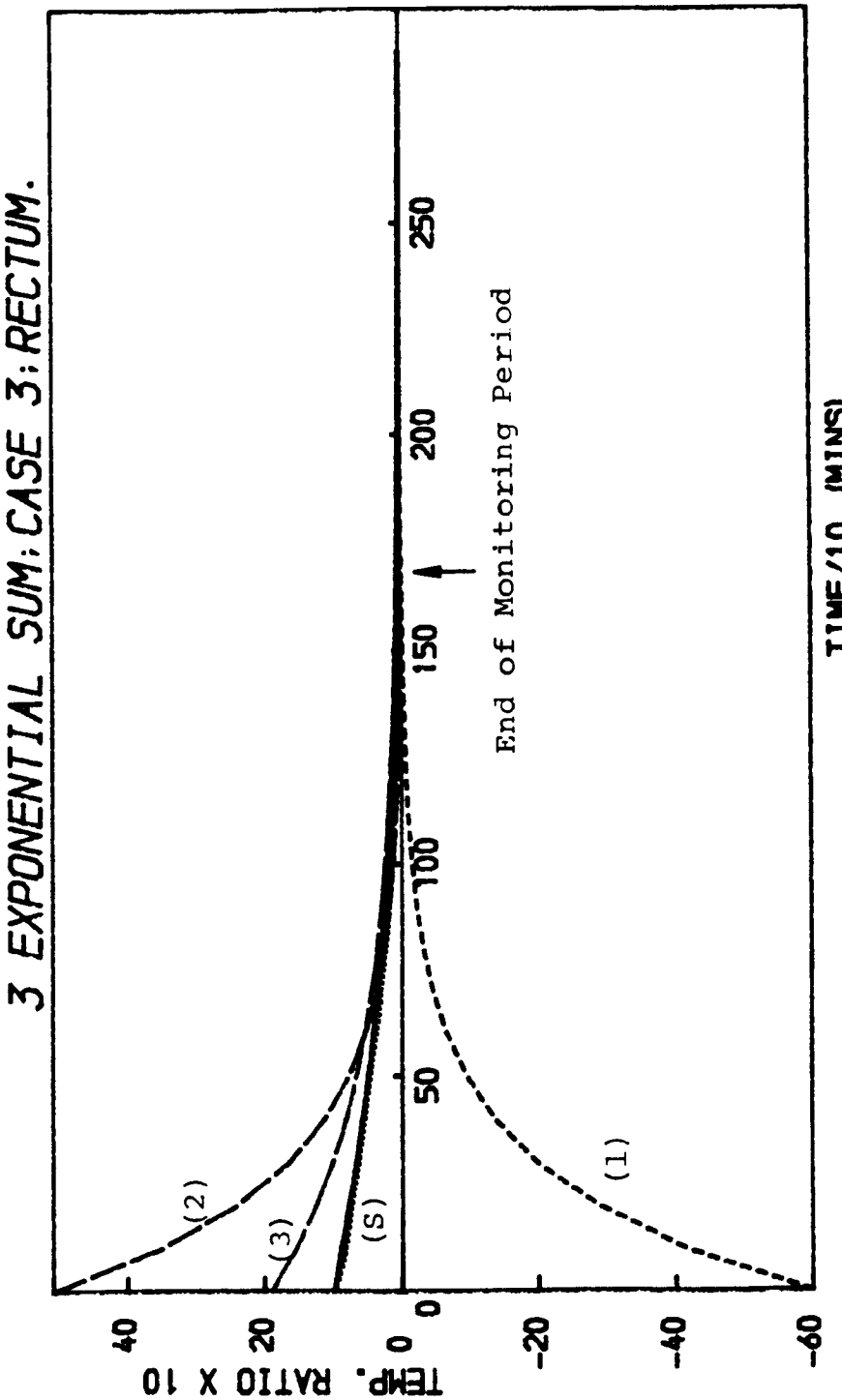


Figure 3.19b: The same case as given in 3.19a showing a slight improvement when fitted using a 3-exponential equation (RMS = 0.0044).

demonstrate that the double-exponential equation is less able than the triple one to describe the data (for comparision note that both a and b of the Figure represent the same rectal data of case No.6). The second two figures represent the exceptional cases in which two exponential terms are sufficient to fit the data, although even in these cases the triple equation gives a better fit (RMS 0.0048 and 0.0044 respectively).

The main conclusion drawn from the above is that the best fit was found to be given by the triple-exponential function of the form given in Equation 3.1

$$R = P_1 e^{P_2 t} + P_3 e^{P_4 t} + P_5 e^{P_6 t} \quad \text{.....Equation 3.1}$$

where R = temperature difference ratio (Equation 2.2)

P_1 to P_6 are parameters (Table 3.21)

and t is the time after death in hours.

Examples of curve-fitting of the data to triple-exponential equations are shown in Figures 3.18a, 3.19a and 3.20-3.22. In each of these figures there are curves labelled 1-3 which represent the three exponential terms of the equation in the same order i.e. one negative term (term 1) representing the processes that modify cooling and two positive terms (terms 2 and 3) indicating cooling processes. The absolute values of these terms diminish as the post-mortem interval increases, and the rate of this diminution depends on the rate of cooling of the body site concerned, for example, when the cooling was slow (Figures 3.20a and 3.22a-c) these terms require a

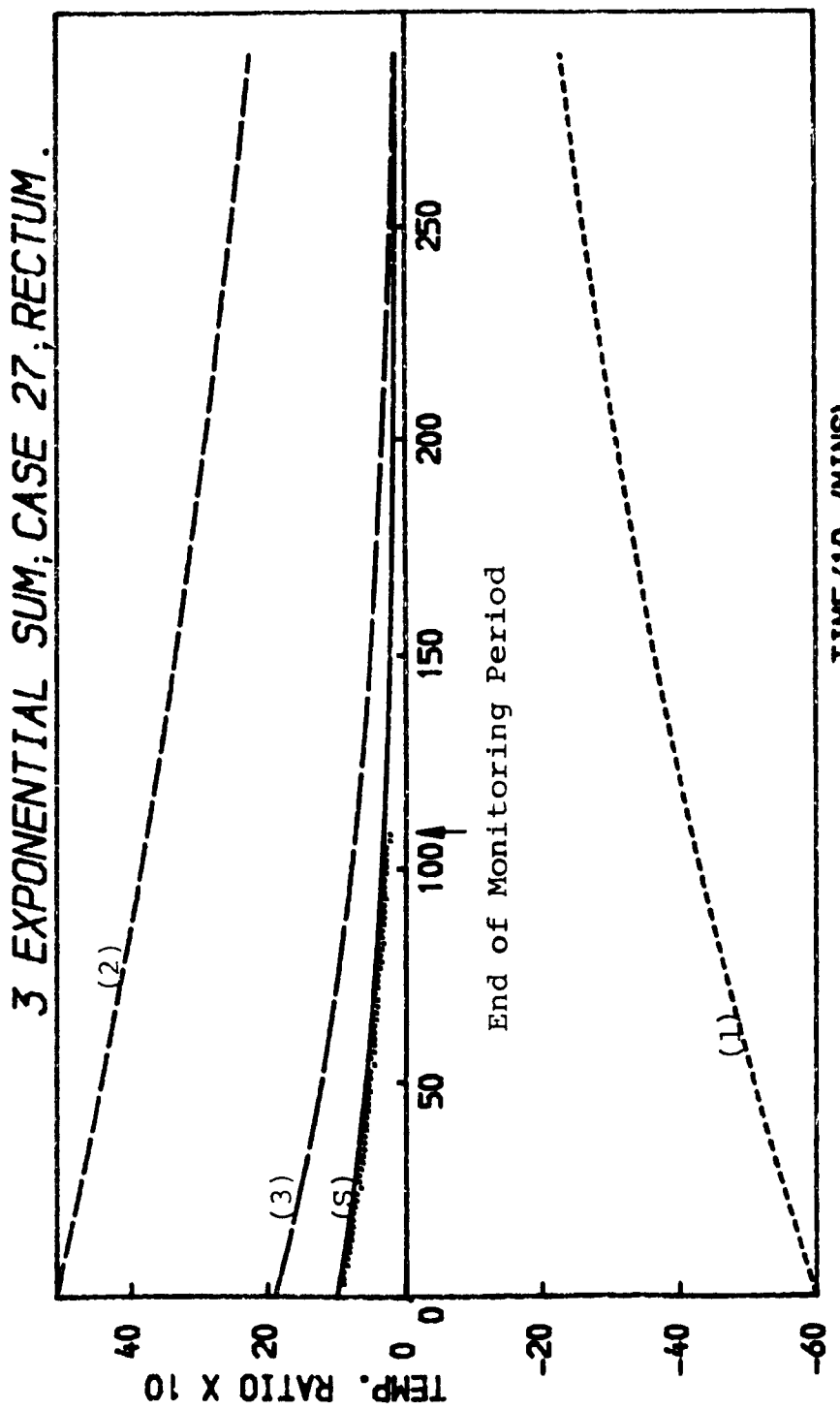


Figure 3.20a: Example of a rectal cooling curve fitted by a 3-exponential equation (S) for a case in the naked body group which cooled slowly. This is reflected by the gentle gradients of the three terms (1-3).

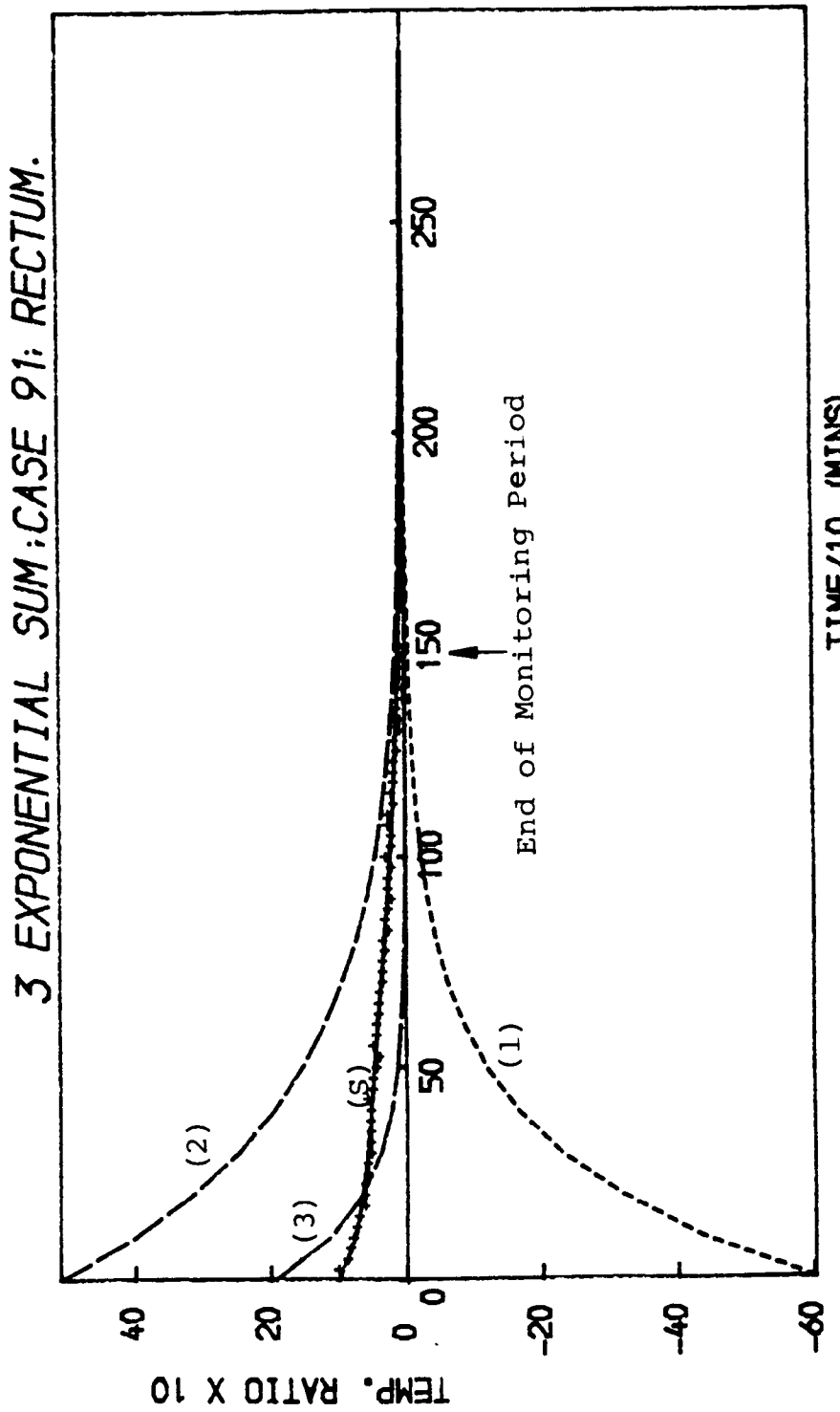


Figure 3.20b: Example of a rectal cooling curve fitted by a 3-exponential equation (S) for a case in the covered body group which cooled rapidly. This is reflected by the steep slopes of the three terms (1-3).

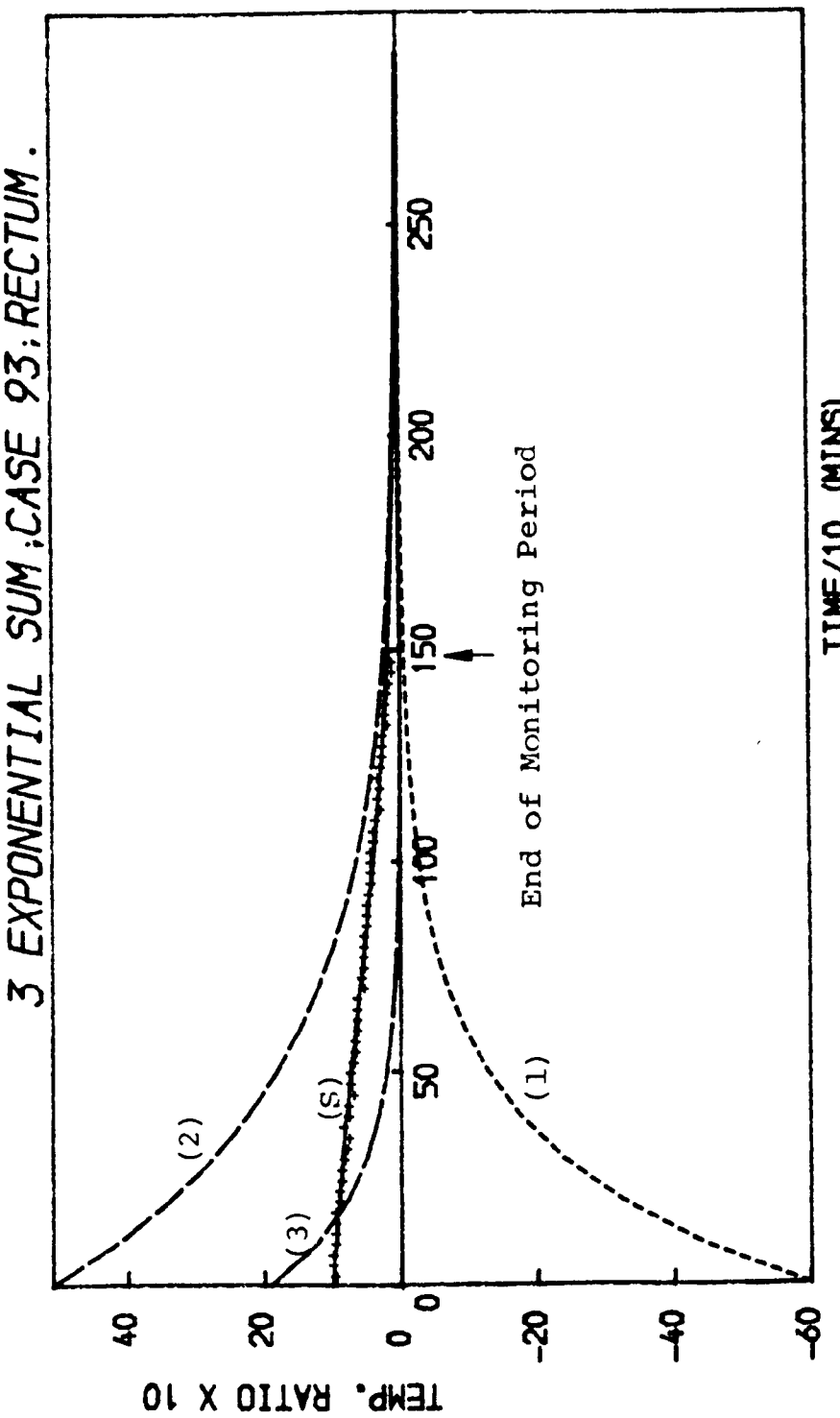


Figure 3.20c: A case similar to that shown in 3.20b except for the presence of a plateau during the initial cooling period.

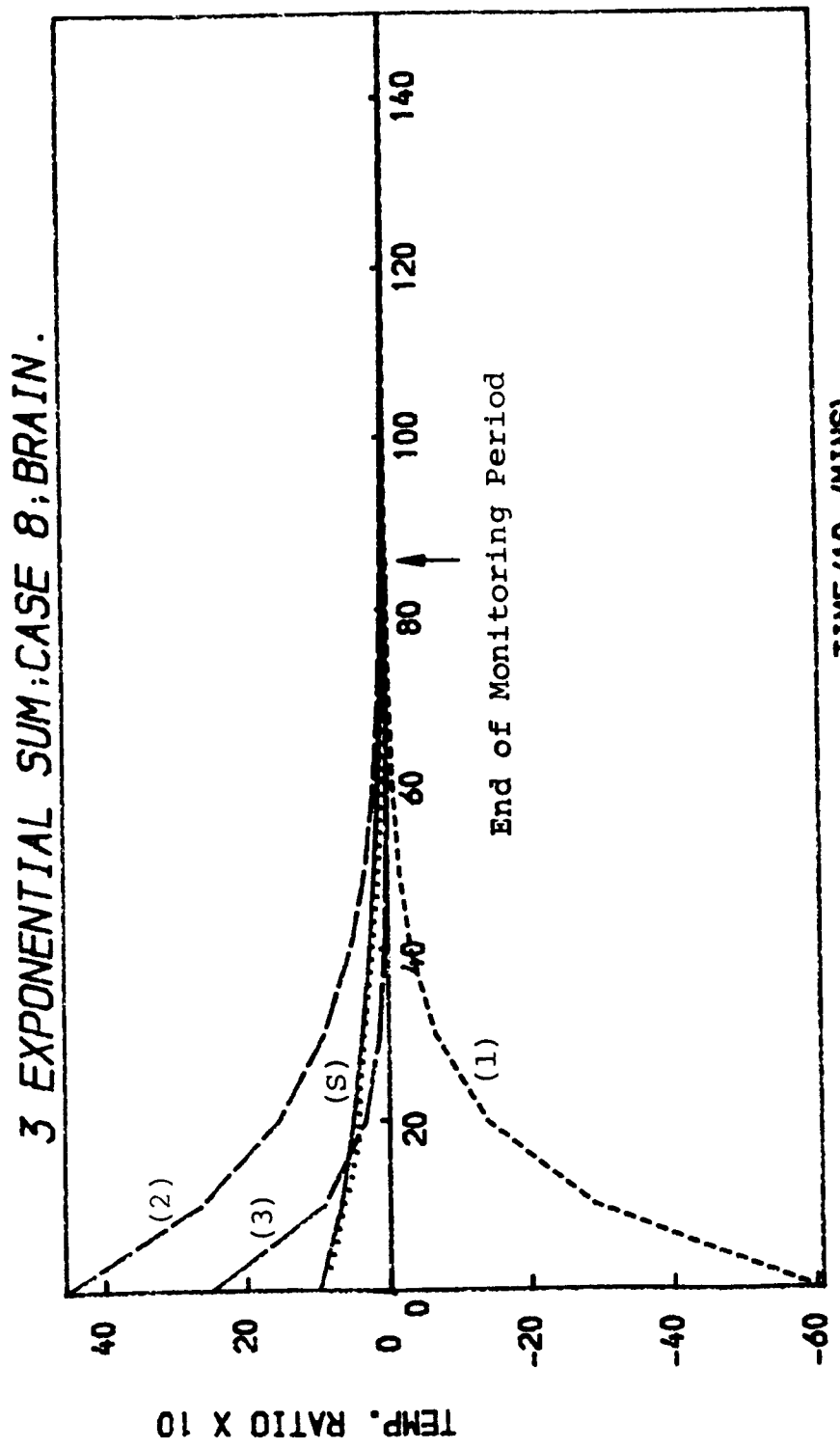


Figure 3.21a: Example of a brain cooling curve fitted by a 3-exponetial equation (S) for a case in the naked body group which cooled rapidly. This is reflected by the steep slopes of the three terms (1-3).

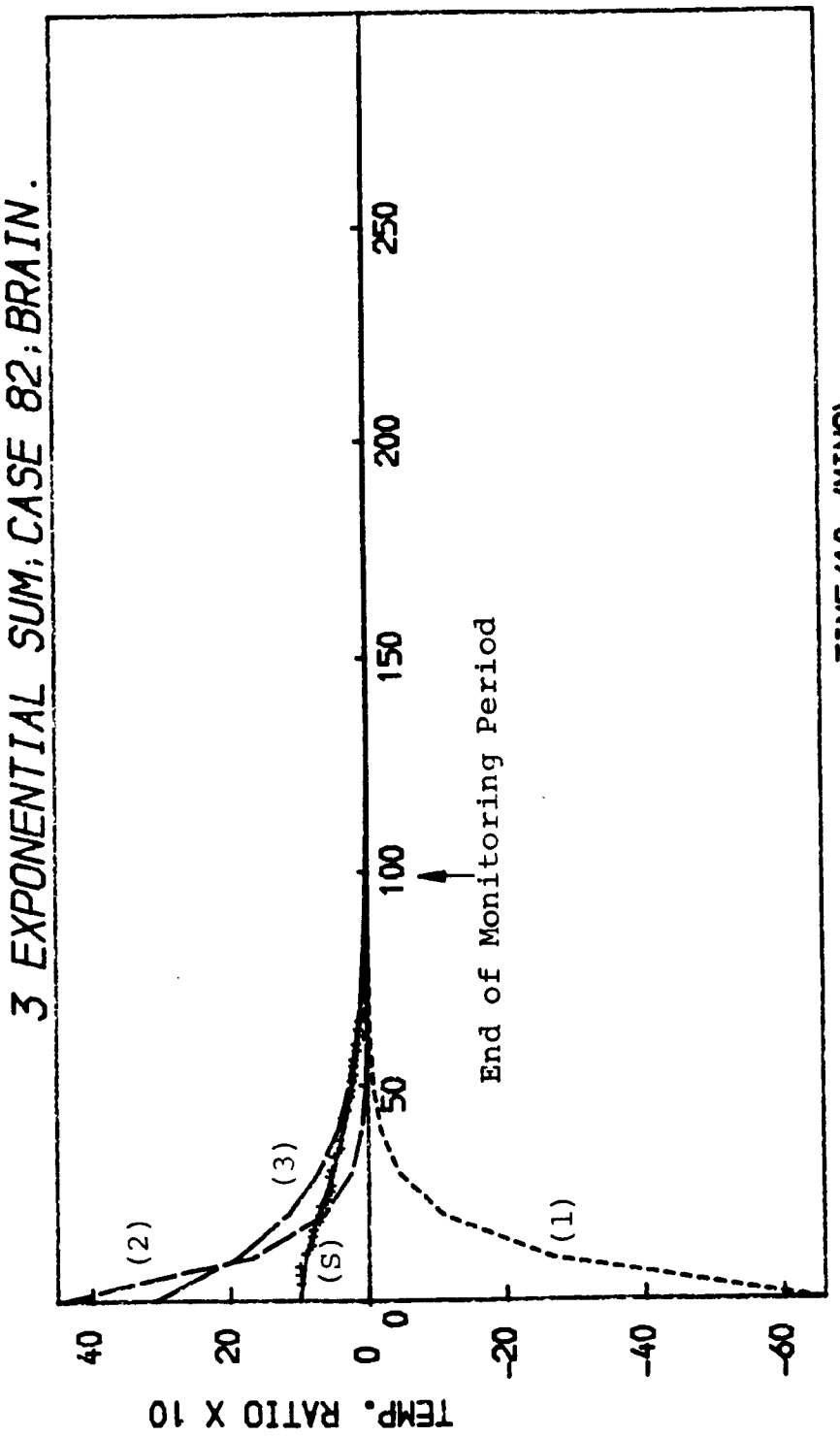


Figure 3.21b: Example of a brain cooling curve fitted by a 3-exponential equation (S) for a case in the covered body group which cooled rapidly. This is reflected by the steep slopes of the three terms (1-3). However, note the presence of a plateau in the initial cooling period.

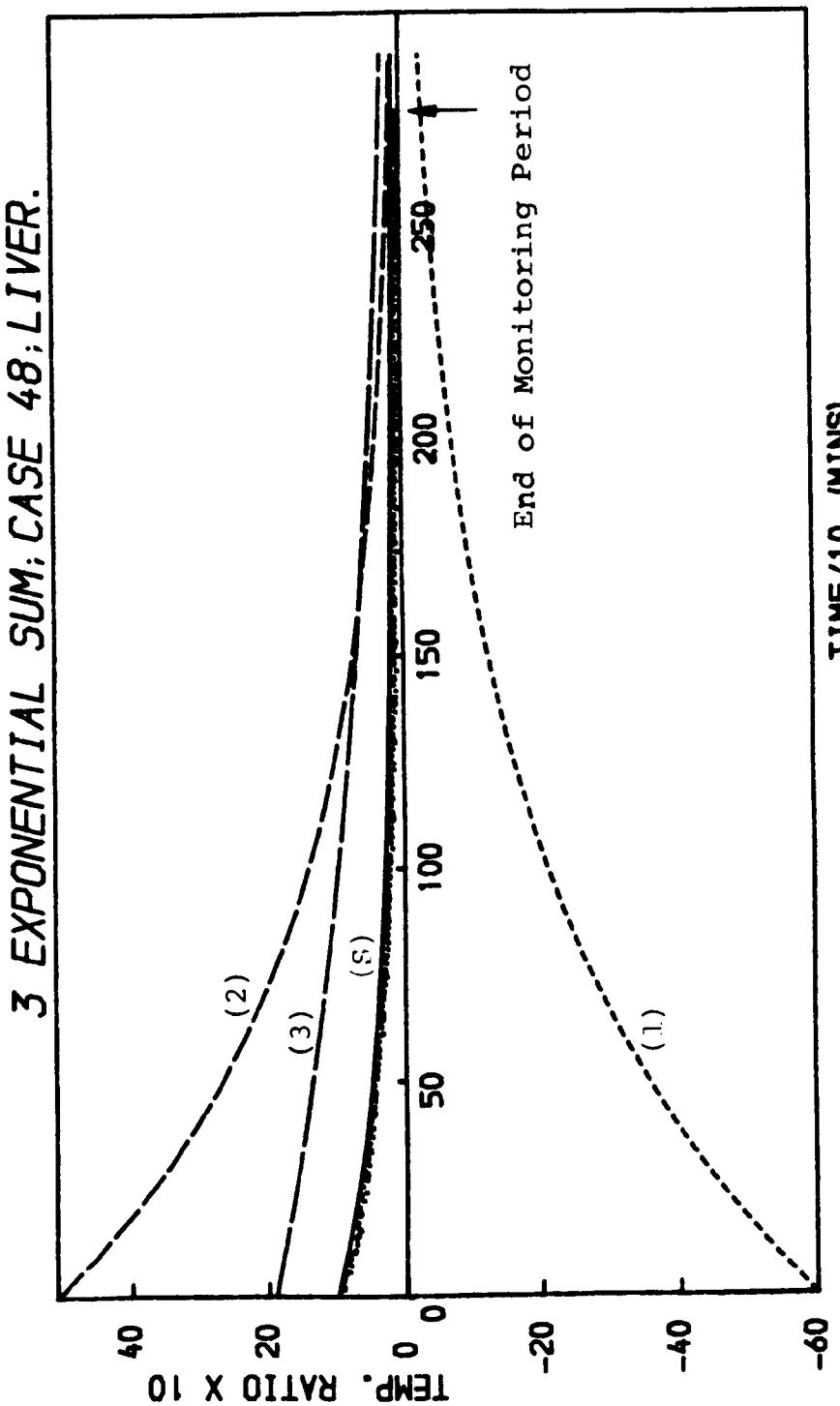


Figure 3.22a: Example of a liver cooling curve fitted by a 3-exponential equation (S) for a case in the naked body group which cooled slowly. This is reflected by the gentle gradients of the three terms (1-3).

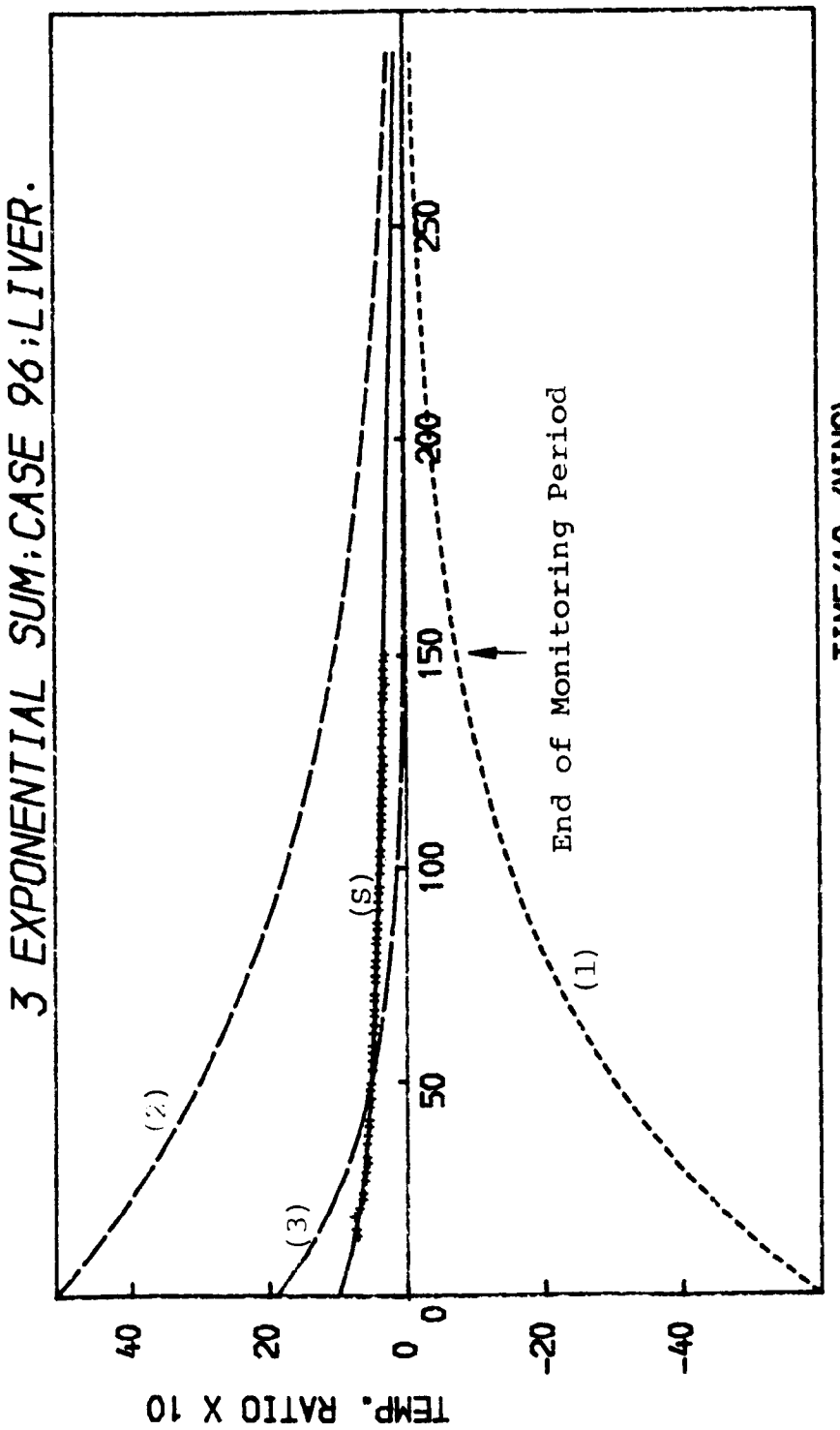


Figure 3.22b: Example of a liver cooling curve similar to that shown in 3.22a but for a case in the covered body group.

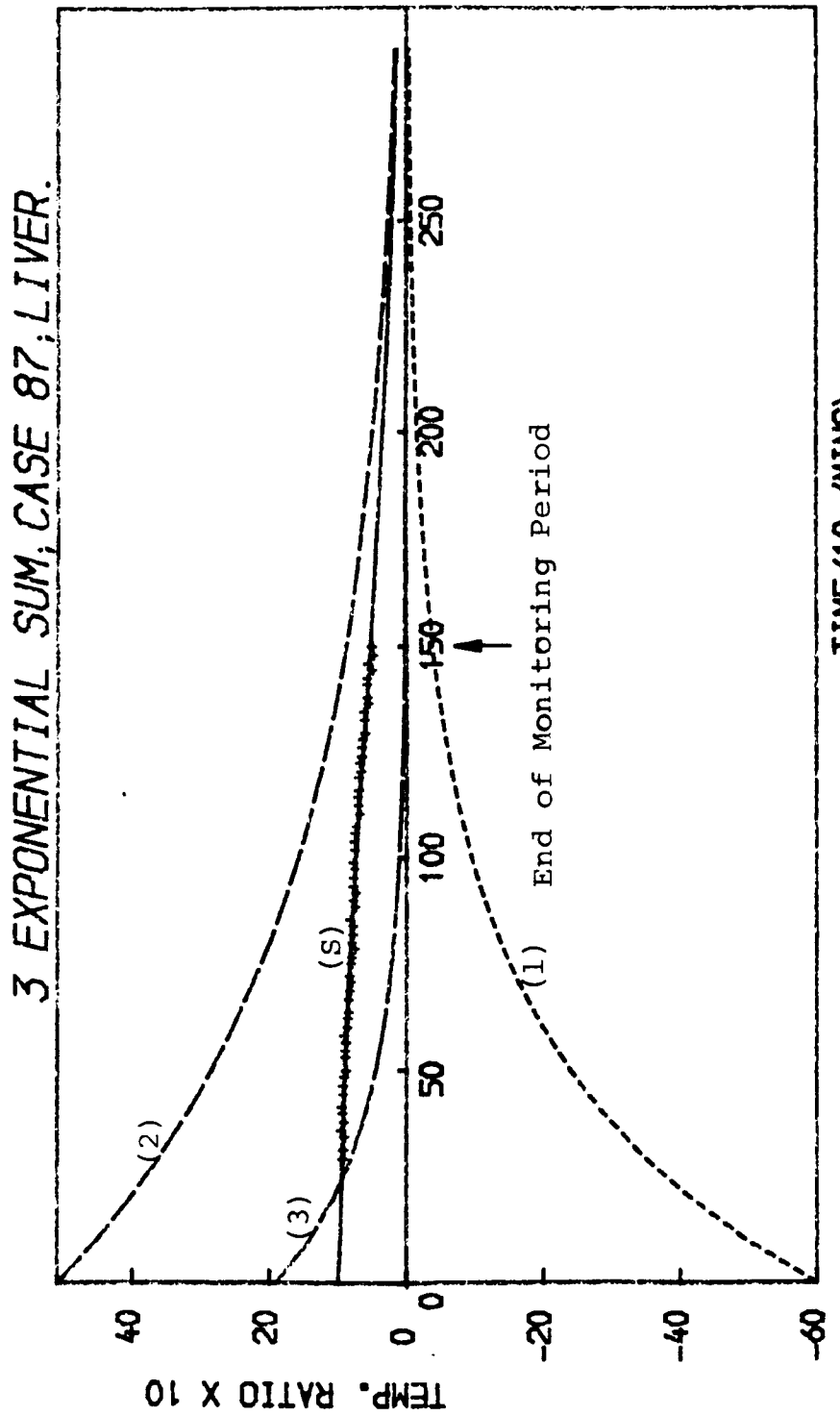


Figure 3.22c: A case similar to that shown in 22b except for the presence of a plateau during the initial cooling period.

longer time to approach the zero line (which represents the environment) than when the cooling is relatively rapid (Figures 3.18b, 3.19b, 3.20b-c and 3.21a-b). The theoretical basis of these terms will be discussed later (Paragraph 4.8.3). The fitted curve represents the sum of the three terms and is denoted by S. Lastly, the observed data are represented by (.) or (+) symbols. Note that the X and Y axes are multiplied and divided by 10 respectively according to the TRANSFORM paragraph explained earlier (Paragraph 3.3.4). Note also that in Figures 3.20c, 3.21b and 3.22c temperature plateaux are visible.

3.4.2 Correlation of Parameters to Body Variables

For each curve six parameters were obtained which described the curve accurately throughout the whole time course of the monitoring period and behaved logically over a period of 60 hours. Cases were primarily divided into either naked or covered groups. The parameters derived from curve-fitting Class 2 were classified according to the above groups as well as being subdivided by body site. The parameters P_1, P_3 and P_5 are intercept parameters (i.e. they represent the value of each term when x = 0) and the parameters P_2, P_4 and P_6 are the exponent ones. It was found that the intercept parameters did not vary from one curve to another as long as these curves belonged to the same body group and site, that is, these parameters were constant for related cases of a

given group. This was not the case for the exponent parameters, which were notably variable. These parameters did, in fact, reflect individual variations of cooling as well as differences in cooling observed between body sites. Accordingly the exponent parameters were considered as the cooling parameters. The effects of the body variables such as 'cooling size factor (Z)', weight, surface area and hip and head diameters on the rate of cooling were evaluated. This was carried out as follows:

1. Using linear regression analysis P_2, P_4 and P_6 for related cases were correlated to each of the body variables mentioned above. Minitab, a statistical programme package available on the mainframe computer, was used for this purpose. Examples of correlation coefficients derived from this analysis are given in Table 3.16.
2. The cooling parameters were also correlated to the cooling size factors and the body weights by a non-linear regression analysis using the BMDP programme. Non-linear correlation coefficients are shown in Table 3.17.
3. The cooling parameters of related cases were plotted versus the body variables such as the cooling size factor. Thus the correlation was visually assessed. Examples of these plots are shown in Figure 3.23.

It was concluded from the above procedures that

Table 3.16: Coefficients of linear correlation* of parameters P_2, P_4 and P_6 with the "Cooling Size Factor(Z)" for the naked body group.

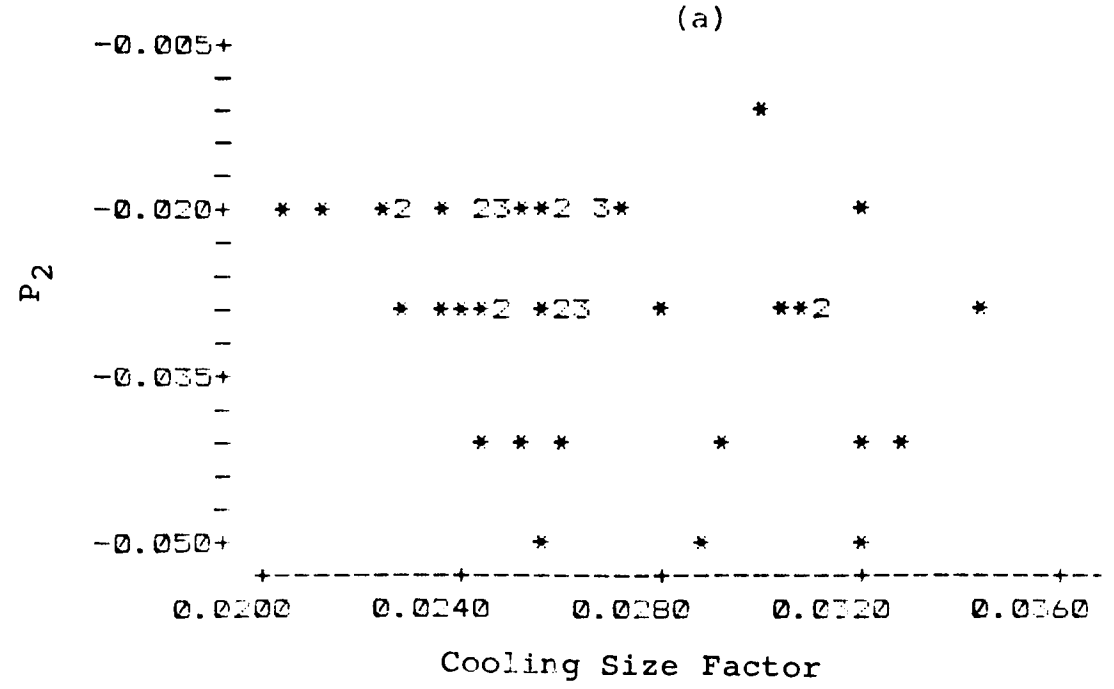
BODY SITE	P_2		P_4		P_6	
	Intercept (I)	Slope (S)	Intercept (I)	Slope (S)	Intercept (I)	Slope (S)
Brain	-0.071	0.769	-0.082	1.13	-0.012	-1.46
Liver	-0.037	0.291	-0.031	0.191	-0.043	0.48
Rectum	0.002	-0.995	-0.021	0.153	-0.044	0.083

*Correlation Equations are P_2, P_4 or $P_6 = I + S.Z$

Table 3.17 Coefficients of non-linear correlations* of parameters P_2, P_4 and P_6 with the "Cooling Size Factor(Z)" for the naked body group.

BODY SITE	P_2		P_4		P_6	
	Intercept (I)	Slope (S)	Intercept (I)	Slope (S)	Intercept (I)	Slope (S)
Brain	-0.07	-0.219	-0.073	-0.204	0.134	-0.435
Liver	-0.012	0.193	-0.017	0.06	-0.006	-.387
Rectum	- 0.60	- 0.858	- 0.087	- 0.353	- 0.037	0.003

*Correlation Equations are P_2, P_4 or $P_6 = \frac{I}{Z^S}$



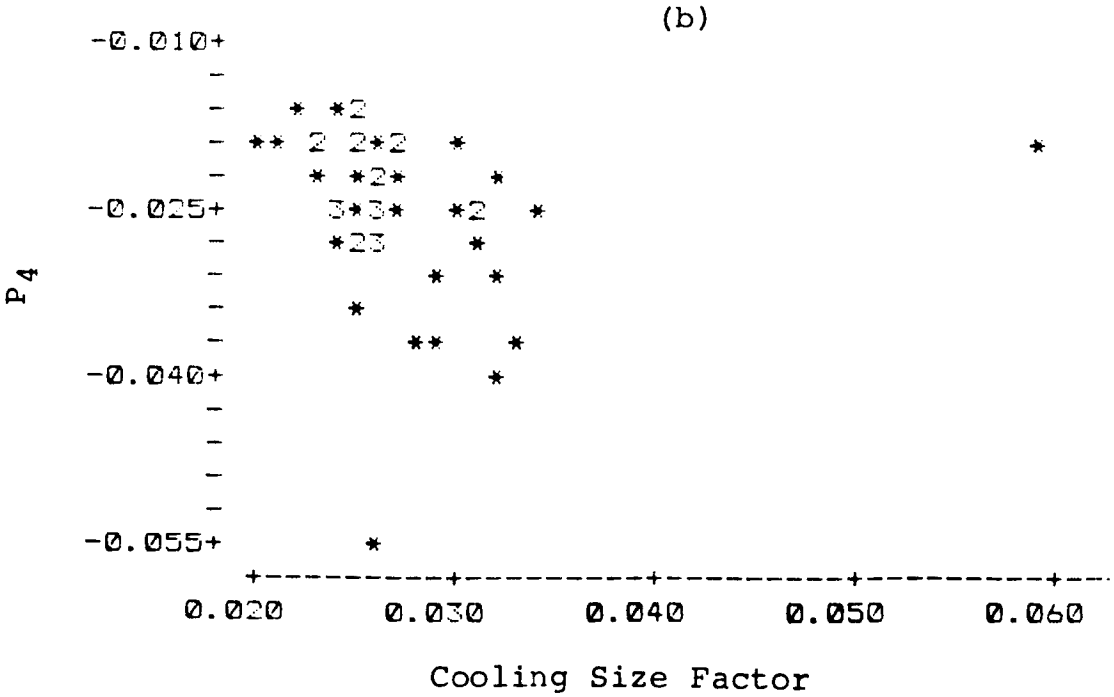


Figure 3.23: Scatter diagrams for cooling parameters versus 'cooling size factor (Z)'.

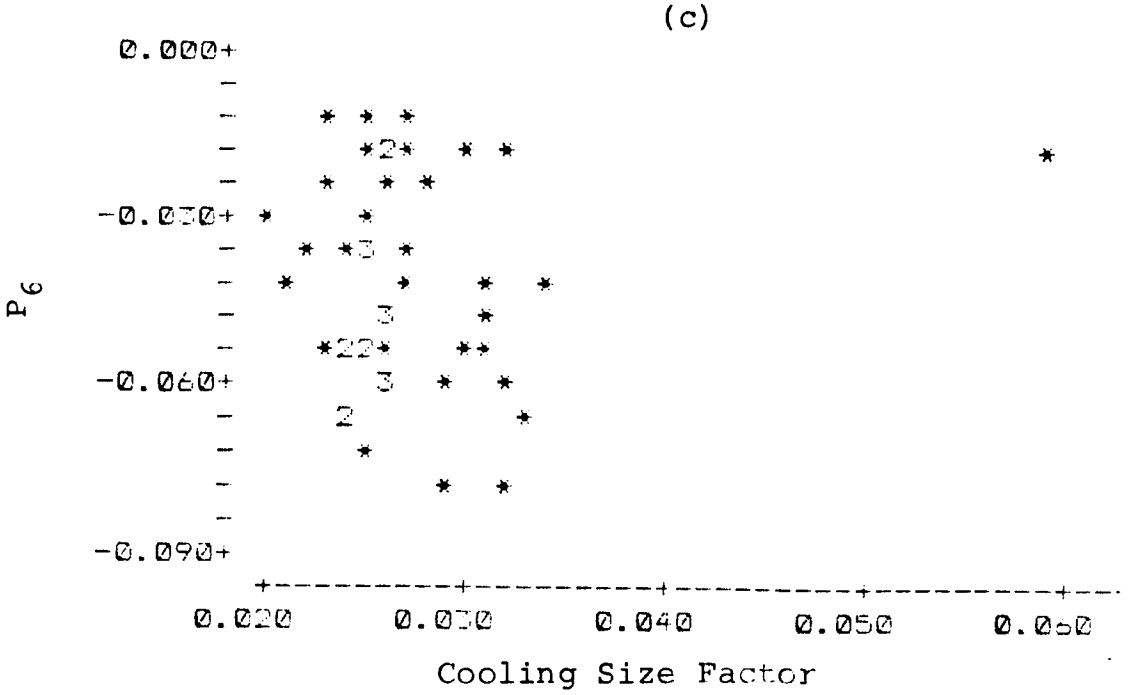


Figure 3.23: Scatter diagrams for cooling parameters versus 'cooling size factor (Z)'.

there were no good correlations between the cooling parameters and any one of the body variables. Therefore these variables could not be used to predict the cooling parameters accurately.

The naked and covered groups for each body site were subdivided into two categories namely fat and thin bodies. Body variables on which this subdivision was based are given in Table 3.18. Equations used to calculate the surface area and the cooling size factor were explained earlier (Paragraphs 2.7.2 and 2.7.3). The cooling size factor is inversly related to the weight of the body and therefore fat bodies have smaller cooling size factors than thin bodies. The effort was made to correlate the cooling parameters of these categories with the body variables, using the methods described above, so that the parameters could be precisely predicted from the weight of the body.

This was not successful: it was found that there were fat bodies whose parameters appeared similar to those observed in thin bodies and vice versa. For example, while 20 cases in the naked group which were classified as fat according to the above criteria (Table 3.18) showed slow rectal cooling and 19 thin cases showed rapid cooling, the cooling behaviour of the remainder (31 cases) was unrelated to the weight. In all other groups, the cooling parameters could not be related to the body size or weight.

Table 3.18: Body Variables of cases classified under fat and thin body groups (N = 29 and 19, respectively). All cases were monitored as naked bodies.

	Fat Bodies				Thin Bodies			
	Weight (kg)	Height (m)	Surface (m^2)	Size Cooling Factor	Weight (kg)	Height (m)	Surface (m^2)	Size Cooling Factor
Range	65 to 117	1.6 to 1.96	1.73 to 2.37	0.02 to 0.027	45 to 61	1.52 to 1.83	1.44 to 1.75	0.028 to 0.033
Mean	81.2	1.77	1.98	0.0247	52	1.69	1.59	0.031
S.D.	15	0.10	0.213	0.0019	5.3	0.09	0.1	0.0017

3.4.3 Average Cooling Formulae

Average cooling curves are required for estimation of the post-mortem interval in the field and therefore parameters of related cases of each group and category were averaged as described in Paragraph 2.7.4. In summary this was carried out by two methods. The first was a simple numerical averaging of the parameters whereas the second method involved two steps: temperature difference ratios for groups of related cases were averaged (Tables 3.19 and 3.20) and then ratios thus obtained were refitted using the BMDP programme. Both methods were used for the naked cases. Also, the ability of the formulae derived from these methods to predict post-mortem intervals accurately was assessed as described in Paragraph 3.5.

It was found that average formulae obtained by both methods gave similar estimates of the time of death. However, the second method resulted in slightly more accurate estimates than the first and only this method was used subsequently for covered bodies and for the fat/thin categories. A summary of the average parameters is given in Table 3.21. It is important to realise that the average formula contains six constants (the parameters) and two variables, the temperature difference ratio (R) which should be known from actual temperature measurements in the field (Equation 2.2) and the time after death (t) which is to be calculated (i.e. it is unknown).

Table 3.19: Average temperature difference ratios for covered bodies versus time; (N = 43).

POST-MORTEM INTERVAL	Temperature Difference Ratios					
	Rectum		Liver		Brain	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
0.0	1	0	1	0	1	0
0.5	0.97	0.019	0.99	0.012	0.94	0.029
1.0	0.94	0.026	0.98	0.022	0.88	0.052
1.5	0.91	0.047	0.96	0.031	0.82	0.07
2	0.89	0.057	0.95	0.041	0.77	0.085
4	0.80	0.084	0.90	0.067	0.59	0.13
6	0.72	0.097	0.86	0.085	0.45	0.152
8	0.65	0.104	0.81	0.098	0.34	0.165
10	0.58	0.11	0.76	0.108	0.25	0.168
12	0.51	0.11	0.71	0.115	0.19	0.164
14	0.45	0.11	0.67	0.13	0.18	0.15
16	0.4	0.11	0.61	0.13	0.15	0.15
18	0.35	0.11	0.56	0.13	0.12	0.14
20	0.3	0.107	0.52	0.13	0.097	0.14
22	0.26	0.108	0.45	0.15	0.078	0.14
24	0.22	0.102	0.44	0.13	0.06	0.13
28	0.16	0.09	0.36	0.12	-	-
32	0.12	0.08	0.3	0.12	-	-
36	0.086	0.07	0.25	0.11	-	-
40	0.06	0.06	0.20	0.104	-	-
50	0.03	0.04	0.12	0.084	0.08	0.13

Table 3.20: Average temperature difference ratios for naked bodies versus time; (N = 74).

POST-MORTEM INTERVAL	Temperature Difference Ratios							
	Rectum						Liver	
	Fat Bodies		Thin Bodies		All Bodies			
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0.0	1	0	1	0	1	0	1	0
0.5	0.97	0.01	0.946	0.024	-	-	0.96	0.018
1.0	0.948	0.02	0.897	0.044	0.93	0.037	0.92	0.034
1.5	0.922	0.03	0.853	0.062	-	-	0.88	0.046
2.0	0.899	0.034	0.812	0.08	0.863	0.066	0.84	0.058
4	0.81	0.056	0.662	0.13	0.75	0.109	0.72	0.089
6	0.72	0.066	0.528	0.15	0.645	0.13	0.62	0.106
8	0.65	0.077	0.41	0.142	0.54	0.14	0.53	0.113
10	0.57	0.081	0.31	0.126	0.45	0.412	0.46	0.116
12	0.50	0.081	0.23	0.106	0.367	0.14	0.4	0.117
14	0.43	0.08	0.169	0.088	0.3	0.13	0.35	0.116
16	0.373	0.078	0.12	0.07	-	-	-	-
18	0.32	0.074	0.087	0.057	-	-	0.27	0.113
20	0.27	0.07	0.061	0.045	0.16	0.1	0.23	0.11
22	0.23	0.066	0.044	0.035	-	-	0.2	0.11
24	0.2	0.06	0.043	0.026	0.1	0.082	0.176	0.107
28	0.14	0.05	0.022	0.016	-	-	0.134	0.103
32	0.096	0.04	0.011	0.011	0.037	0.05	0.1	0.1
36	0.067	0.036	0.006	0.007	0.021	0.04	0.08	0.09
40	0.045	0.029	9.003	0.005	0.015	0.029	0.06	0.09
50	0.17	0.016	0.0007	0.002	0.006	0.015	0.028	0.077

Table 3.21: Parameters of average cooling curves of brain, liver and rectum for naked and covered body groups and for thin and fat categories of rectal cases

Body Site	Remarks**	Parameters *					
		P_1	P_2	P_3	P_4	P_5	P_6
Brain (Naked)	Am.U.	- 5.99	- 0.301	4.5	- 0.311	2.49	- 0.308
Brain (Naked)	Gm.	- 5.94	- 0.20	4.5	- 0.208	2.44	- 0.161
Brain (Covered)	Cf.U.	- 5.9	- 0.101	4.5	- 0.119	2.4	- 0.083
Liver (Naked)	Cf.U	- 6.00	- 0.104	5.1	- 0.09	1.9	- 0.133
Liver (Naked)	Gm.	- 6.00	- 0.133	5.1	- 0.122	1.9	- 0.13
Liver (Covered)	Cf.U	- 5.86	- 0.142	5.1	- 0.150	1.76	- 0.054
Rectum (Naked)	Gm.	- 6.01	- 0.188	5.1	- 0.146	1.9	- 0.228
Rectum (Covered)	Cf.U.	- 5.99	- 0.192	5.1	- 0.206	1.89	- 0.085
Rectum (Thin-Naked)	Cf.U.	- 6.00	- 0.265	5.1	- 0.202	1.9	- 0
Rectum (Fat-Naked)	Cf.U.	- 5.25	- 0.129	5.93	- 0.109	0.323	-0.269

* Parameters are in Equation 3.1

** Am. = Arithmetic mean
Gm. = Geometric mean
Cf. = Curve-fitting
U = Used for assessment

Note that, for the brain, both arithmetic and geometric means of the parameters were calculated in an attempt to reduce the error in time estimates. The average cooling formula of the brain in the covered group also accurately represented the cooling of the brain in the naked group. This also explains why there are no data for the brain in Table 3.20.

Average cooling curves of the three body sites (i.e. brain, liver and rectum) for covered and naked body groups are shown collectively in Figures 3.24 and 3.25. It is clear from these figures that the brain is the quickest to cool in both naked and covered body situations. In the covered group the liver cools more slowly than the rectum, while in the naked group both the liver and the rectum cool almost at the same rate. However the rectum showed slightly slower cooling than the liver at the beginning of the monitoring period.

The effects of covering the torso on the cooling of each site are shown in Figures 3.26-3.28. These figures indicate that the brain is the least affected by covering of the torso. Nevertheless, cooling of the brain in the covered group is slightly slower at the end of monitoring than in the naked group. In the latter group the brain temperature approaches that of the environment in about 30 hours, on average, while in the covered group the curve is flattened at the end and the time required to approach the environment (zero line in the figure) is delayed. The liver is found to

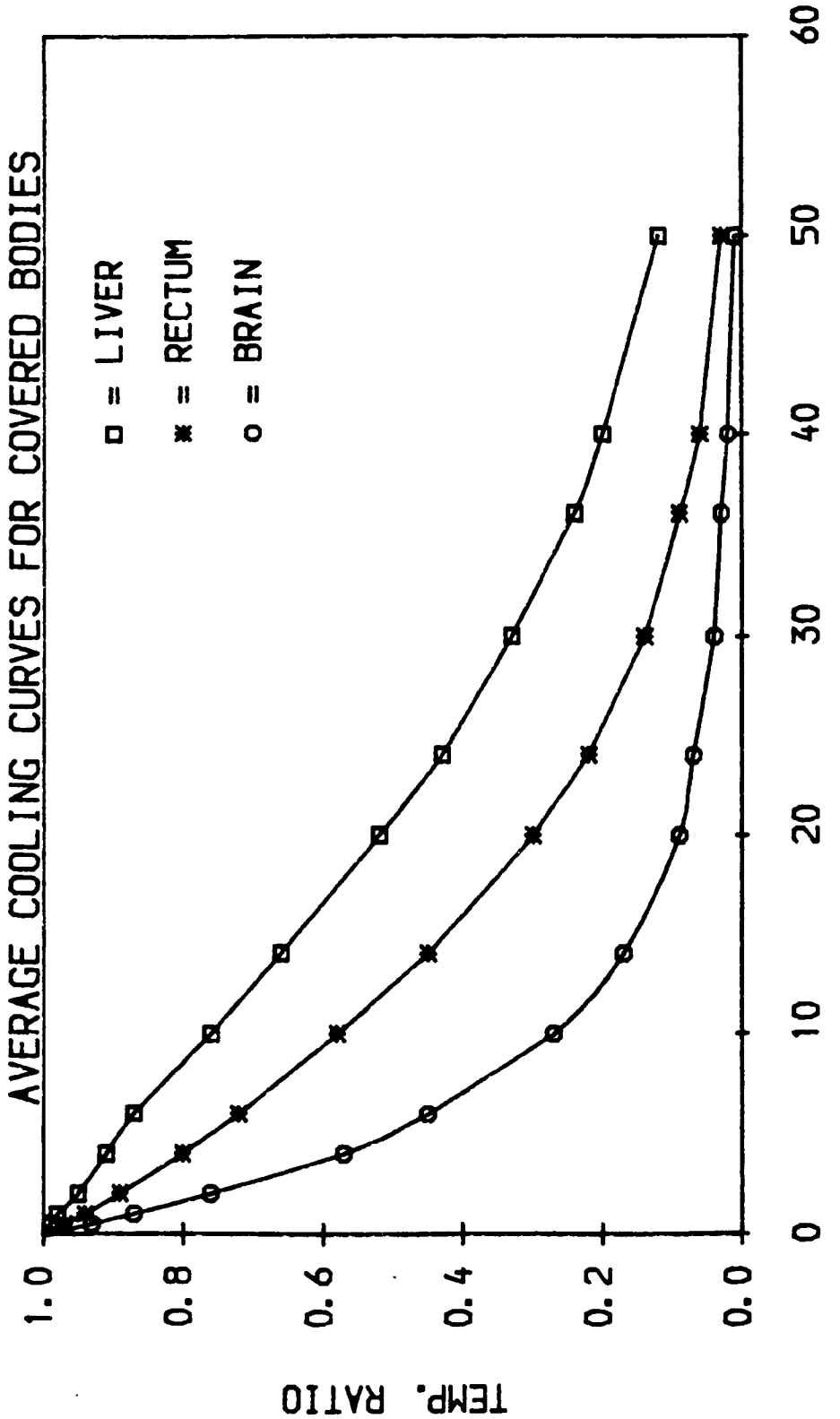

AVERAGE COOLING CURVES FOR COVERED BODIES
□ = LIVER
✱ = RECTUM
o = BRAIN
TEMP. RATIO
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
HOURS AFTER DEATH

Figure 3.24:

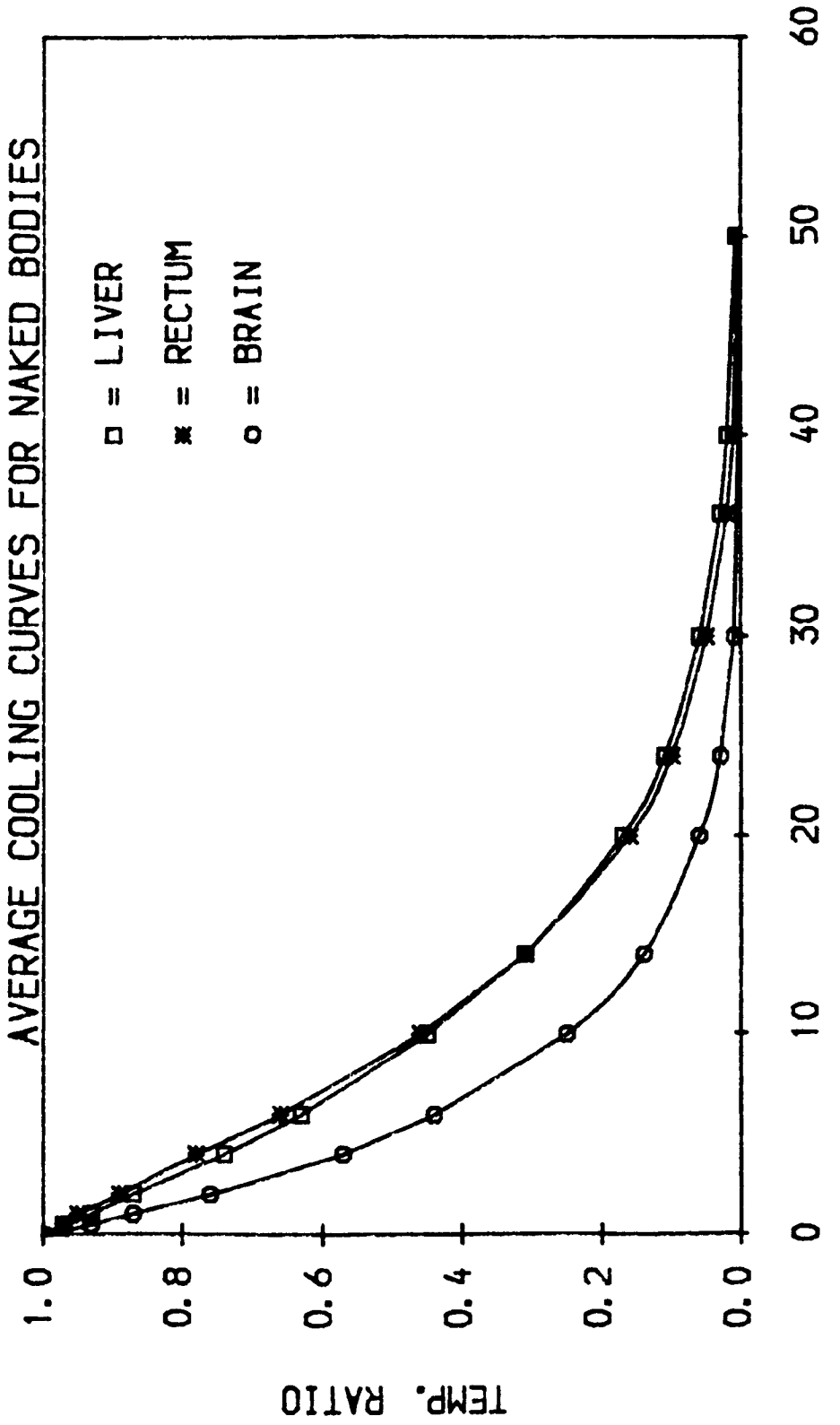

AVERAGE COOLING CURVES FOR NAKED BODIES
□ = LIVER
✱ = RECTUM
o = BRAIN
TEMP. RATIO
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
HOURS AFTER DEATH

Figure 3.25:

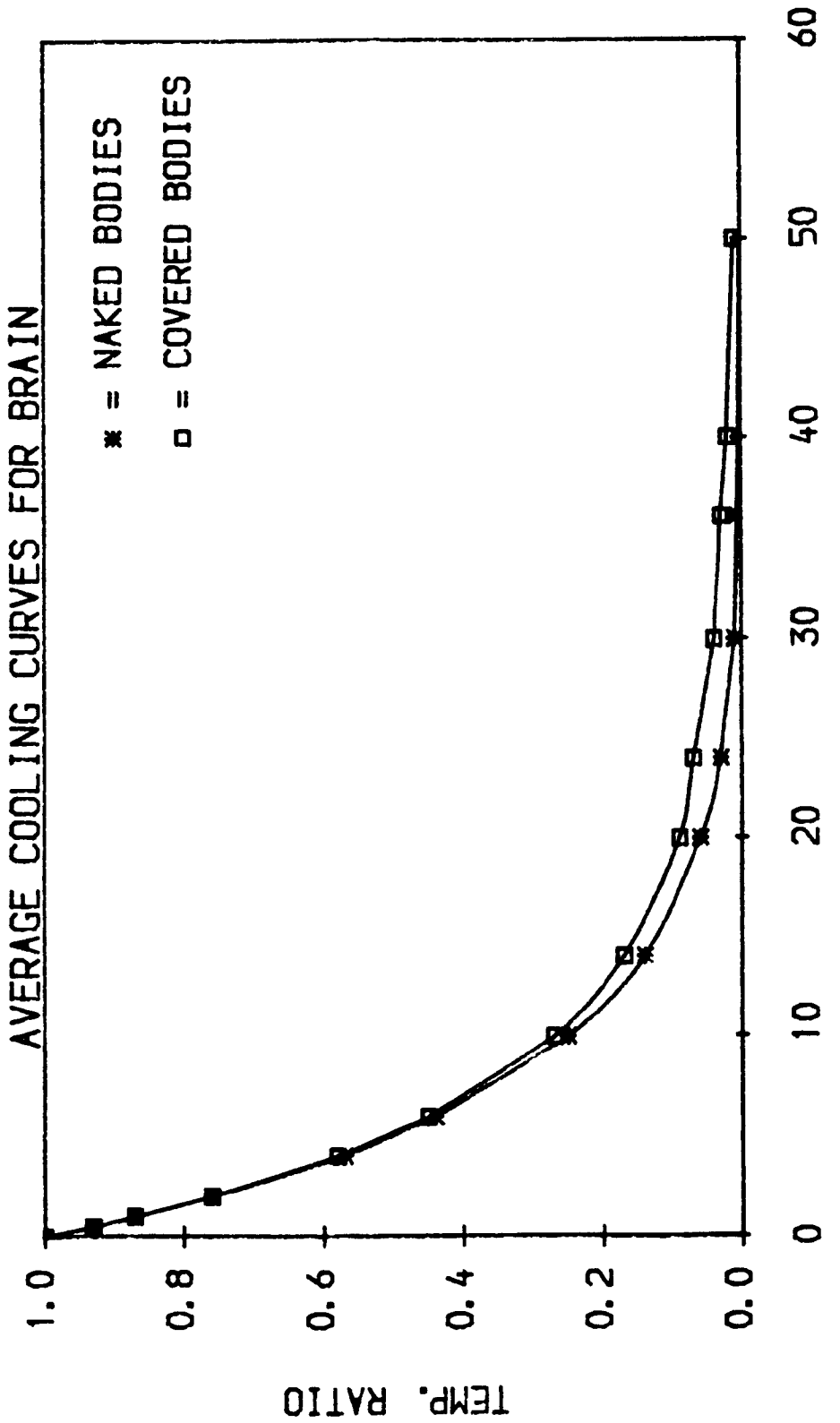

AVERAGE COOLING CURVES FOR BRAIN
* = NAKED BODIES
□ = COVERED BODIES
TEMP. RATIO
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
HOURS AFTER DEATH

Figure 3.26:

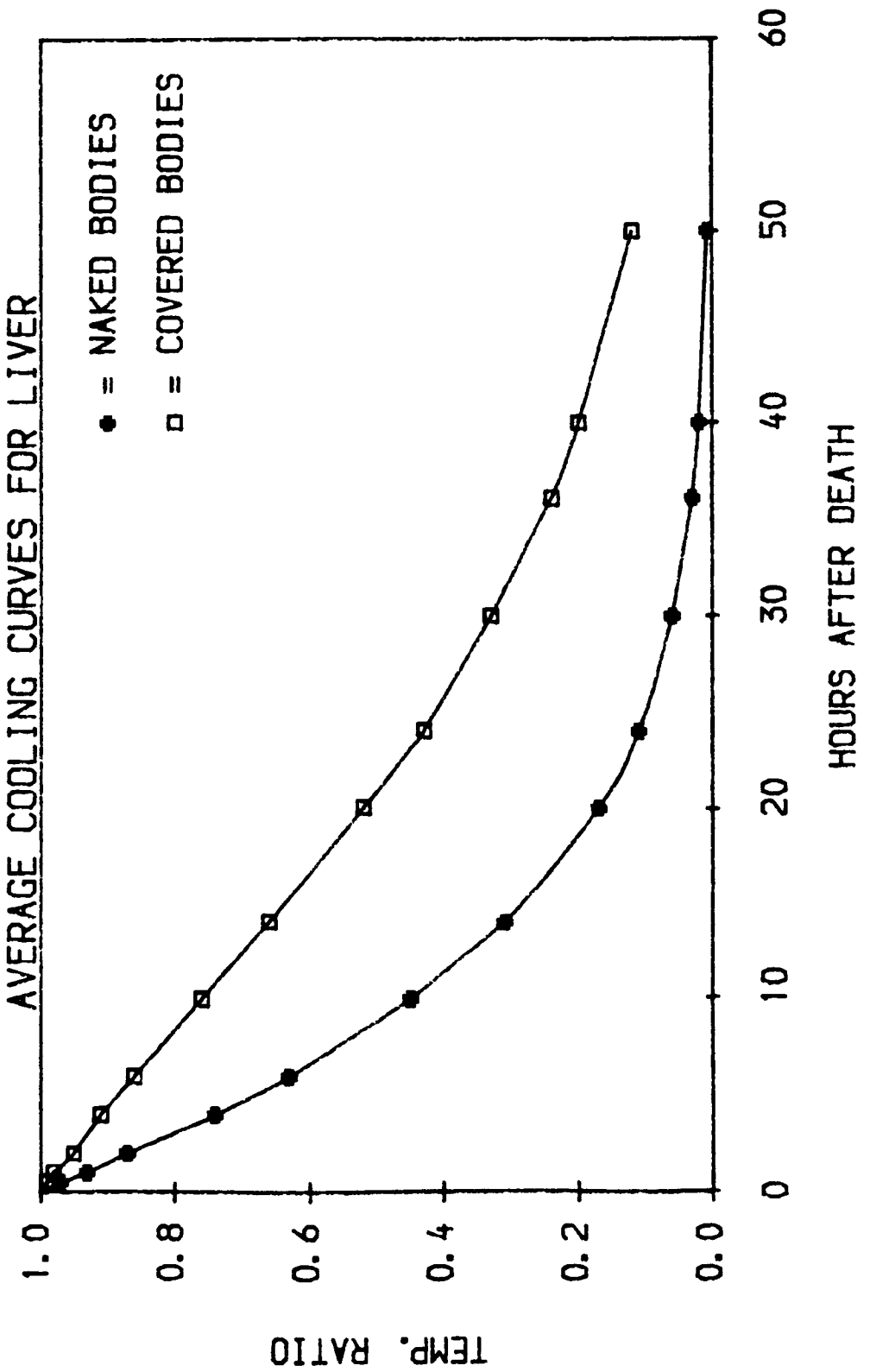

AVERAGE COOLING CURVES FOR LIVER
● = NAKED BODIES
□ = COVERED BODIES
TEMP. RATIO
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
HOURS AFTER DEATH

Figure 3.27:

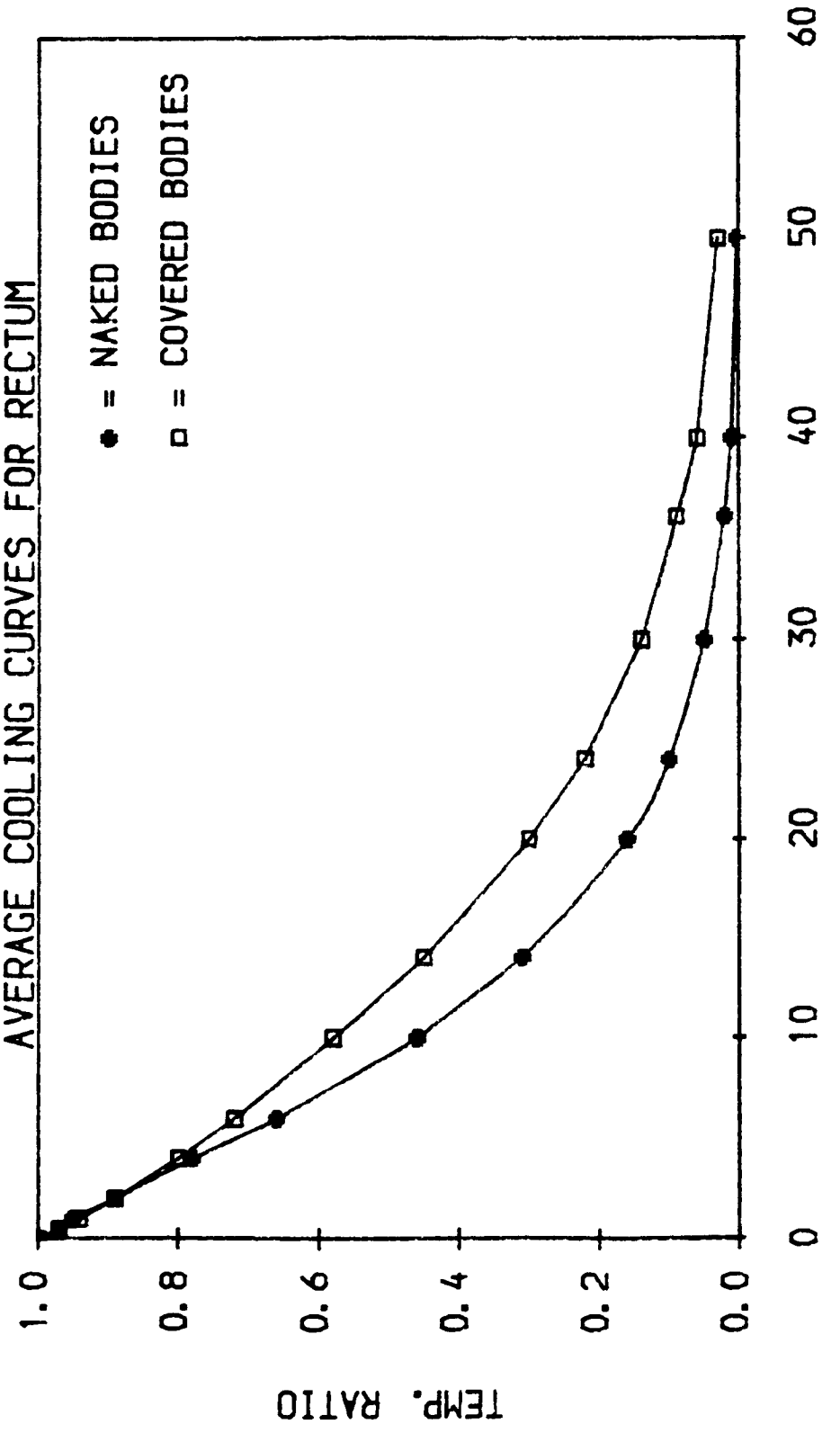

AVERAGE COOLING CURVES FOR RECTUM
● = NAKED BODIES
□ = COVERED BODIES
TEMP. RATIO
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
HOURS AFTER DEATH

Figure 3.28:

be the most affected by covering: the average curve of the covered group is higher than that of the naked group throughout the whole monitoring period. The rectum is also affected by covering but to a lesser extent than the liver. However, in the first 5 hours, the rectum cools almost at the same rate in both covered and naked body conditions. Also, the curve in the naked group is steeper than in the covered group particularly at the mid-stage of cooling (from about 10-30 hours).

The comparison between average rectal cooling curves of fat and thin bodies is shown in Figure 3.29. It is concluded that thin bodies cool faster than fat ones, on average. However it is important to emphasise that this pattern was not observed in 44% of the rectal curves, as explained earlier (Paragraph 3.4.2).

The age of the deceased was found to have no effect on the cooling. This was true for all sites and groups studied. It should be noted, however, that the cases were all of adult or old people. Effects of other factors such as the posture of the body and the movement of air on the post-mortem cooling were not examined in this research.

3.5 Assessment of the Method

3.5.1 Accuracy of Average Formulae

The ability of the average cooling formulae to predict the time of death was tested by comparing the

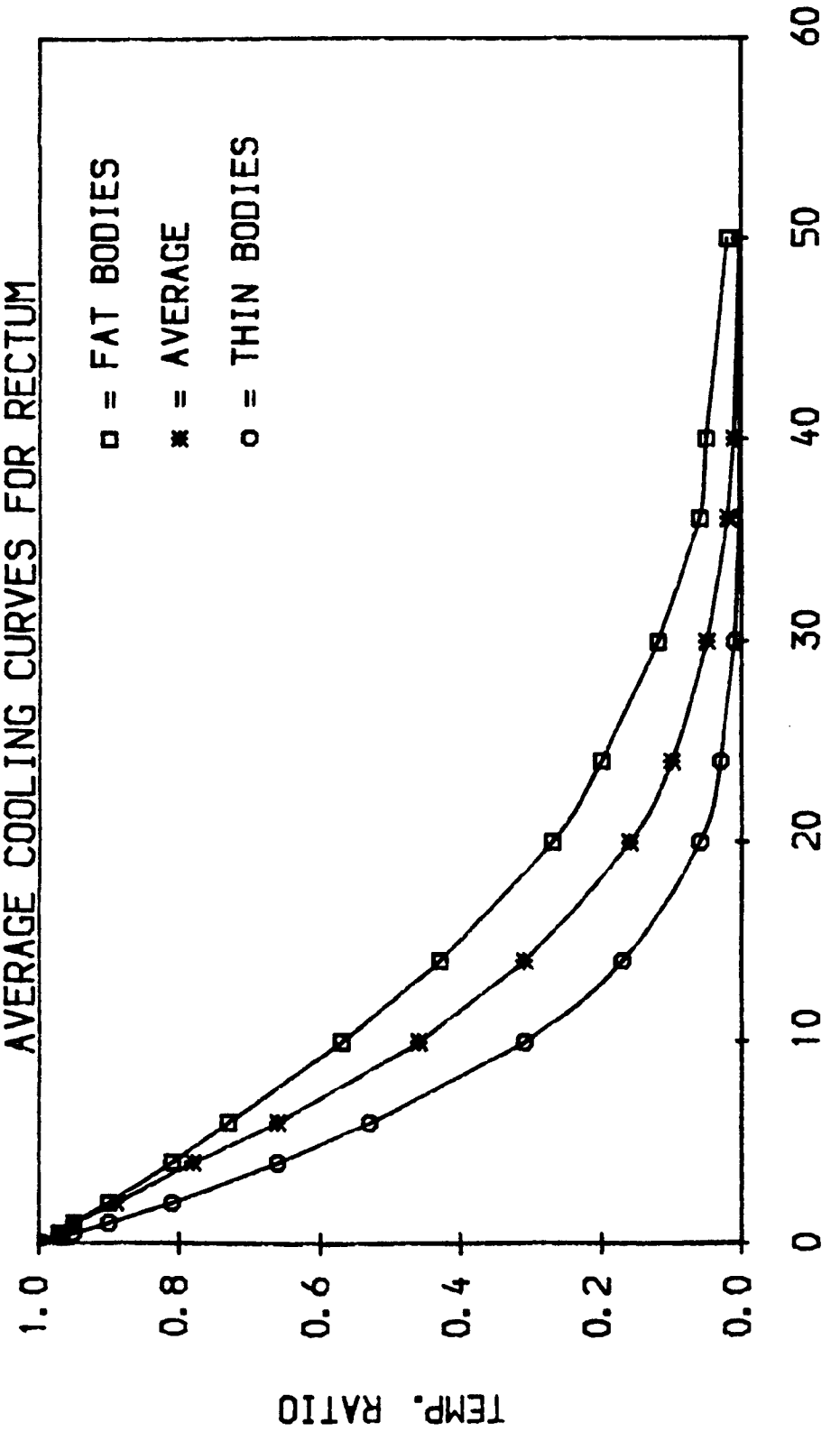

AVERAGE COOLING CURVES FOR RECTUM
□ = FAT BODIES
* = AVERAGE
o = THIN BODIES
TEMP. RATIO
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
HOURS AFTER DEATH

Figure 3.29:

average cooling curve with curves for individual cases in which both (R) and (t) were experimentally recorded and processed. Thus an average formula was used to calculate times for values of (R). As actual times corresponding to these values of R were known, differences between the actual times and the predicted times were calculated throughout the entire monitoring period. This was carried out for each body site in all cases.

The assessed formulae, in this section, were those derived by the second method of averaging of the parameters (Paragraphs 2.7.4 and 3.4.3). These parameters were denoted by (Cf) in Table 3.21. The mean and standard deviation of the differences thus obtained were calculated for selected times after death and for related cases in each body group. These are given in Tables 3.22 and 3.23, which also show the number of cases in which the assessement was performed. The former table contains the differences between the actual times and the predicted times (also known as the deviation of estimated times from actual times) obtained in the covered group at selected post-mortem intervals. The latter table contains the same type of information but for naked bodies. Also, it contains information concerning fat and thin categories.

Obviously these differences represent the errors in time estimates when the average formulae are used.

Table 3.22: Deviation of estimated time from actual time using the average formulae for the covered group

ACTUAL TIME (hours)	Error in Estimated Time (hours)					
	Rectum *		Liver *		Brain **	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
0.5	0.21	0.27	- 0.03	0.49	0.26	0.44
1	0.47	0.74	0.08	0.92	0.23	0.62
2	0.46	1.56	- 0.39	1.95	0.15	0.89
4	0.3	2.6	0.11	2.74	- 0.02	1.54
8	0.09	3.7	- 0.06	3.5	- 0.02	2.7
12	0.35	4.2	- 0.38	4.1	0.59	4.2
18	1.38	3.2	- 0.1	4.63	4.3	4.7
24	0.98	6.2	0.5	5.4	6.0	5.3
30	3.72	6.1	2.67	6.5	-	-

* N = 43
** Assessment was made in both naked and covered groups; (N = 117).

Table 3.23: Deviation of estimated time from actual time using the average formulae for the rectum and liver in naked bodies; (N = 74).

ACTUAL TIME (hours)	Error in Estimated Time (hours)					
	Rectum				Liver	
	Fat Bodies		Thin Bodies			
	Mean	S.D.	Mean	S.D.	Mean	S.D.
0.5	- 0.07	0.28	0.27	0.28	0.01	0.5
1	0.11	0.4	0.07	0.43	0.11	0.61
2	0.0	0.7	0.68	2.8	0.2	0.99
4	0.1	1.1	0.38	1.4	- 0.2	1.44
8	0.1	2.1	0.76	1.76	- 0.42	2.55
12	0.01	2.7	1.0	2.19	- 0.45	3.72
18	- 0.45	3.66	0.79	3.07	- 0.21	5.4
24	- 0.53	5.6	0.1	6.4	3.65	6.35
30	- 1.18	4.4	0.2	4.4	4.2	7.9
50	- 3.1	6.1	-	-	-	-

Values of mean and standard deviation of these errors were plotted versus the post-mortem interval: examples of these plots are shown in Figures 3.30-3.35. These correspond to the brain, liver and the rectum in the covered and naked groups. The average formula of the brain in the covered group was assessed in relation to all cases in both covered and naked groups. This was because cooling of the brain was found to be relatively unaffected by the covering of the torso.

The following conclusions are drawn from these tables and figures:

1. The mean error in time estimates is noticeably small, particularly in the liver and the rectum (Note that the curves are near to the zero lines in Figures 3.31-3.35 for the whole or most of the interval). For the brain (Figure 3.30) the average error is also very small up to 10 hours after death, then it increases gradually to a value of 6 hours at 24 hours post-mortem. This increase in the error at the end stage of the monitoring period is also observed, although to a lesser extent, in the liver (Figures 3.32 and 3.33) and the rectum (covered group Figure 3.34).
2. There are marked individual variations for all body sites in all groups. This is indicated by large values of the standard deviation (shown as vertical bars in the figures). These values increase with the post-mortem interval in all situations except

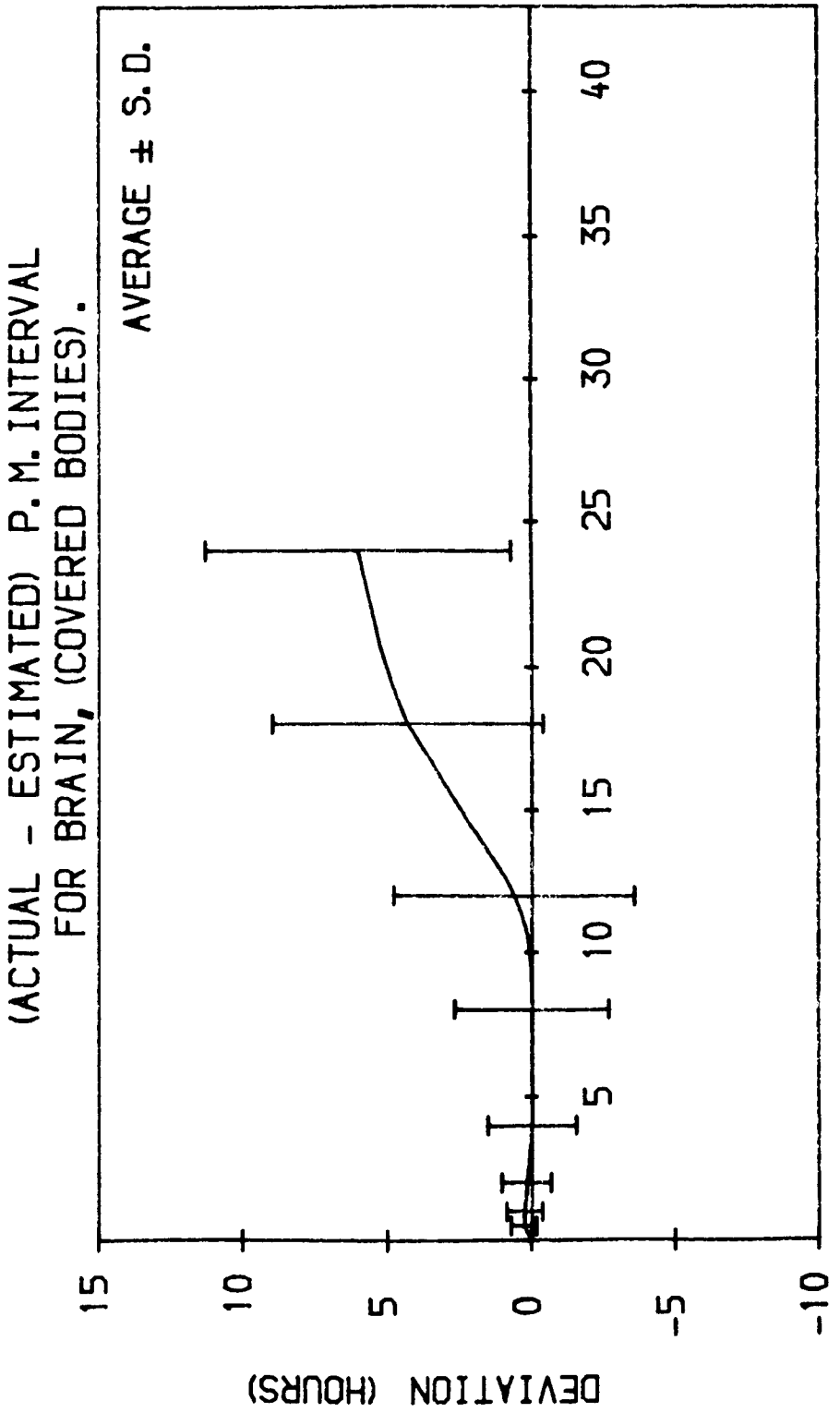


Figure 3.30: Deviation of estimated time from actual time after death, using average formula for brain in covered bodies.

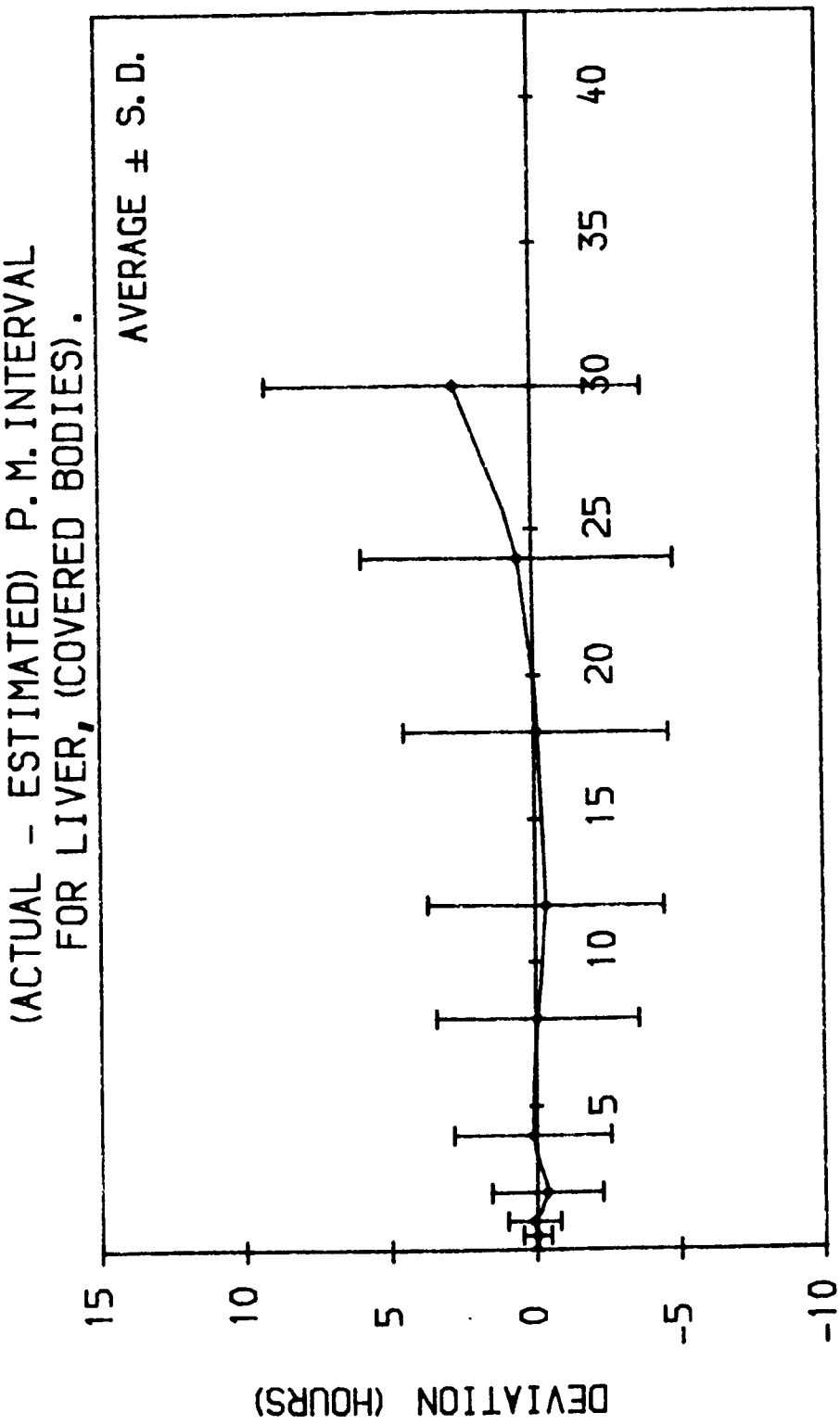


Figure 3.31: Deviation of estimated time from actual time after death, using average formula for liver in covered bodies.

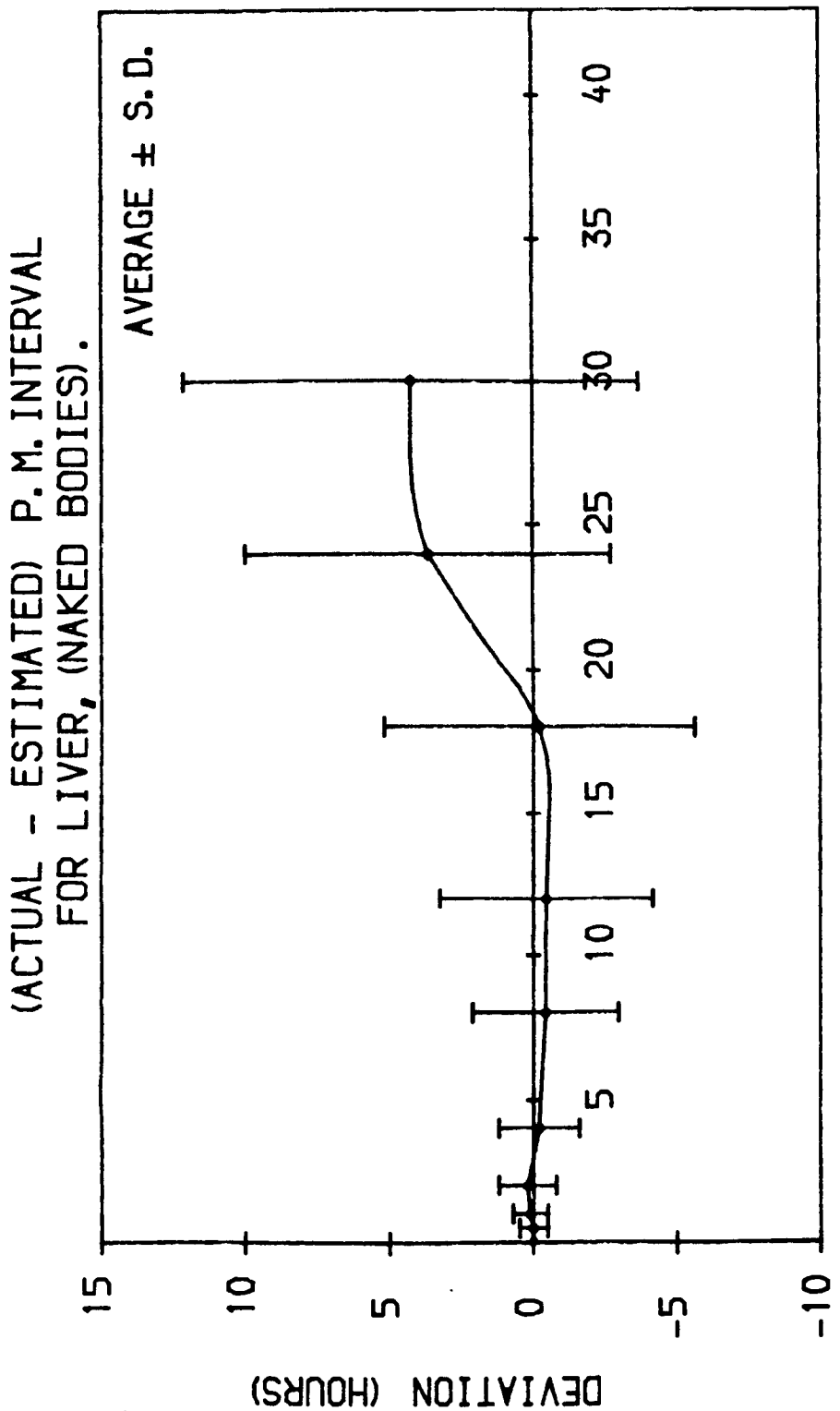


Figure 3.32: Deviation of estimated time from actual time after death, using average formula for liver in naked bodies.

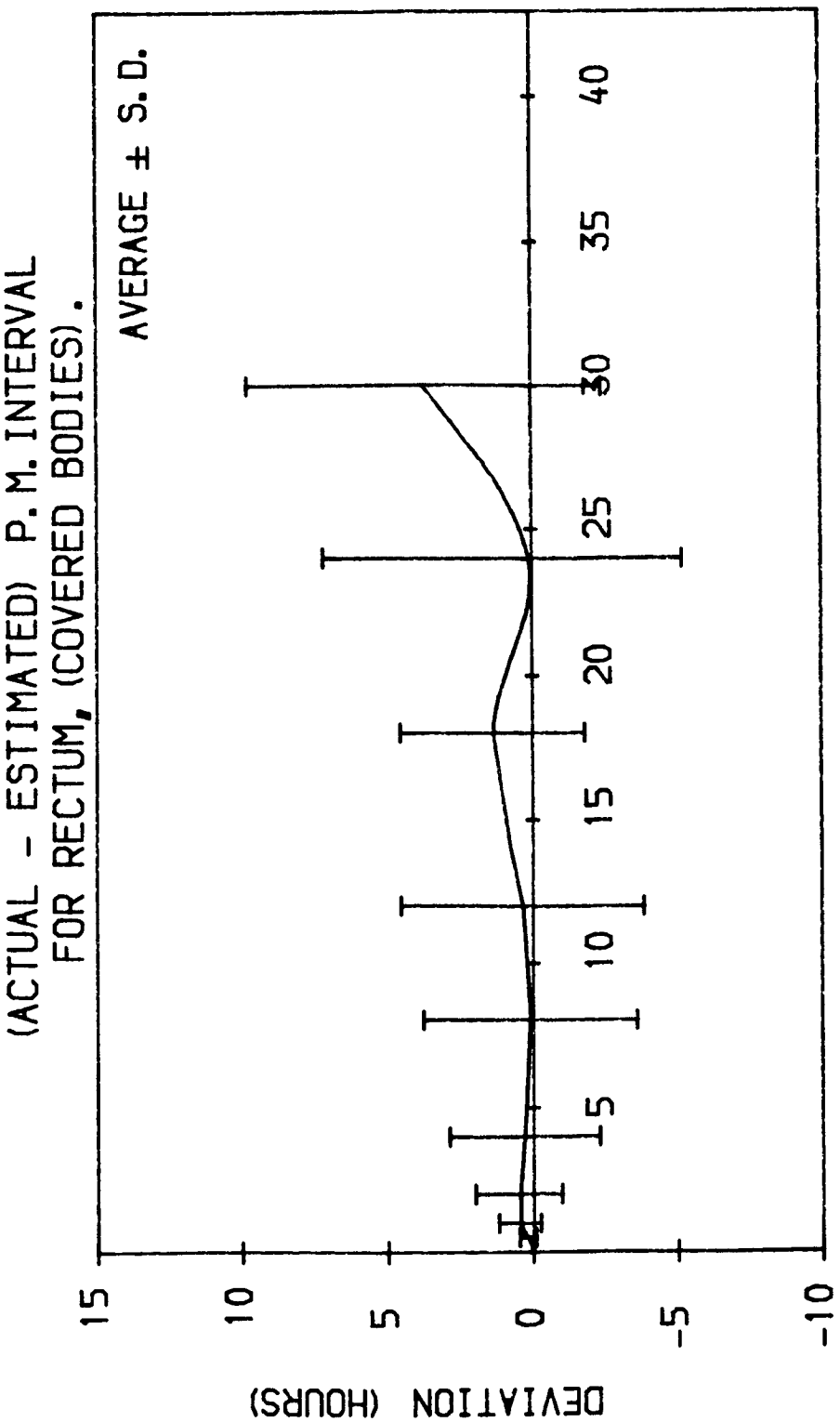


Figure 3.33: Deviation of estimated time from actual time after death, using average formula for rectum in covered bodies.

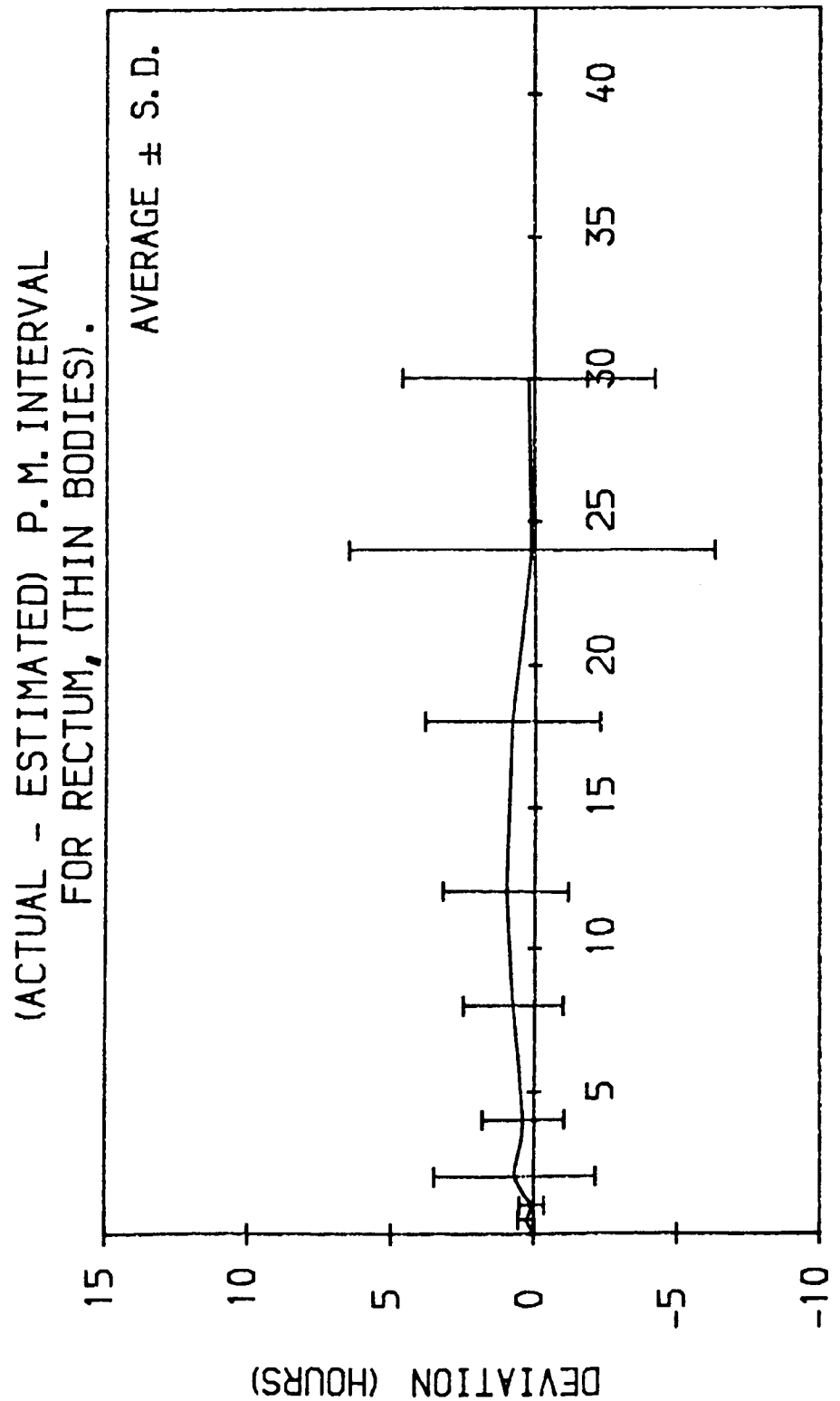


Figure 3.34: Deviation of estimated time from actual time after death, using average formula for rectum in thin naked bodies.

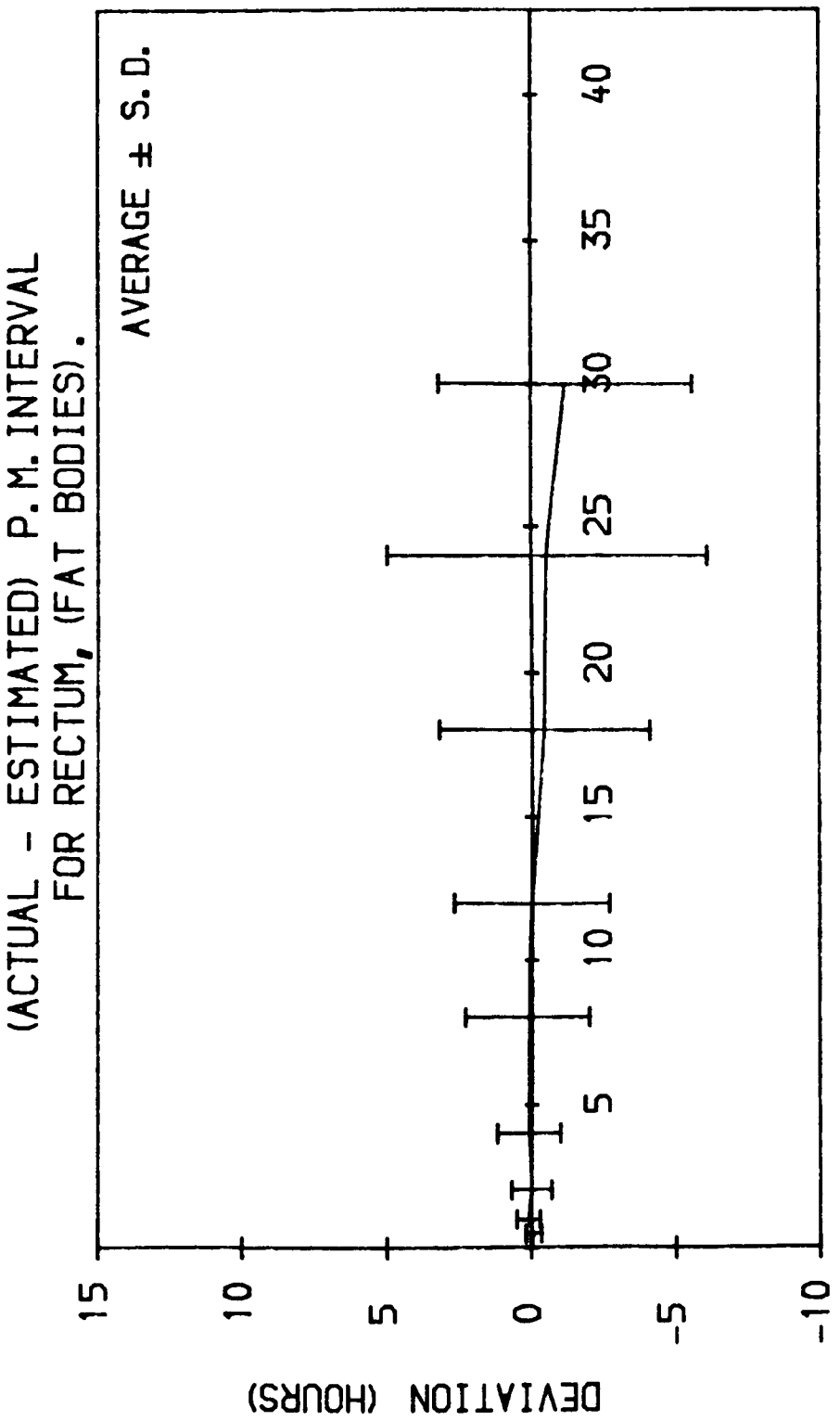


Figure 3.35: Deviation of estimated time from actual time after death, using average formula for rectum in fat naked bodies.

for rectal curves in thin bodies, in which the scatter at 2 hours after death is wider than that at 4 hours post-mortem. In all groups the scatter is least during the first 5 hours or so.

3. It is necessary to emphasize that one standard deviation is shown in these figures. Therefore 68% of cases encountered in practice under similar environmental conditions are likely to be within this range of error.

3.5.2 Assessment of Other Formulae

In addition to the assessment of the average formulae described above, the validity of other formulae for predicting the post-mortem interval were also assessed in a similar manner:

1. The formula derived by simple numerical averaging of the parameters of the brain cooling curve in the naked group (Paragraphs 2.7.4.1 and 3.4.3) was tested in 10 cases. This formula contained arithmetic mean values of the parameters denoted by (Am) in Table 3.21. The errors in time estimates found in this way were greater than those found in the assessment described in Paragraph 3.5.1. Average and standard deviation values of these errors are given in Table 3.24 and are plotted versus time in Figure 3.36. Because this assessment was not encouraging similar formulae for the liver and the rectum were not used or assessed.
2. The equations derived by linear and non-linear

Table 3.24: Deviation of estimated time from actual time using the arithmetic mean formula of the brain cooling curve in the naked bodies. (N = 74)

ACTUAL TIME (HOURS)	ERROR IN ESTIMATED TIME (HOURS)	
	Mean	S.D.
0.05	0.39	0.22
1	0.58	0.26
2	1.1	0.34
4	2.04	0.73
8	3.78	1.79
12	6.0	2.5
18	10.18	2.34
24	11.7	2.8

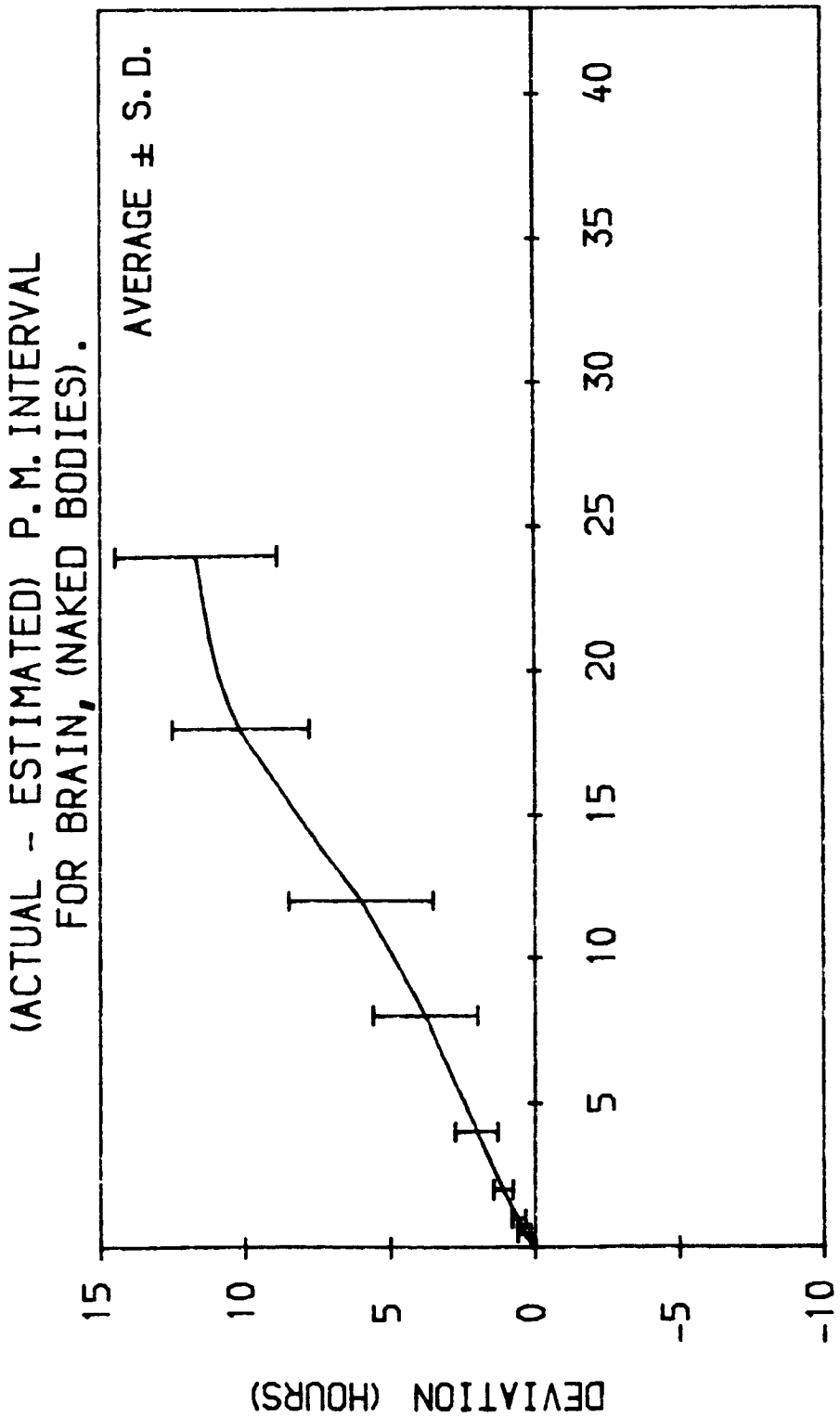


Figure 3.36: Deviation of estimated time from actual time after death, using arithmetic mean formula for brain in naked bodies.

correlation of the cooling parameters, P_2, P_4 and P_6 with body variables (see Tables 3.16 and 3.17) were assessed in 5 cases for each body site. As the correlations were bad (Paragraph 3.4.2) the resulting errors in time estimates were enormous.

As a result of these assessments it was concluded that the best average formulae to be used in practice were those denoted by (Cf) in Table 3.21.

3.5.3 Attempts to Reduce the Error of Time Estimation

It was noted that when the curve-specific parameters, derived from the curve-fitting Class 2 of that particular curve, were used, the time estimates were very precise and estimation errors were within a very narrow limit. This was seen in Figures 3.18b, 3.19b and 3.20-3.21. When the average parameters were used, such precision could not be maintained and the range of errors was broadened. Many attempts were made to overcome this problem. For example, cooling parameters, P_2, P_4 and P_6 were correlated with each other. This was because these parameters were found to reflect individual variations and body site differences in cooling more than the intercept parameters, P_1, P_3 and P_5 as explained earlier (Paragraph 3.4.2). These correlations were not sufficiently good to permit the precise prediction of one of these three parameters when the other two were known.

For the same purpose, another technique was used

in which it was supposed that for a group of related curves (same site, same body group) all parameters were constant except P_4, which was allowed to vary with time and hence with the temperature difference ratio and was denoted PC_4. Accordingly, PC_4 was given by the following equation:

$$PC_4 = [Log_e(R - P_1e^{P_2t} - P_5e^{P_6t})/P_3] /t \quad \text{...Equation 3.2}$$

Values of PC_4 were computed for each curve at several intervals. Average values of PC_4 were correlated to the average values of temperature difference ratios (R) using a non-linear regression method (BMDP programme). This method was assessed with respect to the accuracy of time estimates in 5 cases. Errors in time estimates when this method was used were compared with those resulting from the use of the average formulae (Table 3.21). The time estimation was not improved in these cases. In fact this method also involved average values (of PC_4) and hence the aim of improving the accuracy of the prediction of the post-mortem interval could not be achieved by this approach. It was concluded that the accuracy achieved by the original average formulae was the best to be expected.

3.5.4 Average Curves For Practical Use

The curves shown in Figures 3.30-3.35 are difficult to use in the field. Therefore other curves were drawn which could easily be used in practice.

Each of these was a plot of the average temperature difference ratios (R) of a body site versus post-mortem interval. The scatter of R values was also shown in the plot. The data used to draw these curves was obtained as described earlier (Paragraph 3.4.3). Therefore each of these plots gave an average curve of a body site as well as the errors in time estimates which would probably be encountered in the field. These curves are shown in Figures 3.37-3.43, which correspond to the brain, liver and rectum in the covered and naked groups. Curves for the fat and thin categories, which represent the rectum only, are also shown.

In each of these curves the Y axis value is actually the average value of R ±1 standard deviation (shown by vertical bars in the figures). If the upper and lower ends of the bars are joined, additional curves are obtained which delineate the range from -1 to +1 standard deviation (Figure 3.44). Thus the errors in time estimates could easily be calculated for any point on the graph. An example, to explain how these plots are used in the field, is given in Figure 3.45. In this example, the temperature difference ratio is calculated according to Equation 2.2 and the value of the rectal temperature at the moment of death is obtained from Table 3.13. The range of error in the time estimate, corresponding to the value of the temperature difference ratio observed in the field, is

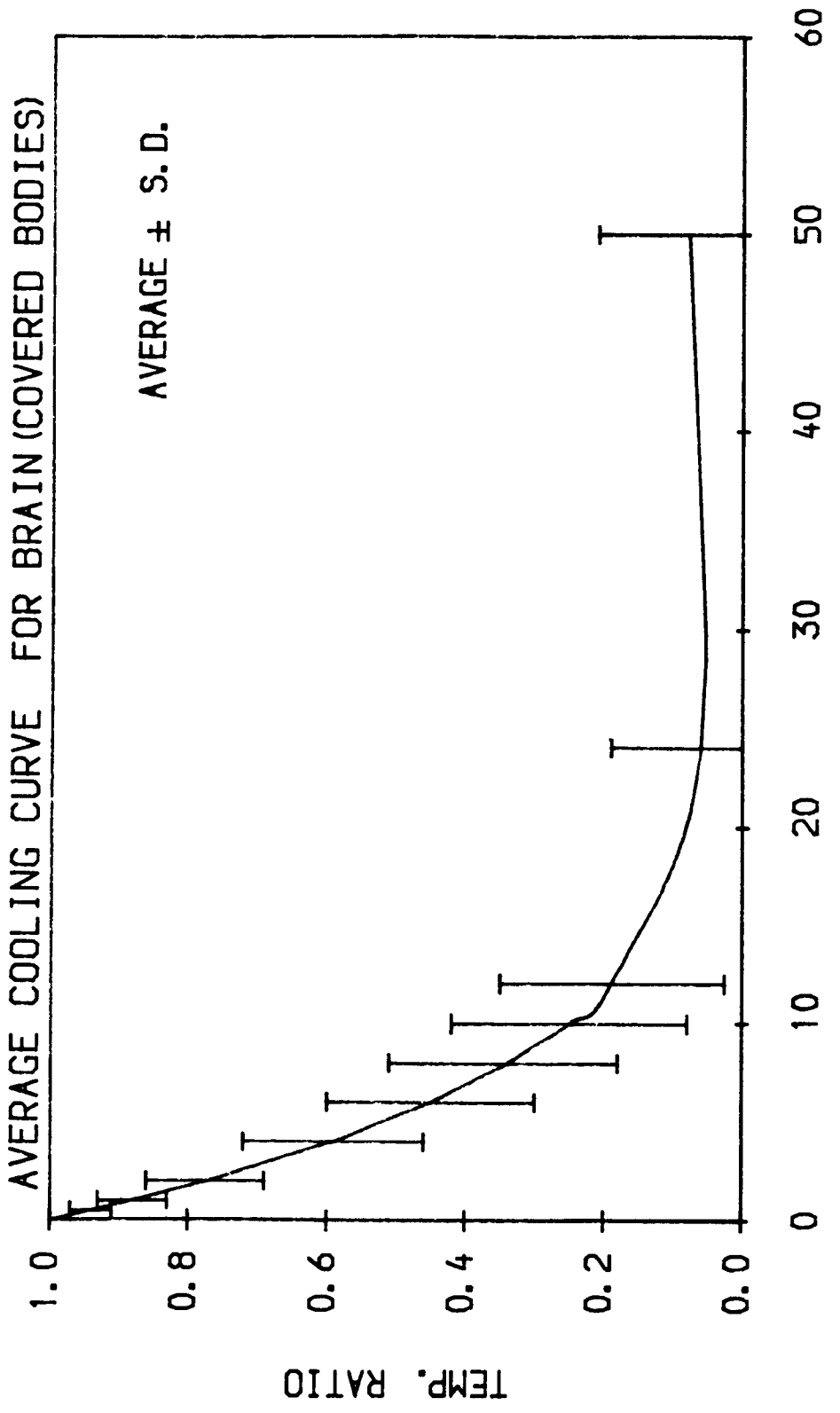


Figure 3.37: Plot of average temperature difference ratio ±1 standard deviation versus post-mortem interval based on temperature measurements by microwave thermography of the brain in the covered body group.

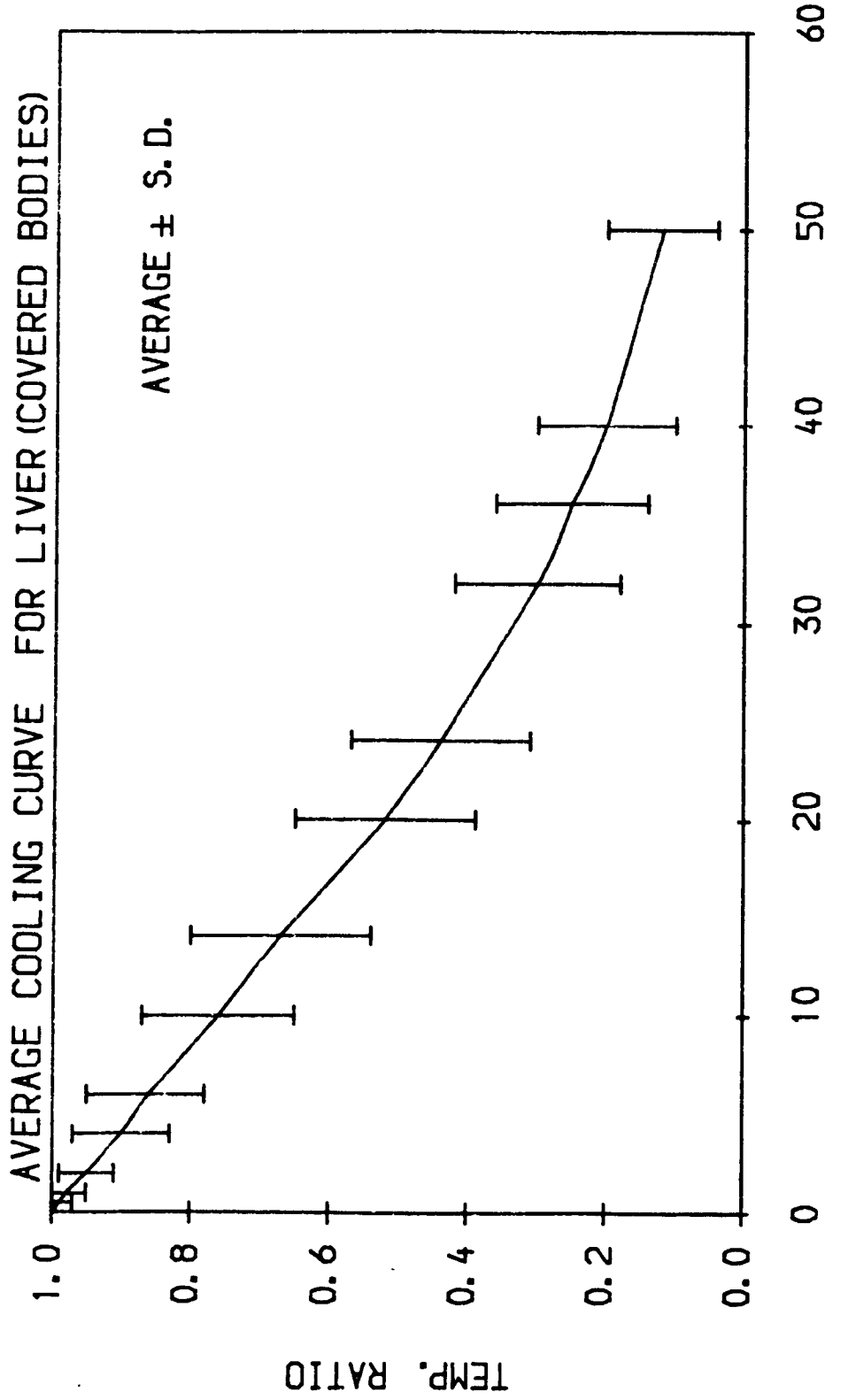


Figure 3.38: Plot of average temperature difference ratio ±1 standard deviation versus post-mortem interval based on temperature measurements by microwave thermography of the liver in the covered body group.

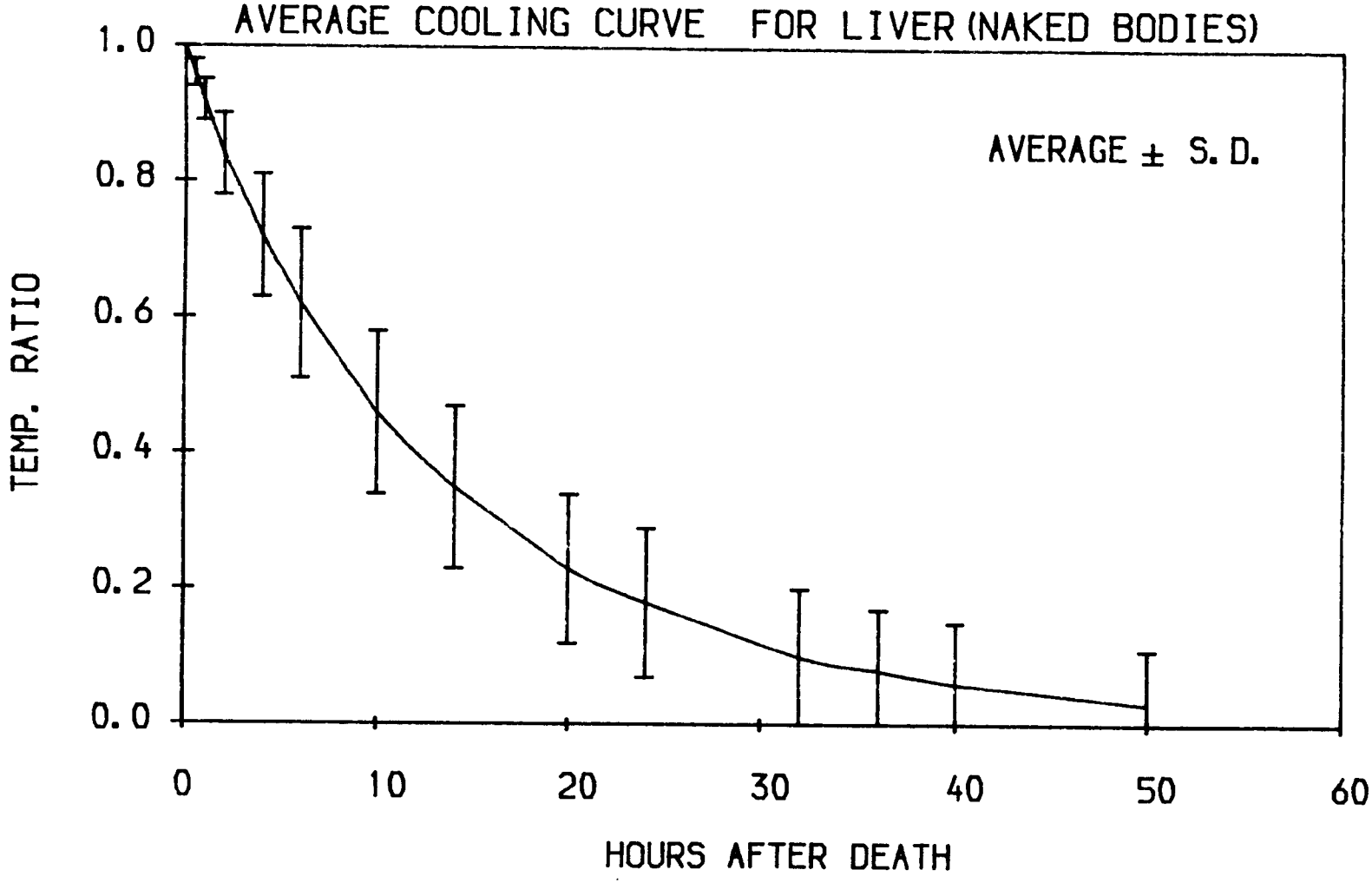


Figure 3.39: Plot of average temperature difference ratio ±1 standard deviation versus post-mortem interval based on temperature measurements by microwave thermography of the liver in the naked body group.

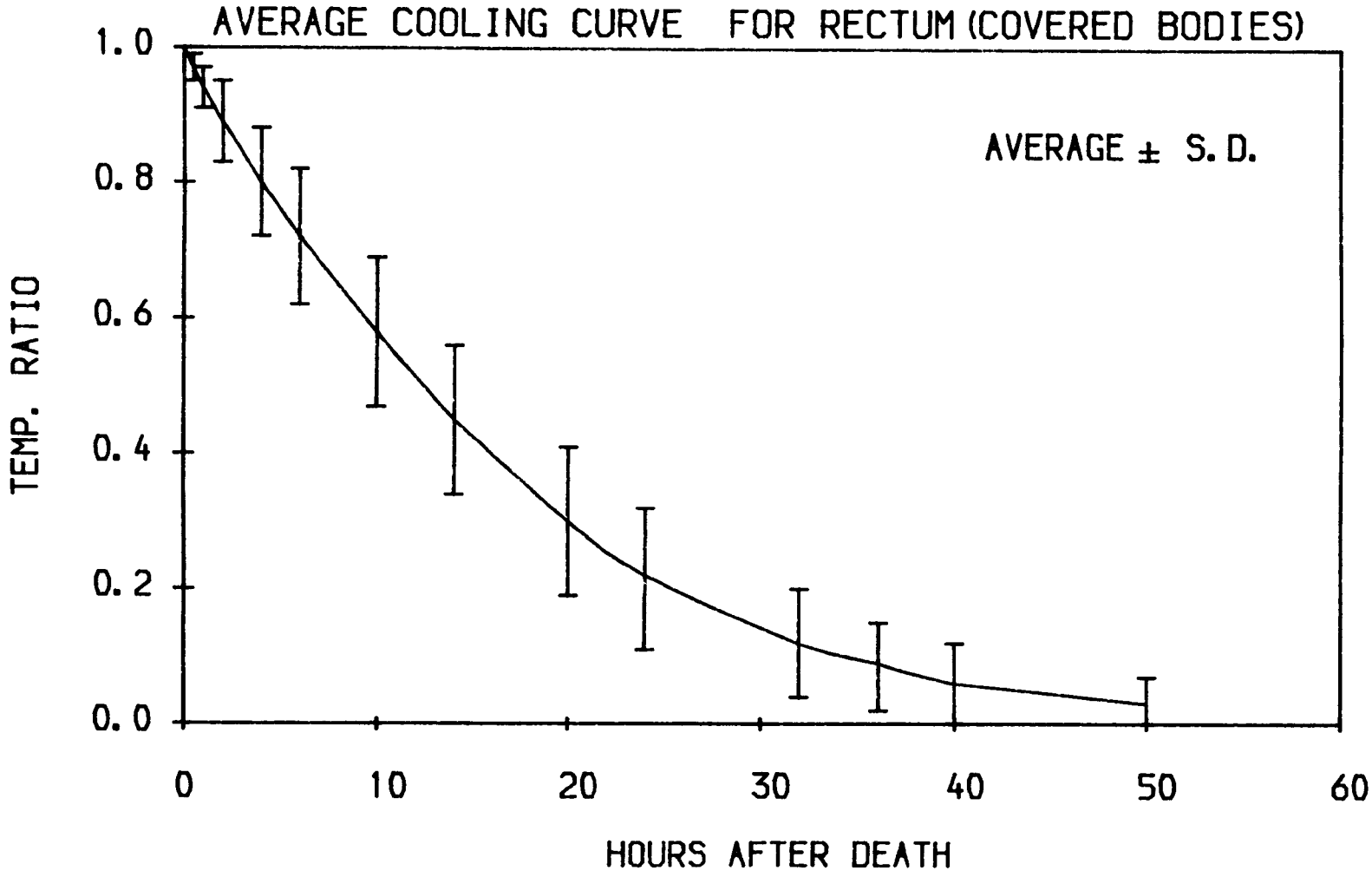


Figure 3.40: Plot of average temperature difference ratio ±1 standard deviation versus post-mortem interval based on temperature measurements by rectal thermocouple in the covered body group.

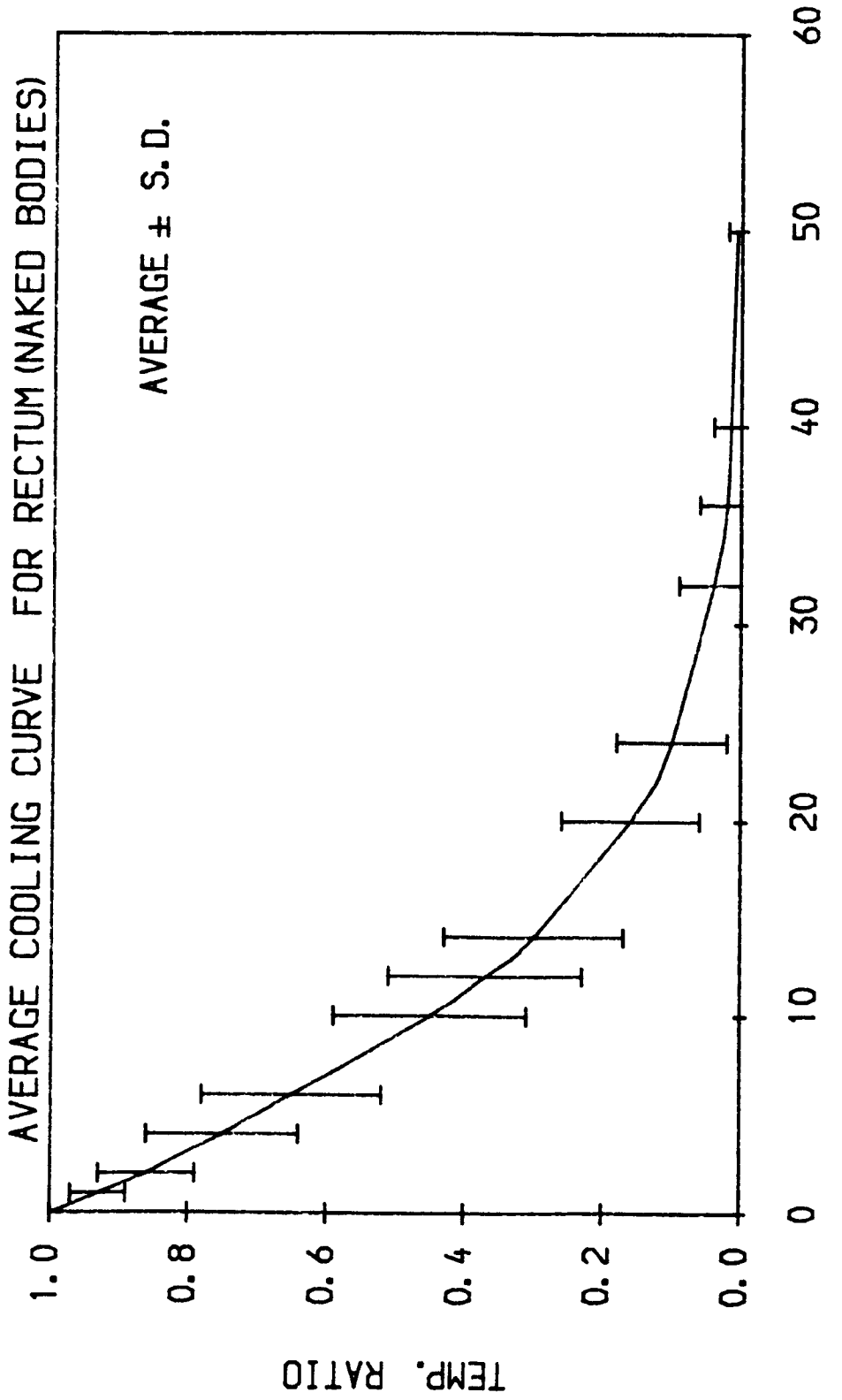


Figure 3.41: Plot of average temperature difference ratio ±1 standard deviation versus post-mortem interval based on temperature measurements by rectal thermocouple in the naked body group.

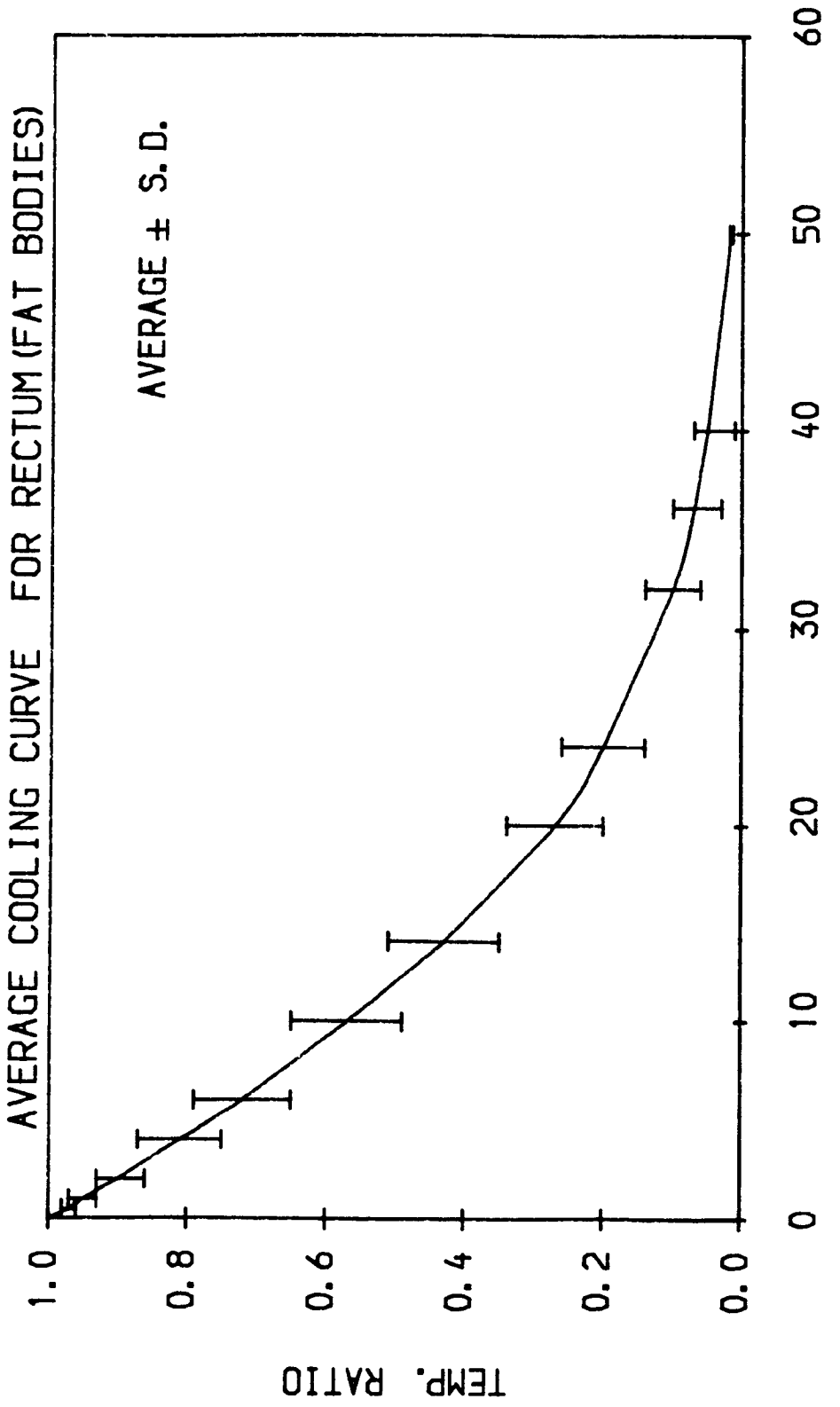


Figure 3.42: Plot of average temperature difference ratio ±1 standard deviation versus post-mortem interval based on temperature measurements by rectal thermocouple in the fat body category.

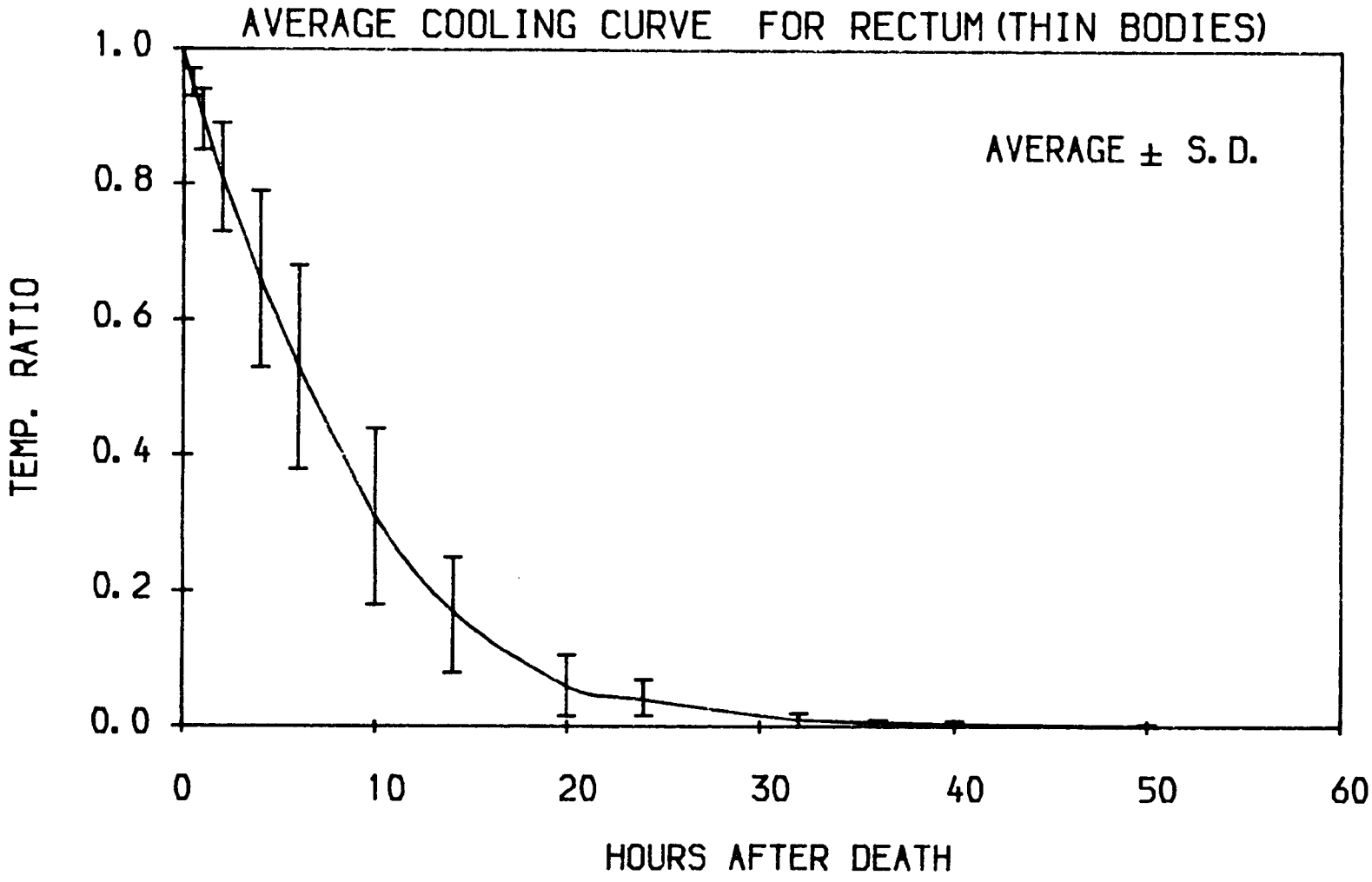


Figure 3.43: Plot of average temperature difference ratio ±1 standard deviation versus post-mortem interval based on temperature measurements by rectal thermocouple in the thin body category.

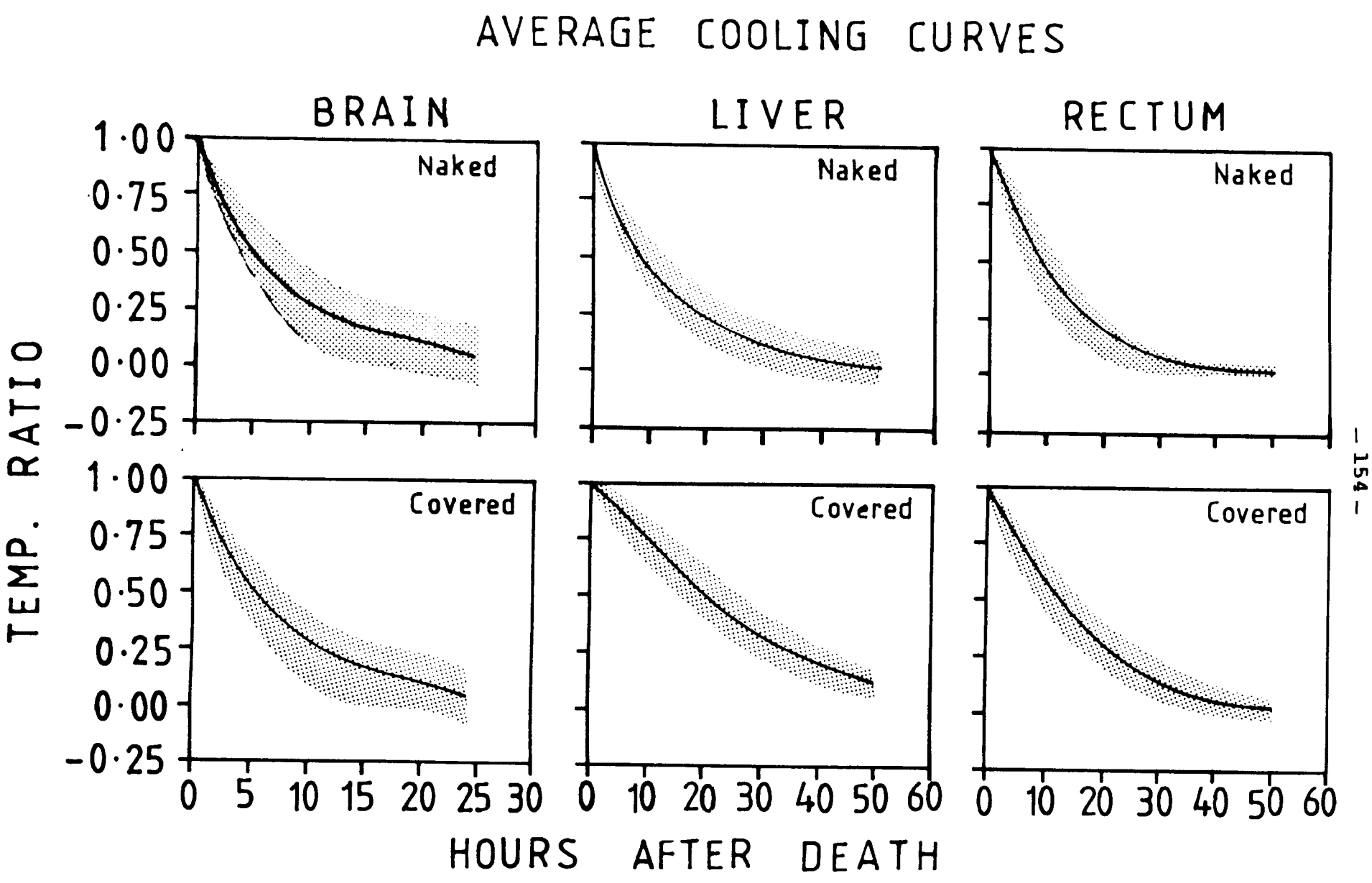


Figure 3.44: Average cooling curves for three sites in naked and covered bodies. The shaded areas indicate the range of error corresponding to ±1 standard deviation.

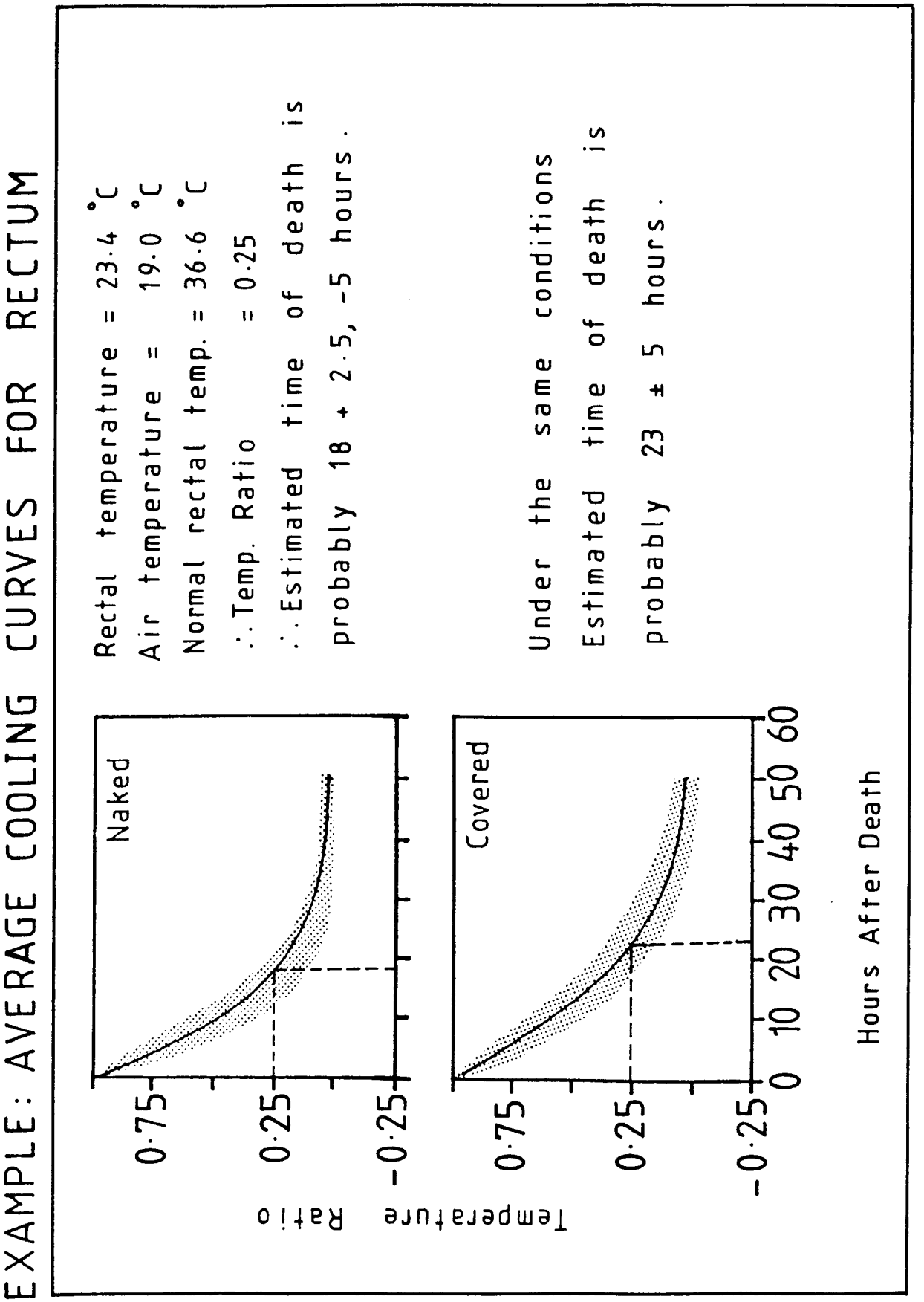


Figure 3.45: Example of a practical application of rectal curves given in Figure 3.44.

equal to the horizontal line enclosed by the shaded area at the hypothetical value of R.

3.5.5 A Programme For Practical Use

The triple-exponential equation used in this study can easily be solved by a microcomputer or even by a small pocket computer. Accordingly the estimation of the post-mortem interval can be greatly simplified by the use of a computer. Therefore the average formulae (in fact those assessed in Paragraph 3.5.1) were implemented in a small computer programme, suitable for use with a BBC microcomputer. A listing of this programme is given in Table 3.25. To enable this programme to calculate the probable errors in the time estimates, the following procedure was performed:

Data of Tables 3.19 and 3.20 were used. At each post-mortem interval shown in these tables and for each body site, the value of the standard deviation was added to and subtracted from the value of the mean temperature difference ratio R. Values resulting from the addition and subtraction were denoted as (U) and (L) respectively (U for upper limit and L for lower limit). U and L values were then fitted to triple-exponential equations using the mainframe programme BMDP as explained earlier (Paragraph 3.3.4). The formulae thus obtained were also implemented in the programme.

Thus 3 formulae were obtained for each site and each group of related cases. The first was the average

Table 3.25: Listing of the programme developed in this study for estimating the post-mortem interval.

```
   10 REM PROGRAMME CALLED "PMTIME"
   20 REM IT CALCULATES THE POST-MORTEM INTERVAL IN HOURS
   30 REM F IS THE TEMPERATUREDIFFERENCE RATIO, B IS THE TEMPERATURE OF THE BODY SITE AND T0
IS THE TEMPERATURE AT THE MOMENT OF DEATH
   40 REM E IS THE ENVIRONMENTAL TEMPERATURE, P1-P6 ARE PARAMETERS OF AVERAGE EQUATIONS, L1-L
6 ARE PARAMETERS OF LOWER LIMIT EQUATIONS AND U1-U6 ARE PARAMETE RS OF UPPER LIMIT EQUATIONS

   50 O%=&2020A
   60 INPUT"ENTER NAME OF DECEASED IS "NAME$
   70 REPEAT INPUT"WHICH TEMPERATURE WILL YOU ENTER? PRESS: R IF RECTAL, L IF LIVER, B IF BRA
IN "R$
   80 RECTAL=LEFT$(R$,1)="R":BRAIN=LEFT$(R$,1)="B":LIVER=LEFT$(R$,1)="L"
   90 S$=" "
  100 IF RECTAL OR LIVER THEN INPUT"IS THE BODY NAKED OR CLOTHED? PRESS: N IF NAKED OR C IF C
LOTHED "S$
  110 NAKED=LEFT$(S$,1)="N":CLOTHED=LEFT$(S$,1)="C"
  120 PROCCOEFFS
  130 INPUT"TEMPERATURE OF SELECTED SITE IN DEGREE CENTIGRADE =" B
  140 INPUT"TEMPERATURE OF ENVIRONMENT IN DEGREE CENTIGRADE =" E
  150 F=(B-E)/(T0-E)
  160 IF F>1 THEN  250
  170 ESTTIME=FNSOL(F)
  180 LOWTIME=FNLSOL(F)
  190 UPPERTIME=FNUSOL(F)
  200 MODE7
  210 CLS
  220 VDU2
  230 PROCPRINT
  240 VDU3
  250 IF F>1 PRINT"ESTIMATION OF POST-MORTEM INTERVAL IS NOT POSSIBLE BECAUSE ENTERED VALUE
F BODY TEMPERATURE EXEEDS ITS VALUE AT THE MOMENT OF DEATH, BUT THE POST-MORTEM ENTERVAL IS P
ROBABLY VERY SHORT"
```

```
  260 END
  270 DEF PROCCOEFFS
  280 IF RECTAL AND NAKED THEN P1=-6.0:P2=-0.18765:P3=5.1:P4=-0.145848:P5=1.9:P6=-0.227934:T0
=36.62:L1=-13.52:L2=-0.273394:L3=9.2919:L4=-0.222092:L5=5.2282:L6=-0.338187:U1=-5.4:U2=-0.13
512:U3=6.1:U4=-0.120523:U5=0.29958:U6=-0.075756
  290 IF BRAIN THEN P1=-5.9:P2=-0.10134:P3=4.5:P4=-0.119334:P5=2.4:P6=-0.083088:T0=26.64:L1=-
17.6341:L2=-0.493416:L3=11.7687:L4=-0.408574:L5=6.865382:L6=-0.599813:U1=-5.904:U2=-0.036402
U3=4.5048:U4=-0.056176:U5=2.3992:U6=-0.0197
  300 IF LIVER AND NAKED THEN P1=-6.0:P2=-0.103896:P3=5.1:P4=-0.089526:P5=1.9:P6=-0.133404:T0
=27.52:L1=-11.7:L2=-0.19874:L3=7.9:L4=-0.164954:L5=4.8:L6=-0.241688:U1=-6:U2=-0.03044:U3=5:U 4
=-.03925:U5=2:U6=-.020437
  310 IF RECTAL AND CLOTHED THEN P1=-5.99:P2=-.19152:P3=5.1:P4=-.206418:P5=1.89:P6=-.084774: T
0=32.2:L1=-13.48:L2=-.18611:L3=5.41:L4=-.235115:L5=9.07:L6=-.148488:U1=-6:U2=-.161948:U3=5.4
U4=-.165597:U5=1.6:U6=-.06362
  320 IF LIVER AND CLOTHED THEN P1=-5.86:P2=-.142368:P3=5.1:P4=-.149946:P5=1.76:P6=-.053628: T
0=32.7:L1=-5.98:L2=-.262824:L3=5.53:L4=-.279404:L5=1.45:L6=-.065623:U1=-5.9:U2=-.16698:U3=5. 3
:U4=-.173556:U5=1.6:U6=-.041133
  330 ENDPROC
  340 DEF FNPRED(T)=P1*EXP(P2*T)+P3*EXP(P4*T)+P5*EXP(P6*T)
  350 DEF FNSLOP(T)=P1*P2*EXP(P2*T)+P3*P4*EXP(P4*T)+P5*P6*EXP(P6*T)
  360 DEF FNSOL(F):LOCAL S:S=10
  370 REPEAT DS=(F-FNPRED(S))/FNSLOP(S):S=S+DS:UNTIL ABS(DS)<.001
  380=S
  390 DEF FNLPRED(T)=L1*EXP(L2*T)+L3*EXP(L4*T)+L5*EXP(L6*T)
  400 DEF FNLSLOP(T)=L1*L2*EXP(L2*T)+L3*L4*EXP(L4*T)+L5*L6*EXP(L6*T)
  410 DEF FNLSOL(F):LOCAL S:S=10
  420 REPEAT DS=(F-FNLPRED(S))/FNLSLOP(S):S=S+DS:UNTIL ABS(DS)<.001
  430=S
  440 DEF FNUPRED(T)=U1*EXP(U2*T)+U3*EXP(U4*T)+U5*EXP(U6*T)
  450 DEF FNUSLOP(T)=U1*U2*EXP(U2*T)+U3*U4*EXP(U4*T)+U5*U6*EXP(U6*T)
  460 DEF FNUSOL(F):LOCAL S:S=10
  470 REPEAT DS=(F-FNUPRED(S))/FNUSLOP(S):S=S+DS:UNTIL ABS(DS)<.001
```

```
  480=S
  490 DEF PROCPRINT
  500 PRINT"REPORT OF POST-MORTEM INTERVAL":PRINT"Name of the deceased is: "NAME$:PRINT"Enter
ed site temperature was: ";B:PRINT"Environmental temperature was: ";E
  510 PRINT"Calculated temperature difference ratio is: ";F
  520 PRINT"THE PROBABLE TIME OF DEATH, IN HOURS, RANGES FROM ";LOWTIME;" TO ";UPPERTIME:PRIN
T"THE ESTIMATED TIME OF DEATH IS MOST PROBABLY ";ESTTIME
  530 PRINT"Notes: The probability of this range is 68%":PRINT"       The range could be wide
r but this is less probable"
  540 PRINT"       The estimated times are prior to  temperature measurements"
  550 ENDPROC
```

cooling formula, the second was the upper limit formula and the third was the lower limit formula. The average post-mortem interval was calculated by the average formula and the range of the probable error in time estimates was calculated by the upper and lower limit formulae.

Also implemented in the programme were Equation 2.2 and temperatures of body sites at the moment of death as estimated earlier (Table 3.13). This enabled the programme to calculate the temperature difference ratio R. Therefore the user should measure and input temperatures of the body site and the environment as encountered in the field.

According to this programme, the user could input temperatures of more than one body site. These should be measured at the same time so that the time after death would be estimated according to different average formulae. If the errors in time estimates resulting from these formulae were averaged the precision and reliability of time prediction was found to be improved. This is shown in Table 3.26.

3.6 Shape of Cooling Curve

3.6.1 Slope And Slope Ratio of the Curve

The manner in which the body cools after death was assessed by the following method:

1. The rate of cooling, as determined by the slope of the curve, was studied in each body site for each case at several successive intervals.

Table 3.26: Deviation of estimated time from actual time using the average formulae separately and together in covered and naked bodies at selected intervals after death.

PM*	Covered Bodies								Naked Bodies							
	Brain		Liver		Rectum		All Sites		Brain		Liver		Rectum		All Sites	
TIME	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
1	0.3	0.4	0.1	0.9	0.5	0.7	0.3	0.7	0.3	0.4	0.1	0.6	0.1	0.4	0.2	0.5
2	0.2	0.9	-0.4	2	0.5	1.6	0.1	1.5	0.2	0.9	0.2	1	0.4	1.4	0.3	1.1
4	-0.1	1.5	0.1	2.7	0.3	2.6	0.1	2.3	-0.1	1.5	-0.2	1.4	0.4	1.2	0.03	1.4
8	0.0	2.7	-0.1	3.5	0.1	3.7	0.0	3.3	0.0	2.7	-0.4	2.6	0.3	2.0	0.03	2.4
12	0.6	4.2	-0.4	4.1	0.4	4.2	0.2	4.2	0.6	4.2	-0.5	3.7	0.5	2.1	0.2	3.3
18	4.0	4.7	-0.1	4.6	1.4	3.2	1.8	4.2	4.0	4.7	-0.2	5.4	0.4	3.3	1.4	4.5
24	6	5.3	0.5	5.4	1	6.2	2.5	5.6	6.0	5.3	3.7	6.4	-0.3	5.5	3.1	5.7

* time after death (hours)

2. The slope contributions made by the three terms of the triple-exponential equation used in the curve-fitting were also evaluated throughout the whole time course of the monitoring period. This was intended to study the effect of each exponential term on the cooling curve.

The slope of the curve was computed according to the following equation, corresponding to the first derivative of Equation 3.1:

$$\frac{dR}{dt} = S = P_1.P_2.e^{P_2t} + P_3.P_4.e^{P_4t} + P_5.P_6.e^{P_6t}$$

.....Equation 3.3

where S was the slope, with respect to time, or rate of cooling,

P_1-P_6 were parameters (constants), and,

t was the time after death in hours.

The slope for each term was given by a similar equation: for example the slope of the first term S_1 is given by:

$$S_1 = P_1.P_2.e^{P_2t}$$

.....Equation 3.4

Similarly S_2 and S_3 are the slopes of second and third terms.

In addition the expression $S_1/(S_2 + S_3)$ was considered and named the "slope ratio" (SR). Therefore, the slope ratio is given in this equation:-

$$SR = (P_1.P_2.e^{P_2t})/(P_3.P_4.e^{P_4t} + P_5.P_6.e^{P_6t})$$

....Equation 3.5

The slope ratio was studied to understand the

proportional effect of each term of the triple-exponential formula on the cooling process. It is necessary at this stage to recall that the first term was found to have a negative value while the second and third terms were positive, as explained in paragraph 3.4.1. This meant that the first term represented factors which modified cooling and the other two terms actually indicated the cooling processes. Therefore, studying the slope ratio can improve our knowledge concerning the formation of the temperature plateau. The greater the value of the slope ratio, the slower will be the rate of cooling: if the ratio = 1 then there is no cooling; if the ratio is greater than 1 then the temperature of the body will increase.

Average values and standard deviations of the slope and the slope ratios of the cooling curves for the brain, liver and rectum in covered, naked, fat and thin body groups were calculated for several successive post-mortem intervals. These are shown in Tables 3.27-32. Also, the slope data thus obtained were plotted versus time after death and these graphs are shown in Figures 3.46-3.53. Similarly, the slope ratio data were plotted versus time and are given in Figures 3.54-3.61. The results inferred from these studies are as follows:-

1. All cooling curves were found to be of compound form. In other words, the rate of cooling was not uniform throughout the whole time course of the

Table 3.27: Slopes of the cooling curves for rectum, liver and brain in covered bodies at different post-mortem intervals.
(Number of Cases = N)

Post-Mortem Interval (hours)	Slope of Cooling Curves*					
	Rectum (N = 21)		Liver (N = 30)		Brain (N = 28)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	- 0.0011	0.00071	- 0.0004	0.00041	- 0.0022	0.0011
1	- 0.00092	0.00046	- 0.00039	0.00033	- 0.0019	0.00069
2	- 0.0008	0.00032	- 0.00039	0.00027	- 0.0016	0.00051
4	- 0.00066	0.00018	- 0.00039	0.0002	- 0.0012	0.00034
6	- 0.00061	0.00012	- 0.00039	0.00016	- 0.00093	0.00023
12	- 0.00051	0.0001	- 0.00041	0.0001	- 0.00038	0.000091
18	- 0.00039	0.00008	- 0.0006	0.00088	- 0.00029	0.000062
24	- 0.00027	0.00005	- 0.00078	0.0025	- 0.000078	0.000079
36	- 0.00012	0.000048	- 0.0036	0.0188	-	-
50	- 0.00005	0.00003	- 0.0256	0.14	-	-

* Slope as in Equation 3.3

Table 3.28: Slopes of cooling curves for rectum, liver and brain in naked bodies at different post-mortem intervals.
(Number of cases = N)

Post-Mortem Interval (hours)	Slope of the Cooling Curves*					
	Rectum (N = 58)		Liver (N = 66)		Brain (N = 62)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	- 0.00144	0.00073	-0.0015	0.00056	-0.0032	0.0018
1	- 0.00118	0.00048	-0.00013	0.00044	-0.0021	0.00058
2	-0.00103	0.00036	-0.0011	0.00034	-0.0188	0.00033
4	-0.00089	0.00025	-0.00091	0.0002	-0.0015	0.00037
6	-0.000814	0.0002	-0.00075	0.00012	-0.00112	0.00024
12	-0.00058	0.000028	-0.00045	0.000085	-0.00036	0.00011
18	-0.00035	0.00001	-0.00029	0.000087	-0.00011	0.00008
24	-0.0002	0.00001	-0.00019	0.000056	-0.00005	0.00005
36	-0.00007	0.000064	-0.00009	0.00008	-	-
50	-0.00003	0.000034	-0.000039	0.000028	-	-

* Slope as in Equation 3.3

Table 3.29: Slopes of the cooling curves for rectum. Thin and fat bodies in cases monitored naked. (Number of Cases = N)

POST-MORTEM INTERVAL (hours)	Slope of the Cooling Curve*			
	Thin Bodies (N = 19)		Fat Bodies (N = 20)	
	Mean	S.D.	Mean	S.D.
0	- 0.00195	0.00093	- 0.00092	0.00039
1	- 0.00152	0.00072	- 0.00084	0.0003
2	- 0.00128	0.00064	- 0.00077	0.00024
4	- 0.00107	0.00033	- 0.00071	0.00018
6	- 0.00096	0.00013	- 0.00067	0.00014
12	- 0.0006	0.00019	- 0.00056	0.000084
18	- 0.00028	0.00012	- 0.00042	0.000065
24	- 0.00012	0.00006	- 0.00028	0.000052
36	- 0.00002	0.000014	- 0.00011	0.000035
50	- 0.000003	0.000012	- 0.000051	0.0001

* Slope as in Equation 3.3

Table 3.30: Slope ratios for rectum, liver and brain in covered bodies.
(number of cases = N)

Post-Mortem Interval (hours)	Slope Ratio*					
	Rectum (N = 21)		Liver (N = 28)		Brain (N = 25)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	-0.917	0.034	-0.951	0.057	-0.81	0.097
1	-0.919	0.031	-0.947	0.053	-0.81	0.086
2	-0.919	0.029	-0.943	0.049	-0.82	0.077
4	-0.913	0.023	-0.935	0.043	-0.824	0.063
6	-0.90	0.017	-0.923	0.038	-0.824	0.071
12	-0.833	0.053	-0.878	0.031	-0.89	0.213
18	-0.749	0.12	-0.822	0.05	-0.82	0.23
24	-0.666	0.174	-0.763	0.083	-0.811	0.254
36	-0.537	0.24	-0.648	0.148	-	-
50	-0.428	0.266	-0.535	0.2	-	-

* Slope ratio as in Equation 3.5

Table 3.31: Slope ratios for rectum, liver and the brain in the naked bodies.
(Number of Cases = N)

Post-Mortem Interval (hours)	Slope Ratio*					
	Rectum (N = 55)		Liver (N = 42)		Brain (N = 42)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	-0.935	0.028	-0.86	0.099	-0.85	0.118
1	-0.937	0.026	-0.863	0.094	-0.859	0.106
2	-0.933	0.028	-0.864	0.091	-0.84	0.088
4	-0.911	0.036	-0.865	0.083	-0.756	0.16
6	-0.873	0.049	-0.863	0.075	-0.682	0.23
12	-0.71	0.118	-0.845	0.064	-0.575	0.36
18	-0.543	0.175	-0.81	0.085	-0.5	0.4
24	-0.392	0.196	-0.76	0.12	-0.506	0.43
36	-0.204	0.198	-0.662	0.2	-	-
50	-0.091	0.171	-0.54	0.26	-	-

* Slope ratio as in Equation 3.5

Table 3.32: Slope ratios for rectum; thin and fat bodies in cases monitored naked.
(Number of cases = N)

Post-Mortem Interval (hours)	Slope Ratio*			
	Thin Bodies (N = 19)		Fat Bodies (N = 20)	
	Mean	S.D.	Mean	S.D.
0	-0.929	0.039	-0.94	0.023
1	-0.929	0.034	-0.937	0.021
2	-0.921	0.037	-0.932	0.021
4	-0.889	0.035	-0.918	0.021
6	-0.834	0.062	-0.897	0.021
12	-0.626	0.128	-0.817	0.037
18	-0.444	0.176	-0.717	0.074
24	-0.315	0.194	-0.618	0.099
36	-0.17	0.186	-0.442	0.143
50	-0.092	0.153	-0.30	0.16

* Slope Ratio as in Equation 3.5

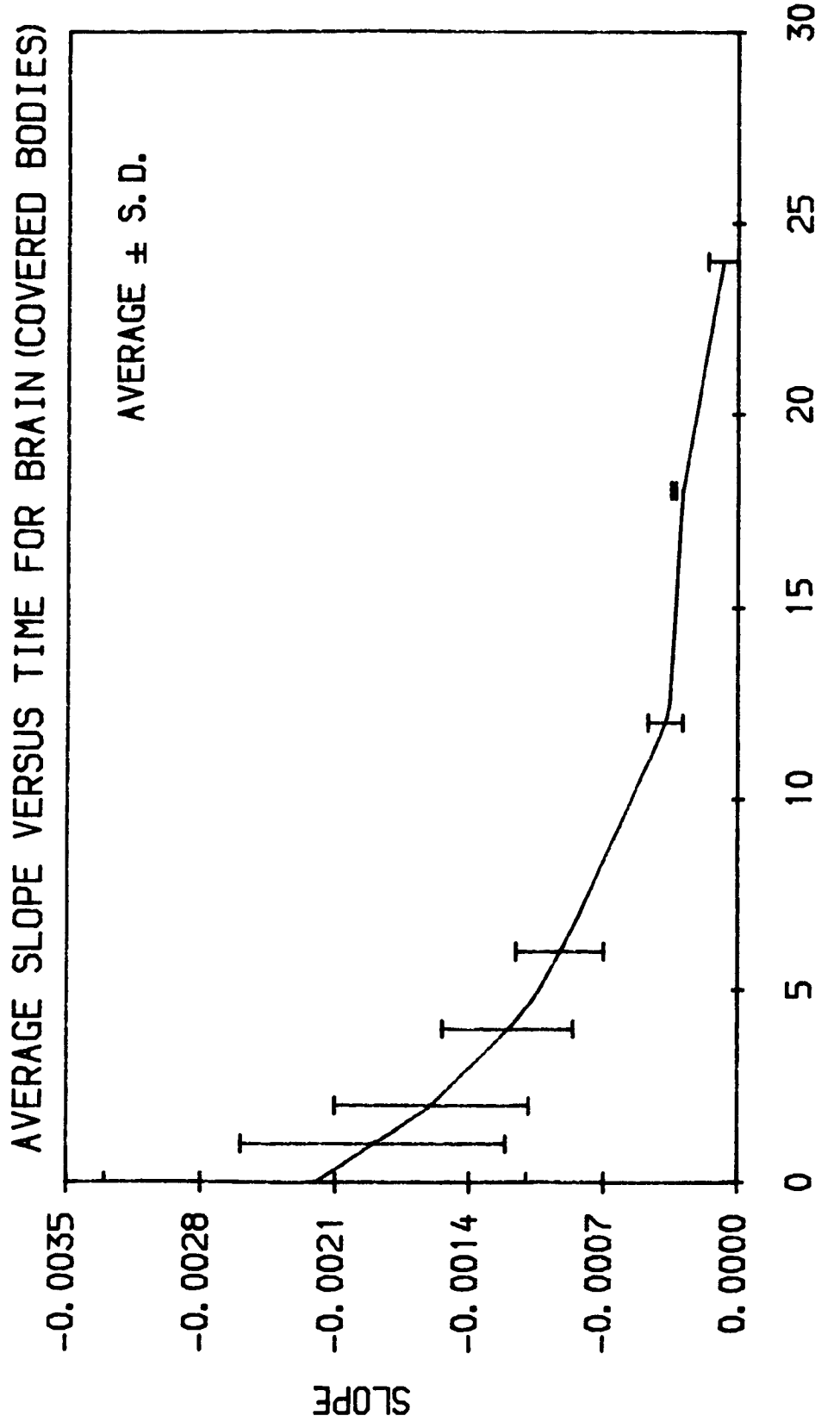


Figure 3.46: Plot versus time of the average slope found in cooling curves for the brain in covered bodies.

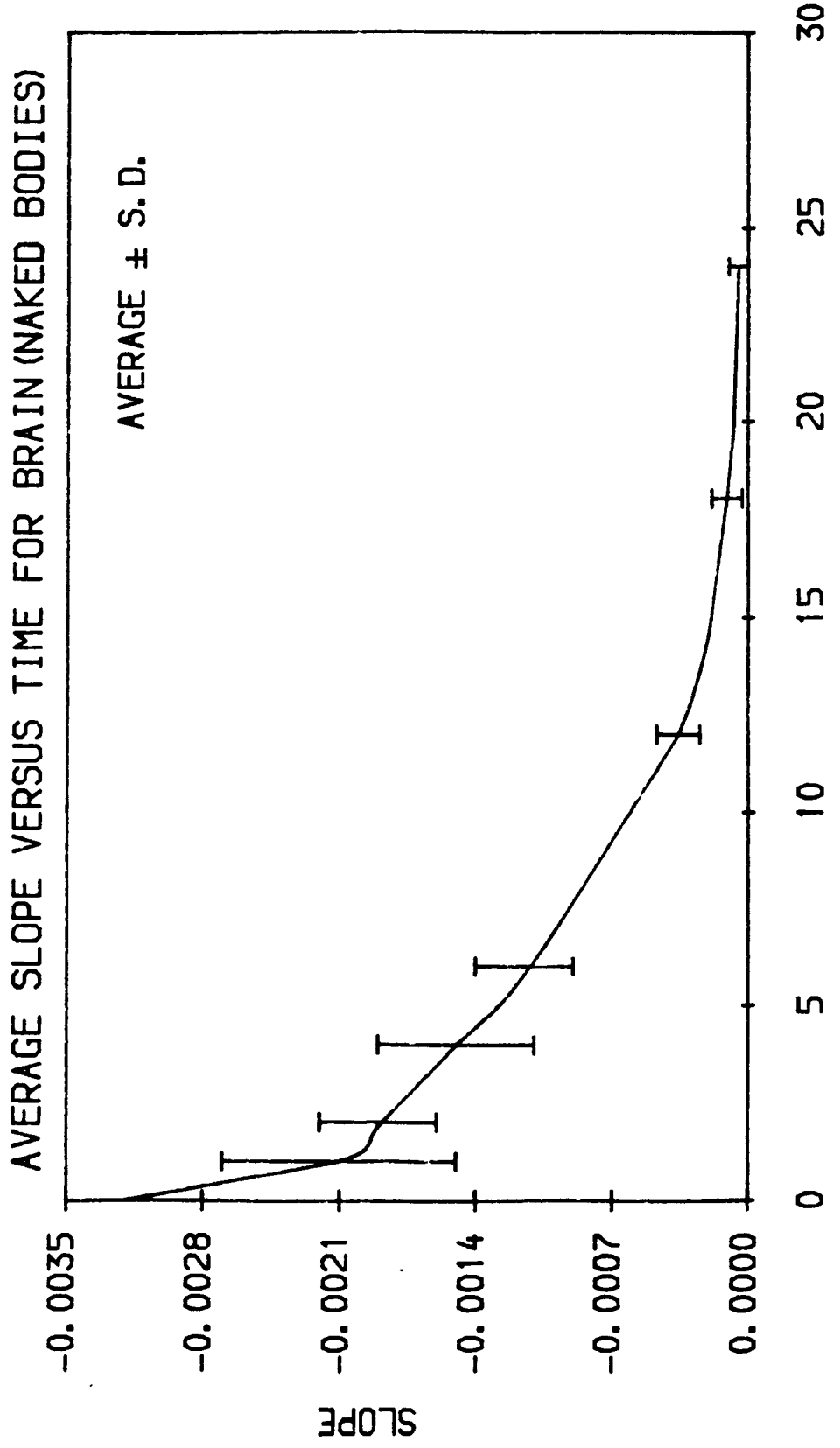


Figure 3.47: Plot versus time of the average slope found in cooling curves for the brain in naked bodies.

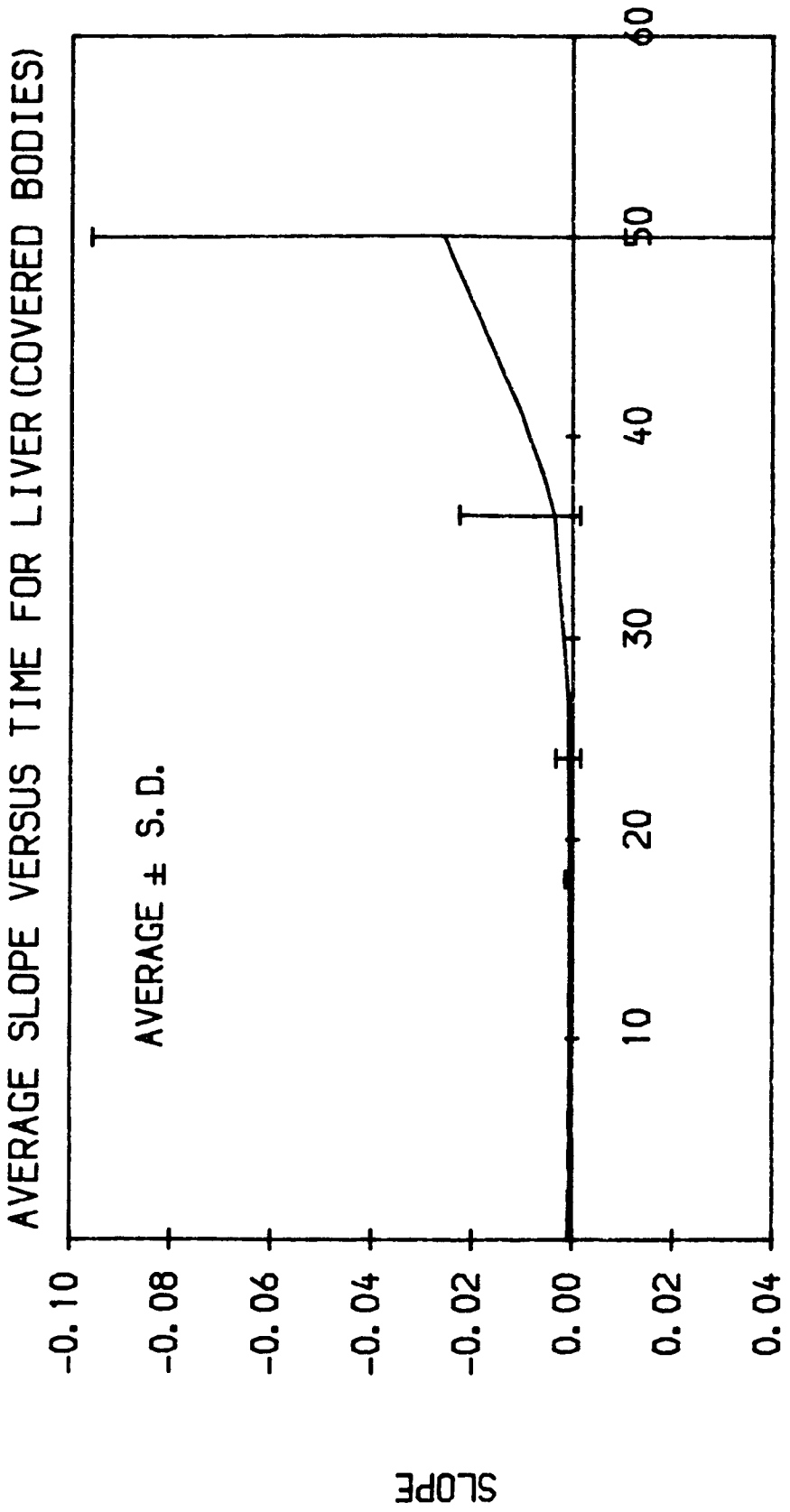


Figure 3.48: Plot versus time of the average slope found in cooling curves for the liver in covered bodies.

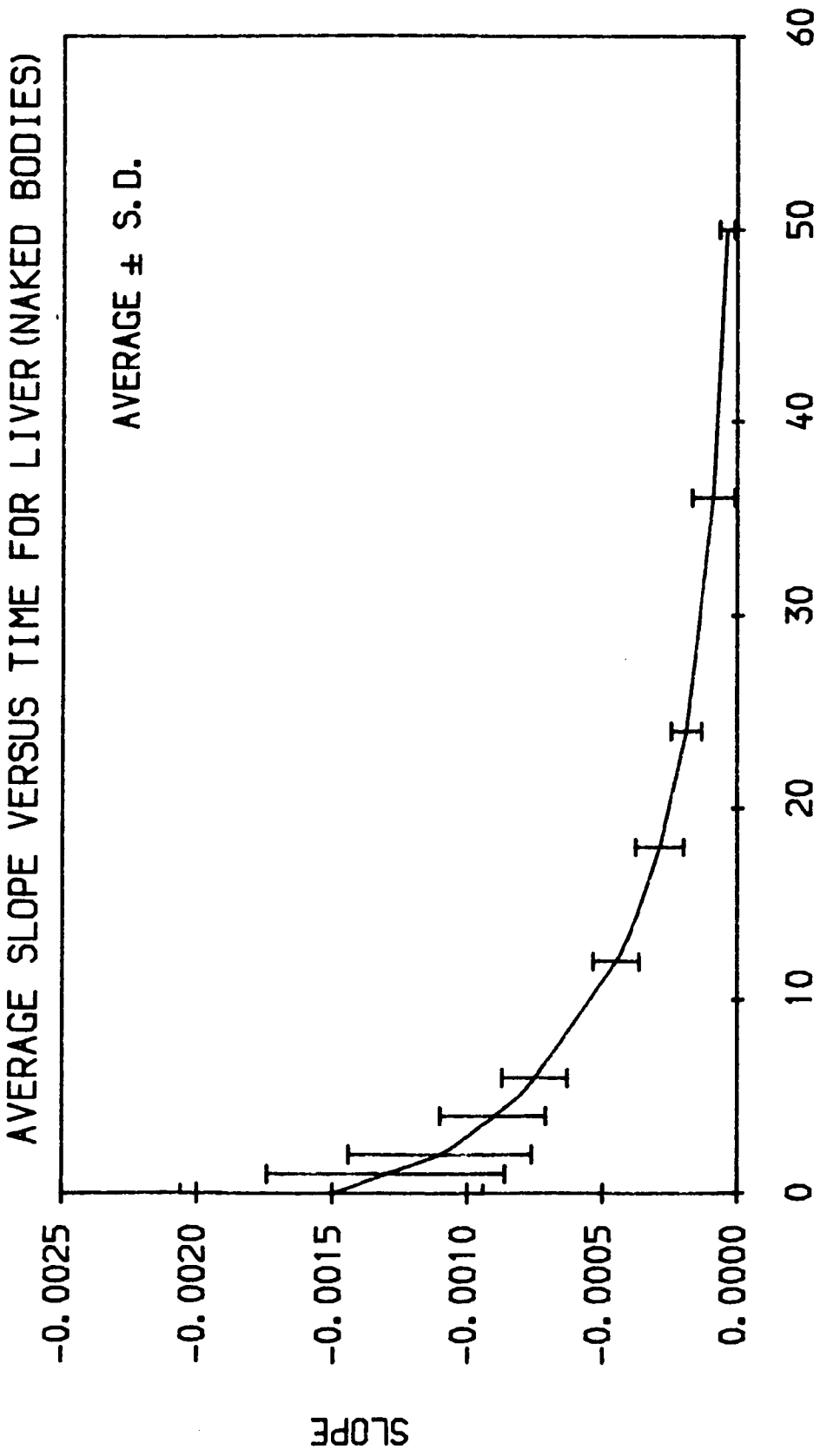


Figure 3.49: Plot versus time of the average slope found in cooling curves for the liver in naked bodies.

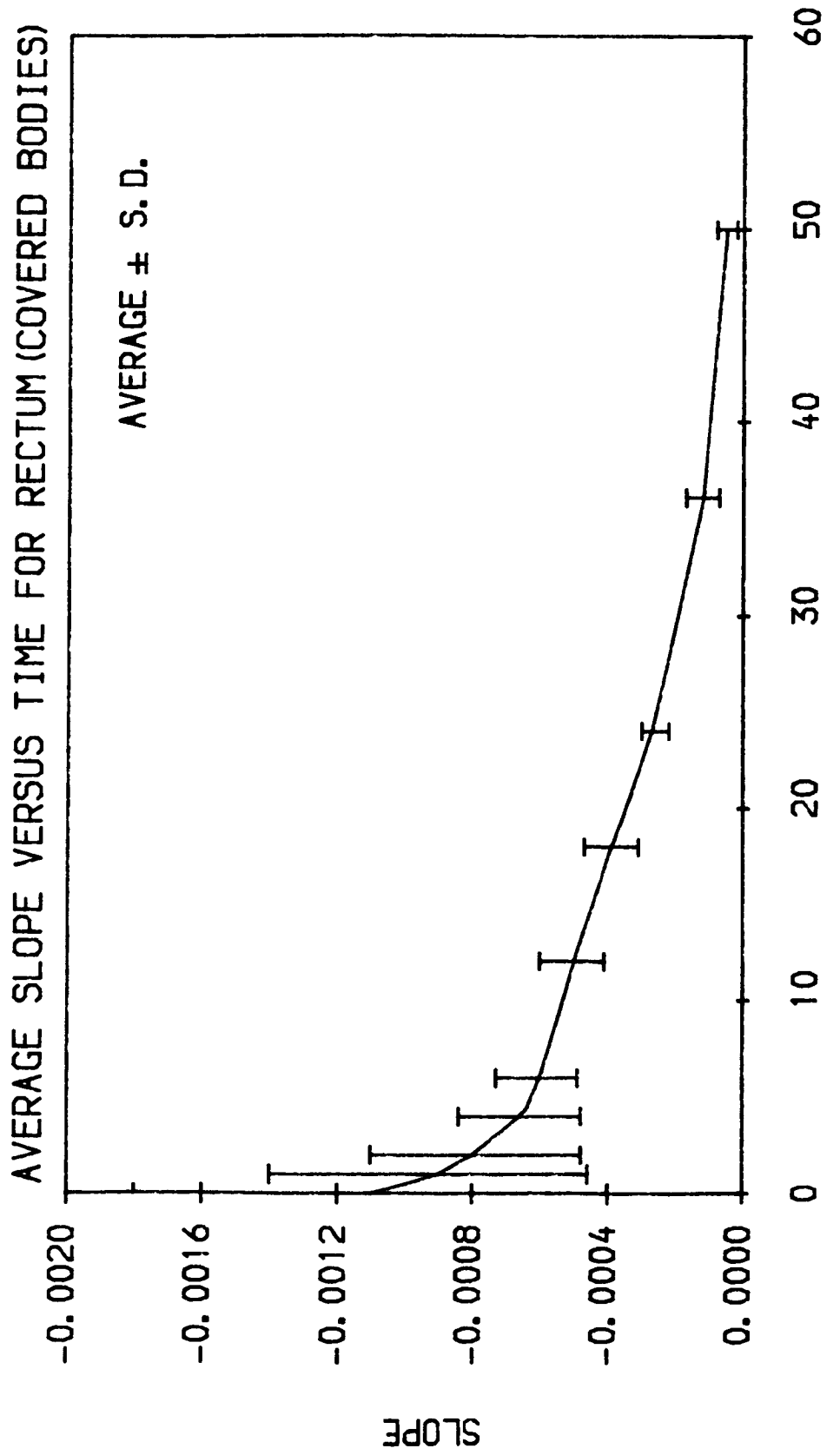


Figure 3.50: Plot versus time of the average slope found in cooling curves for the rectum in covered bodies.

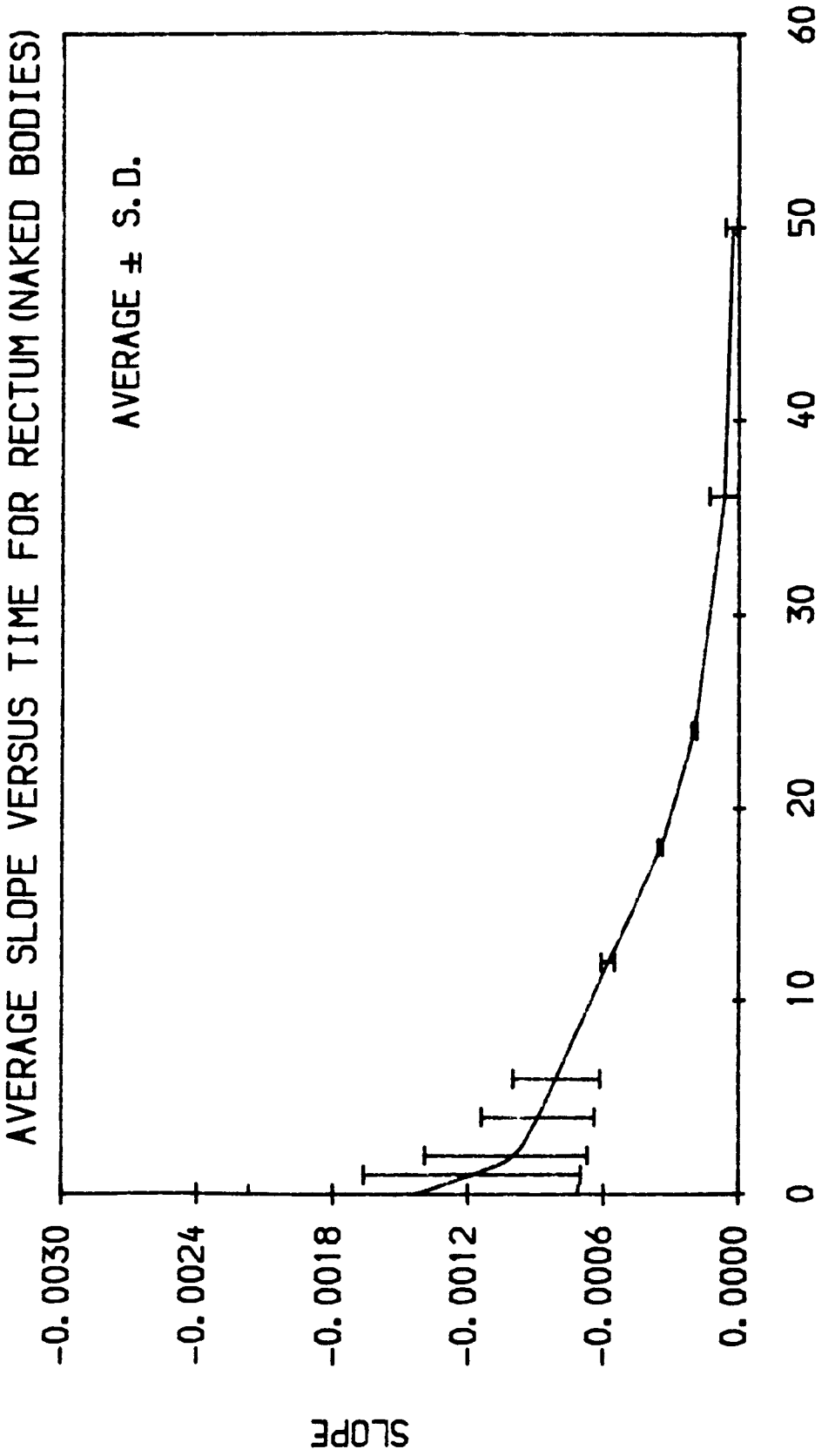


Figure 3.51: Plot versus time of the average slope found in cooling curves for the rectum in naked bodies.

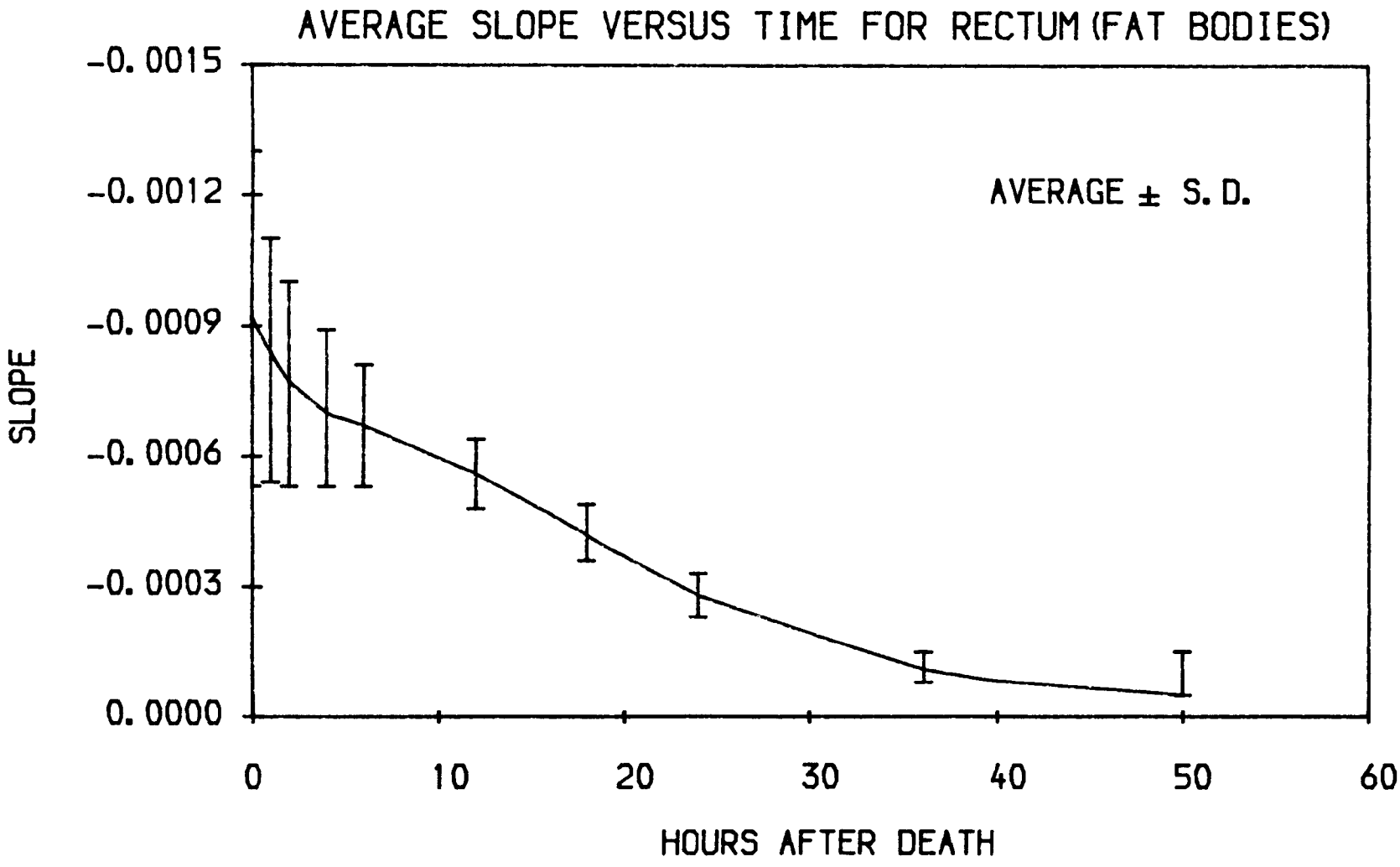


Figure 3.52: Plot versus time of the average slope found in cooling curves for the rectum in fat body category.

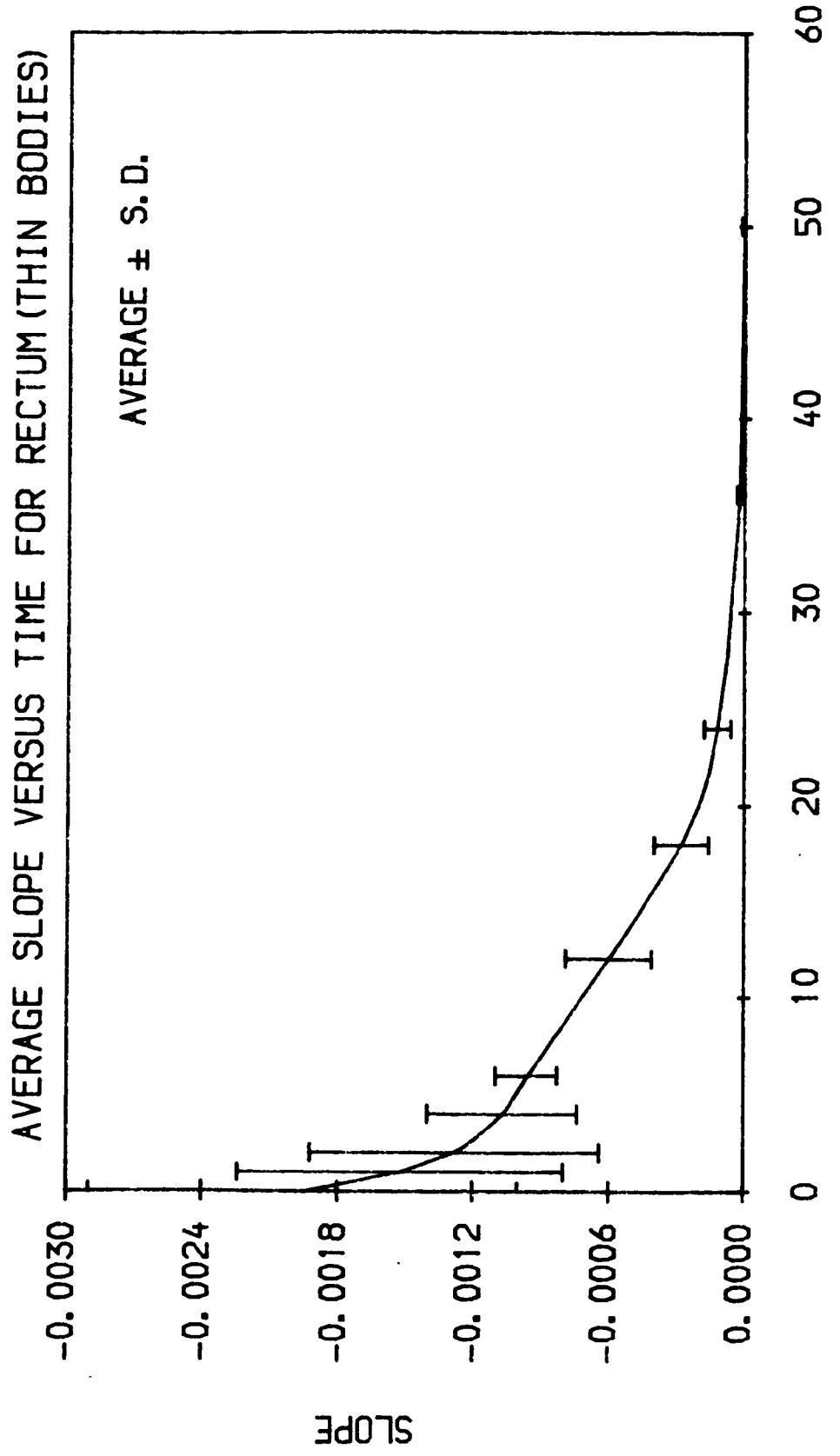


Figure 3.53: Plot versus time of the average slope found in cooling curves for the rectum in thin body category.

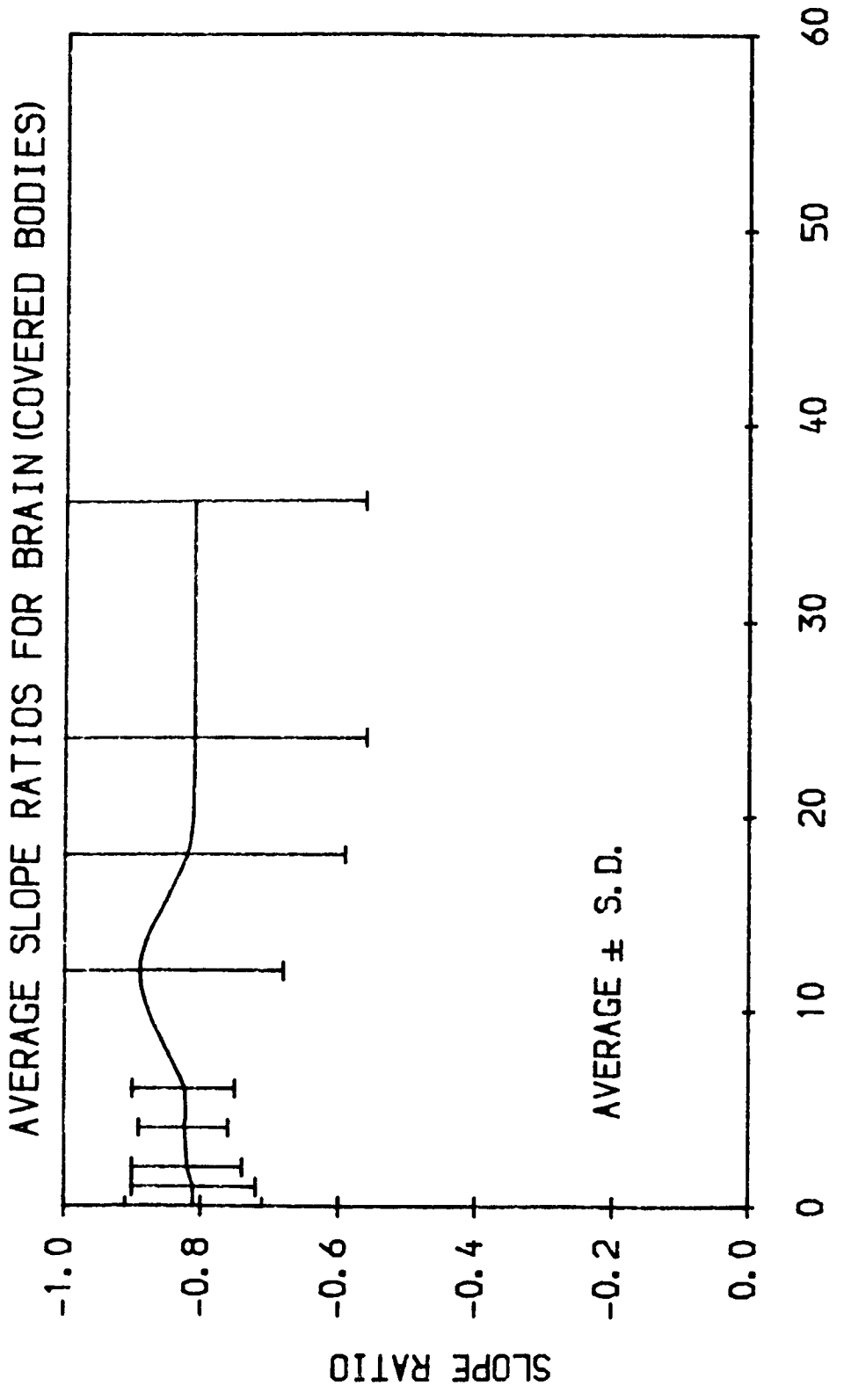


Figure 3.54: Plot versus time of the average slope ratios of terms in the cooling curves for the brain in covered bodies.

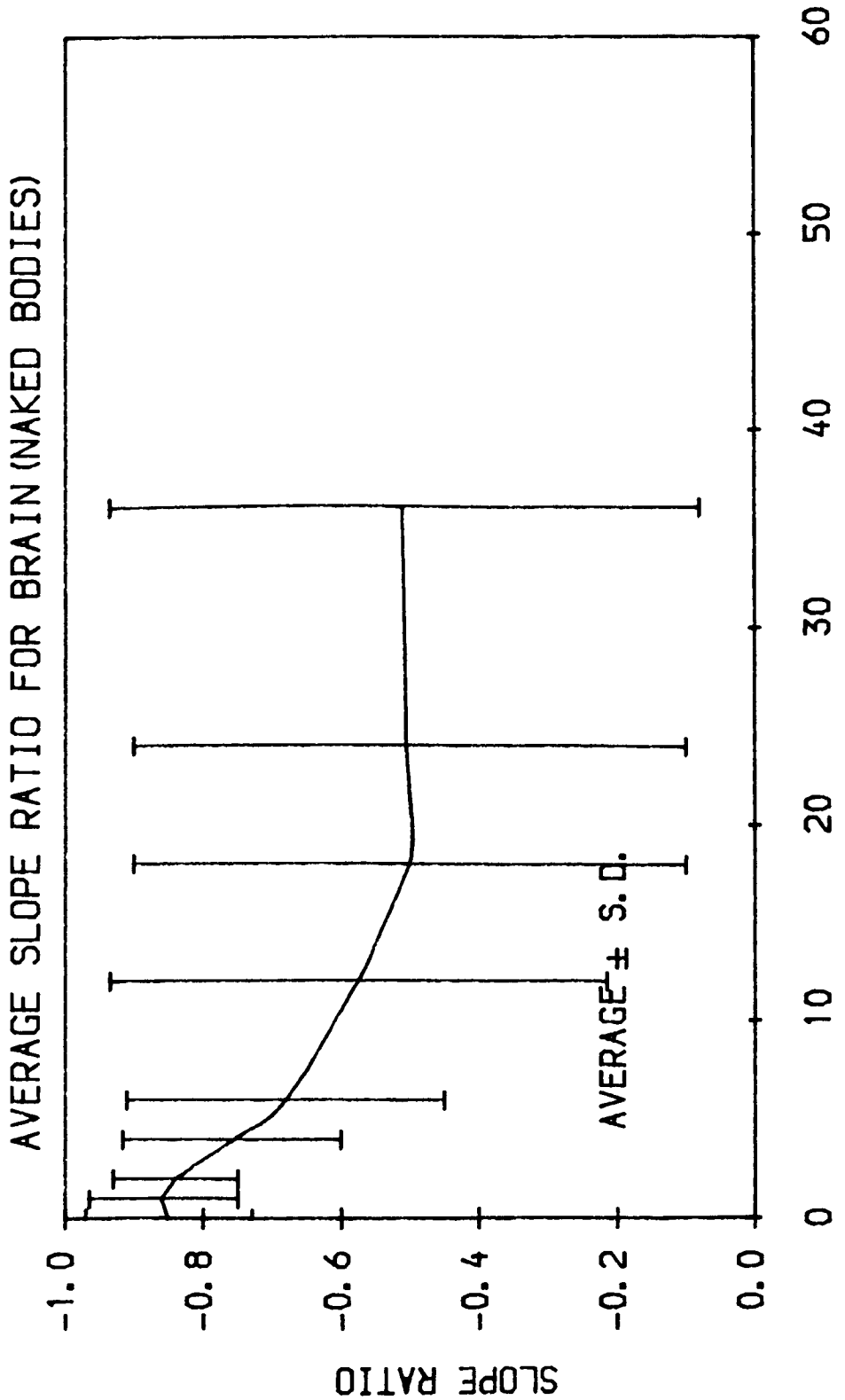


Figure 3.55: Plot versus time of the average slope ratios of terms in the cooling curves for the brain in naked bodies.

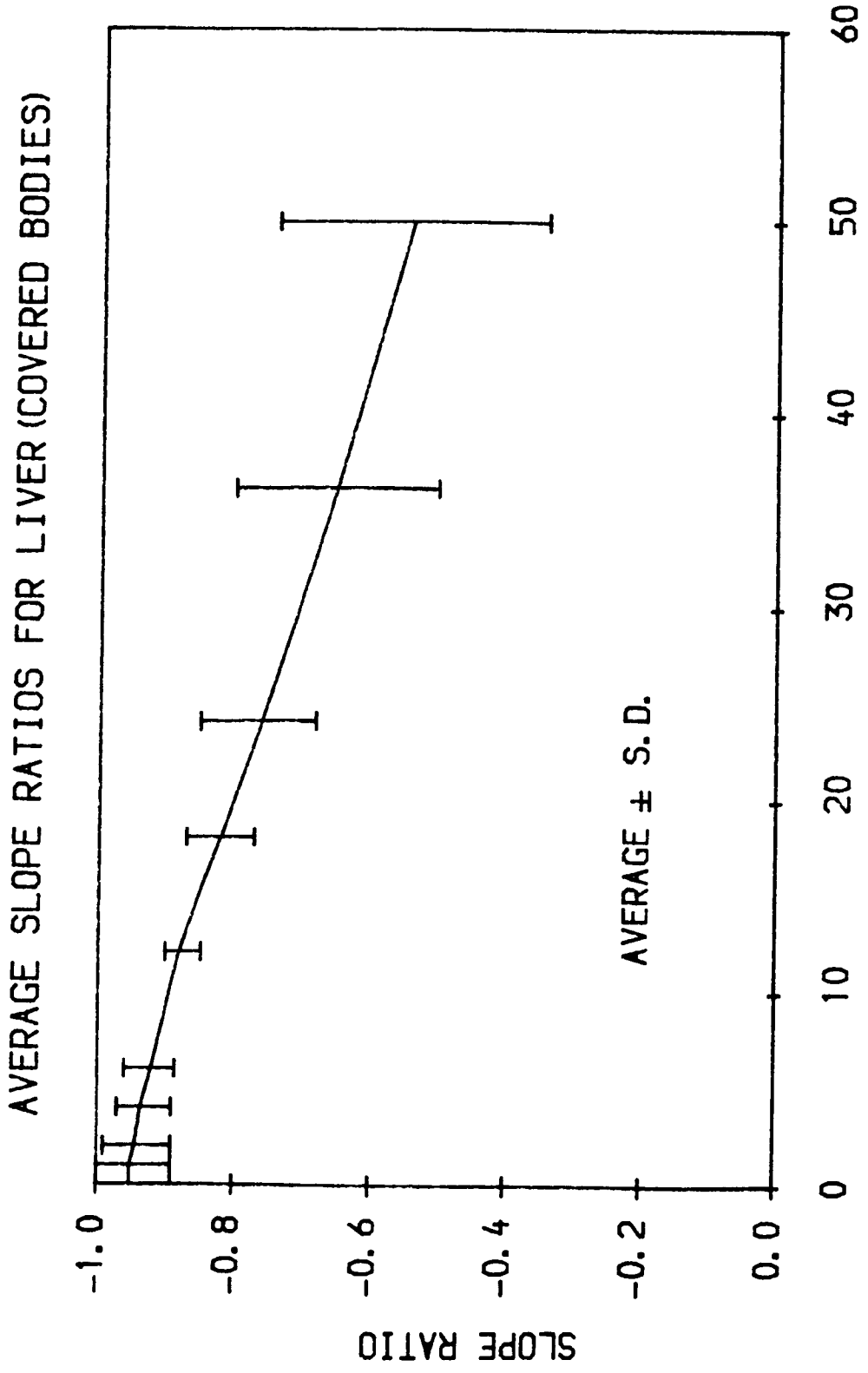


Figure 3.56: Plot versus time of the average slope ratios of terms in the cooling curves for the liver in covered bodies.

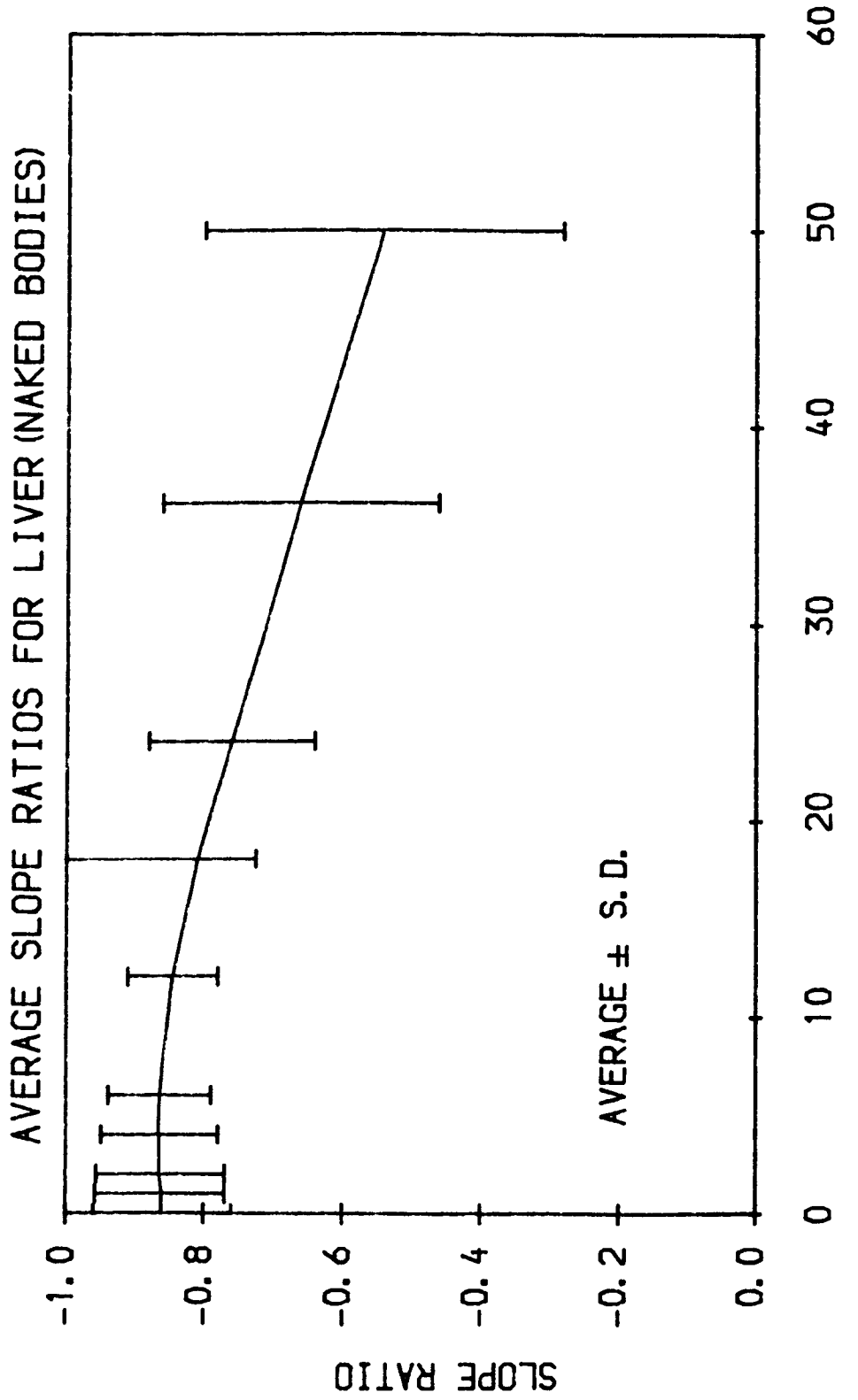


Figure 3.57: Plot versus time of the average slope ratios of terms in the cooling curves for the liver in naked bodies.

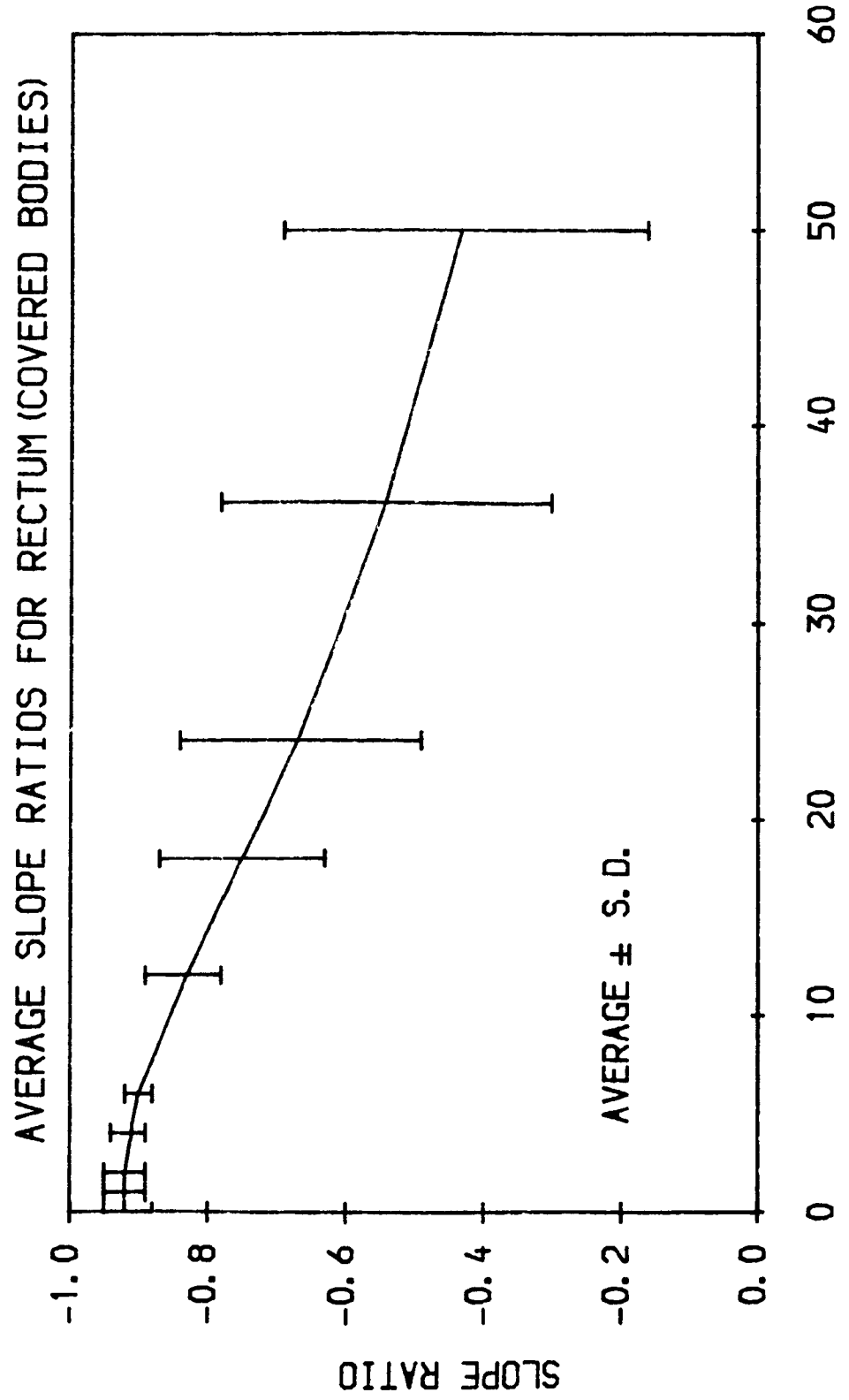


Figure 3.58: Plot versus time of the average slope ratios of terms in the cooling curves for the rectum in covered bodies.

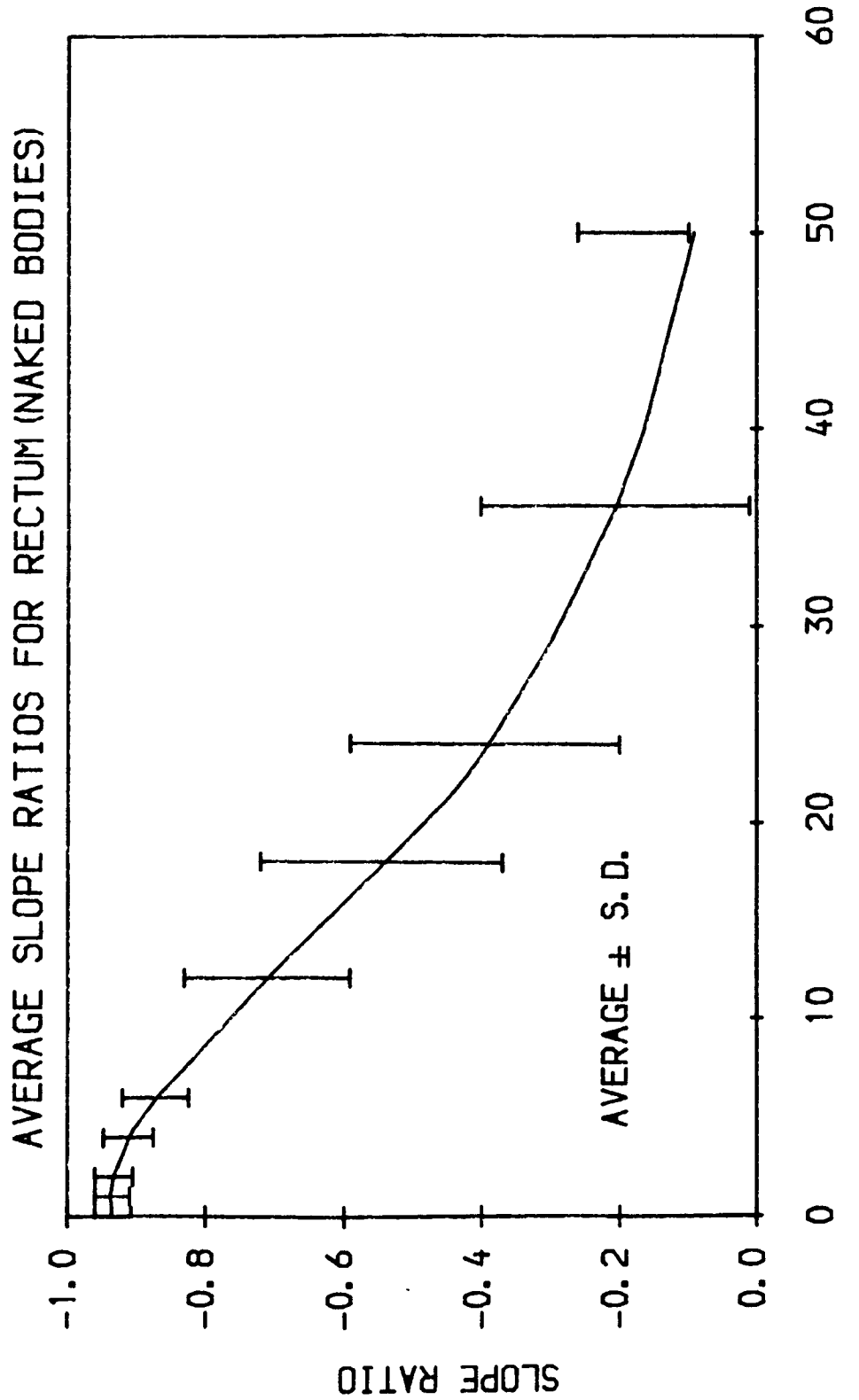


Figure 3.59: Plot versus time of the average slope ratios of terms in the cooling curves for the rectum in naked bodies.

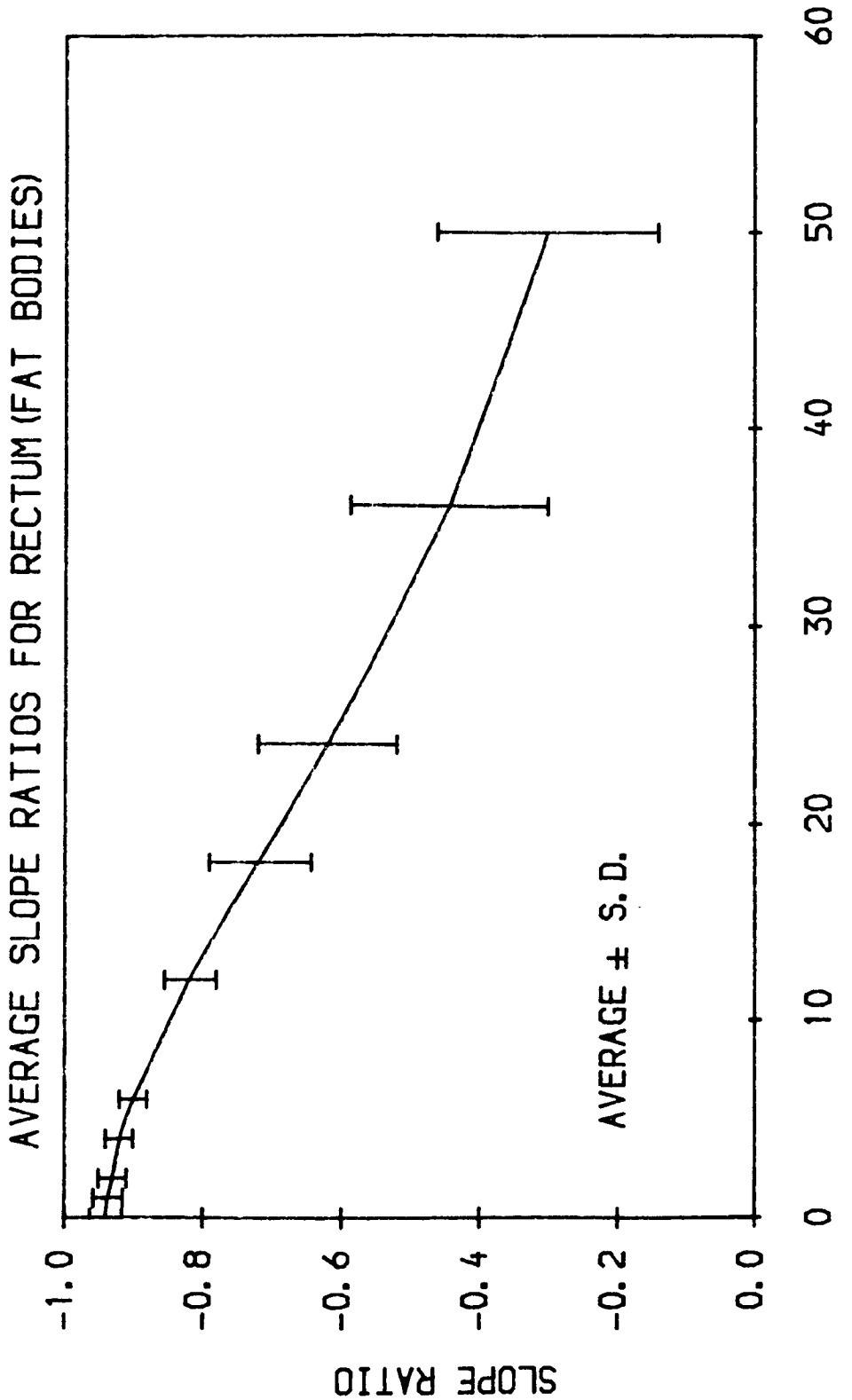


Figure 3.60: Plot versus time of the average slope ratios of terms in the cooling curves for the rectum in fat body category.

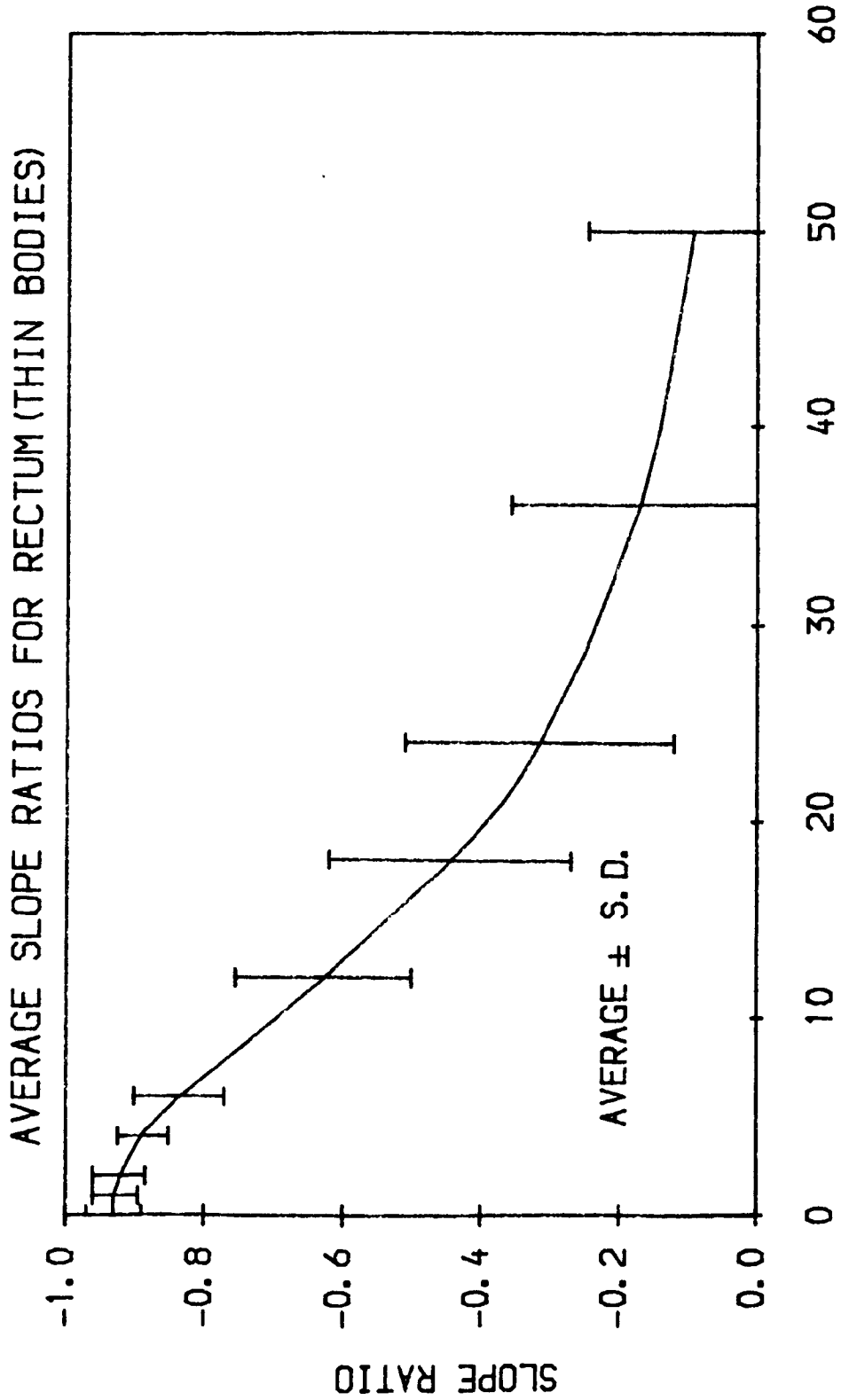


Figure 3.61: Plot versus time of the average slope ratios of terms in the cooling curves for the rectum in thin body category.

monitoring period. This meant that the drop in temperature was not of the linear type.

2. The rate of cooling for all average curves, except that for the liver in the covered body group, was greater in the early interval soon after death than in subsequent periods. In the average cooling curve of the liver in the covered body group the slope was flatter during the first few hours post-mortem than in the subsequent intervals, but in neither situation was the slope nil or positive except in one case. However, in all situations, the slope was different from that of a single-exponential equation. That is, it could not be exactly derived from Newtonian cooling.
3. Average slope values of the brain, liver, and rectal curves were compared (Table 3.27). It was found firstly that in the covered group during the first 6 hours after death, the lowest absolute value, i.e. the slowest cooling rate, was seen in the liver (This is seen in Figure 3.48 where the slope curve is relatively close to the zero line). The brain curve was found to have the greatest absolute slope value (Figure 3.46). At 12 hours post-mortem and thereafter the brain and the rectal curves were noted to have the lowest and the greatest absolute values respectively (Figure 3.46, 3.48 and 3.50). Note that the scale on the Y-axis in Figure 3.48 is higher than those in the other figures.

Secondly, in the naked group (Table 3.28) and also for most intervals up to 6 hours post-mortem, the lowest absolute slope value was seen in the rectal curve and the greatest was that of the brain curve, i.e. the brain had the steepest slope (Figure 3.47). This changed after about 12 hours post-mortem by which time the brain slope was found to be the shallowest and that of the rectal curve became the steepest. In fact the slopes of the rectal and the liver curves were similar, although that of the liver curve was slightly flatter (Figures 3.49 and 3.51).

Thirdly, the average slopes of the rectal curves, for fat and thin bodies were compared (Table 3.29 and Figures 3.52 and 3.53). It was found that, on average, the slope of curves of thin bodies was steeper than that of fat bodies. This was true for the whole monitoring period.

4. The effect of covering the torso on the cooling curve slope for each body site was studied (Compare each site in Table 3.27 with the corresponding entries in Table 3.28). It was clear that in all sites the slopes were steeper for the naked group than those for the covered one. However this difference was slight in the case of the brain but very marked in the liver. The slope of the rectal curve for the covered group was also reduced due to the covering of the torso but to a lesser extent than that of the liver.

5. Average values of the slope ratio for the brain, liver and the rectal curves were compared. It was noted that in the covered group during the first 12 hours, the liver had the greatest absolute value while the brain had the lowest value (Table 3.30 and Figures 3.54, 3.56 and 3.58). After 18 hours the average slope ratio for the brain was greater than those for both liver and rectum. The average slope ratio for the rectum was always less than that of the liver in this group (Figures 3.56 and 3.58).

   By contrast it was found that in the naked body group during the first 6 hours after death the rectal curve had, on average, a greater slope ratio than those of both the liver and the brain curves (Table 3.31 and Figures 3.55, 3.57 and 3.59). At 12 hours post-mortem and thereafter this changed so that the absolute value of the slope ratio for the rectum became less than that for the liver. The lowest absolute value of this ratio was noted in the brain curve throughout the whole monitoring period. Also it was found that the average absolute values of the slope ratio for fat and thin categories were similar. However the latter were slightly less than the former (Table 3.32 and Figures 3.60 and 3.61).

   It is worth noting at this stage that the average curves of the slope ratio in most sites were straight lines or convex in shape (Figures 3.54 - 3.61).

6. The average slope ratios of curves for each body site in naked and covered groups were compared (Tables 3.30 and 3.31). The slope ratio of the brain curve for the covered group, on average, was less than that for the naked group (Figure 3.54 and 3.55). In the case of the liver the slope ratio was, in general, greater for the covered than for naked bodies, but only up to 18 hours after death, after which the reverse was true (Figures 3.56 and 3.57). Also, for the rectum, the slope ratio was greater in the covered group than in the naked group but only for the first 2 hours post-mortem: for the remainder of the monitoring period the slope ratio of the rectal curve for the covered group was, on average, less than that for the naked group (Figures 3.58 and 3.59).

3.6.2 The Initial Temperature Plateau

A plateau in a curve is mathematically indicated by a nil or a small absolute value of the slope of that curve. Also, a rising curve is expressed by a positive value of the slope. In this study the occurrence of the initial temperature plateau was studied in relation to both the slope and the slope ratio of the cooling curve. Accordingly an initial temperature plateau was said to exist if the absolute value of the slope of the cooling curve, at the beginning of the monitoring period, was either nil or of a very small value or if the absolute value of the slope ratio was equal to

unity or very slightly less than that. This should continue for some time to give the shape of the plateau. If the absolute value of the slope was positive or that of the slope ratio was more than unity then the temperature increased after death.

In this way the plateau was studied for the brain, liver and rectum for the covered and naked group and the fat and thin body categories. The incidence of the plateau in these sites and conditions is given in Table 3.33. The occurrence of the plateau in different body sites and conditions was statistically analysed. Results of this analysis are given in Table 3.34. Also relevant are Figures 3.46-3.53 and in particular the scatter shown in them. When only the slope of the curve was considered, the plateau was found to occur only in 21%, on average, of all cooling curves for the three body sites and all body groups. In other words the plateau was not a constant phenomenon that took place in every cooling curve after death.

The effect of the site of temperature measurements on the incidence of the plateau was also studied. This was carried out by comparing the incidence of the initial temperature plateau observed for the curves of the brain, liver and the rectum. It was found that there were significant differences between the three body sites with respect to the occurence of the plateau (P was less than 0.1%, see Table 3.34). In the cases related to the naked group

Table 3.33: Number and percent of the incidence of the initial temperature plateau observed in the slope of the rectal, brain and the liver cooling curves for naked and covered bodies and fat and thin body categories of cases. (N = number of cases)

I T E M	R e c t u m			B r a i n		L i v e r	
	Naked Group		Covered Group	Naked Group	Covered Group	Naked Group	Covered Group
	Thin N = 19	Fat N = 20	N = 21	N = 42	N = 28	N = 43	N = 30
Incidence of plateau	3	7	6	3	2	3	21
Percent	15	33	28	7	7	7	70

Table 3.34: Statistical analysis of the incidence of the temperature plateau using the Chi-squared test.

	C o m p a r i s o n s B e t w e e n –									
Body Sites	Covered and Naked Bodies in:-								Fat and Thin Bodies	
	Rectum		Liver		Brain		All Cases			
Test 1* P%	Test 2**	P%	Test 1*	P%	Test 3***	P%	Test 1*	P%	Test 2**	P%
14.68	<0.1	0.004	>10	29.03	<0.1	No Diff.	–	14.37	<0.1	1.02	>10

* Test 1 = Chi squared
** Test 2 = Yate's Chi squared
*** Test 3 = Fisher's Chi squared

the greatest incidence of the plateau was shown in the rectal cooling curves. In fact 25% of the rectal cooling curves manifested the presence of the initial temperature plateau while the latter was present in only 7% of the brain and the liver curves.

The incidence of the plateau was also compared in fat and thin bodies. It was found that fat bodies showed a higher incidence of the initial temperature plateau (33%) than thin bodies (15%). However this was not statistically significant (P was more than 10%, Table 3.34). Also, it should be noted that the classification of the cases into fat and thin categories was limited to rectal curves only. By experience it was noted that the rectum (or any site) might cool without an initial temperature plateau irrespective of the weight of the body.

Obviously, the plateau was also affected by other factors such as the covering of the body. This factor was studied and found to have a significant effect on the liver (P was less than 0.1%, see Table 3.34). Its effect on the incidence of the plateau in the brain and the rectal curves was not significant (P was more than 10% in both situations, Table 3.34). It was noted that when all curves for the covered and naked groups were compared the result, in general, indicated a significant effect of the covering on the occurrence of the plateau (Table 3.34). This, however, reflected the liver situation rather than that

of the brain or the rectum. It was clear from these results that the rectum was the only site for which cooling curves might show a significant incidence of the initial temperature plateau irrespective of other factors such as the body weight and the presence of covers.

It is important to note that the absolute value of the slope ratio, on average, decreased with post-mortem time. This was true in all body sites and all conditions studied. This meant that the effect of the first term of the triple-exponential formula (the negative term) on cooling of the body was great at the beginning and decreased thereafter. In other words, the average slope ratio showed, in general and particularly in the rectal curves, a definite sigmoid shape when plotted against time (Figure 3.54-3.61). Obviously this did not mean that the effect of the other terms (the positive cooling terms) on the cooling process was slight at the beginning or that they increased with time. The net effect of these two factors on the cooling curve determined the presence or absence of the initial temperature plateau. In fact, despite the large effect of the first term on cooling in the initial stage of monitoring, it was not sufficient to counteract completely or abolish the effect of the other two terms on the cooling process. The slope ratio was always less than unity except in one case. In contrast with the slope, the slope ratio,

on average, decreased slowly in the early stage of cooling and then rapidly.

With regard to an increase in body temperature after death, there was only one liver curve for a naked body which showed an elevation of the temperature (i.e. positive slope and a slope ratio of more than the unity) for the first 1-2 hours after death. This was not encountered in any of the brain or rectal curves.

3.7 Study Of Site Temperature Ratios

The procedure used to calculate the temperature ratios between body sites was described earlier (Paragraph 2.6.3 point 1). These ratios were studied in all cases in the naked body group, including rectum/brain, rectum/liver, and liver/brain ratios. Average values were computed at successive post-mortem intervals up to 24 hours after death (Table 3.35). The ratios were plotted versus time and these graphs are shown in Figure 3.62 which also shows plots of the 68% confidence limit (±1 standard deviation). The plot of the rectal/brain ratio versus time was markedly convex over a long period up to about 21 hours post-mortem; the peak was at 6 hours after death. The plot of the rectal/liver ratio was also convex although very shallow; the peak was also at 6 hours post-mortem. The curve of the liver to brain ratio had a broad convexity with the peak between 12-15 hours after death. It was concluded that the site

Table 3.35: Site temperature ratios versus time in hours.

TIME	Rectal/Brain Ratio		Rectal/Liver Ratio		Liver/Brain Ratio	
(HOURS)	Mean	S.D.	Mean	S.D.	Mean	S.D.
3	1.497	0.317	1.303	0.196	1.154	0.137
6	1.531	0.341	1.311	0.22	1.168	0.128
9	1.516	0.388	1.288	0.247	1.189	0.151
12	1.481	0.415	1.254	0.253	1.196	0.18
15	1.442	0.458	1.205	0.253	1.20	0.235
18	1.378	0.489	1.156	0.254	1.194	0.23
21	1.286	0.466	1.101	0.26	1.158	0.163
24	1.242	0.532	1.057	0.278	1.152	0.164

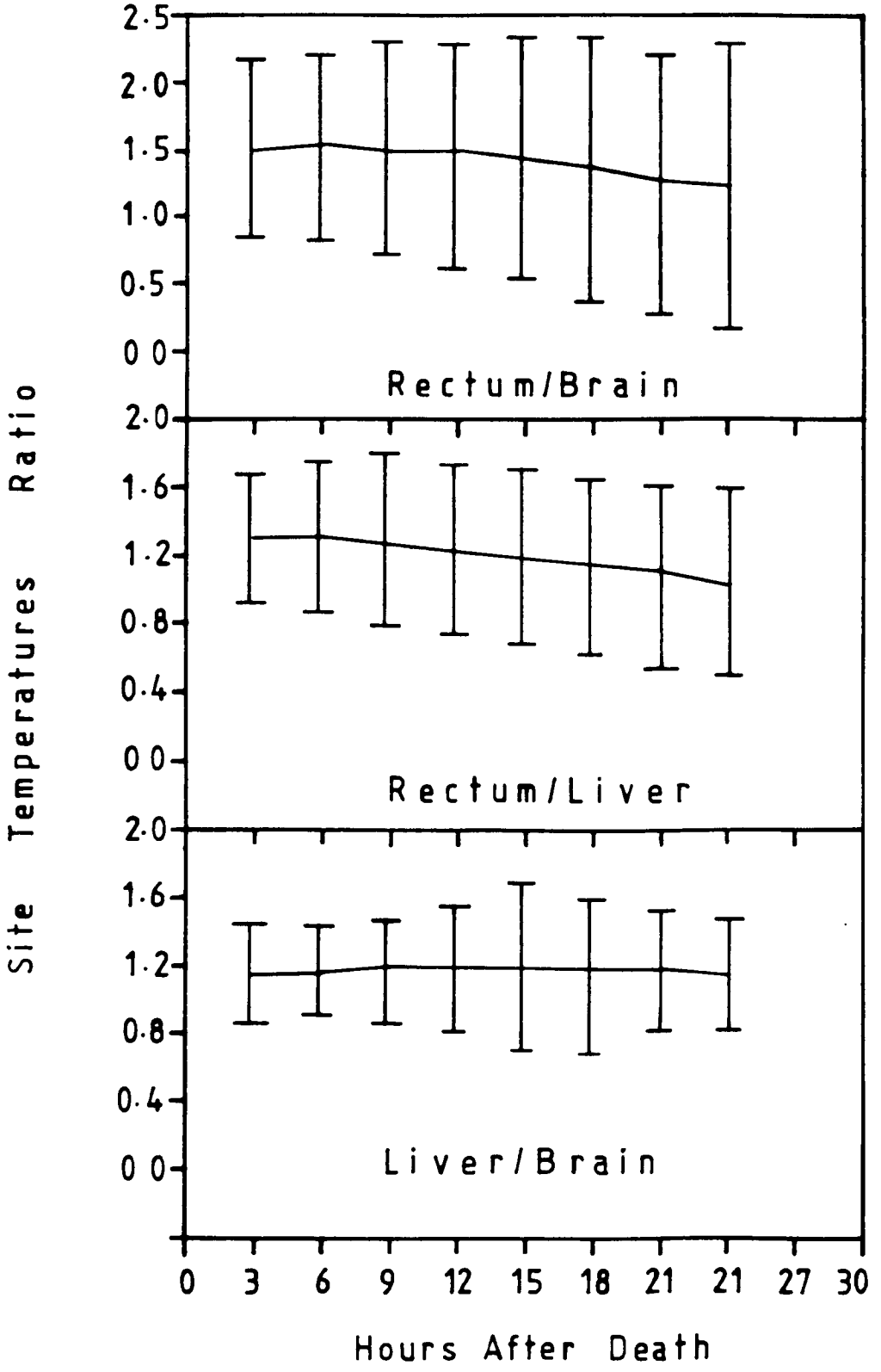


FIGURE 3.62: SITE TEMPERATURE RATIOS VERSUS POST-MORTEM INTERVAL ± ONE STANDARD DEV--IATION.

temperature ratio was of little practical value in the estimation of the post-mortem interval. However it could be used for very approximate estimation in combination with the average formulae devised in this study which were more accurate and far more preferable in practice

3.8 Comparative Studies

In five cases an attempt was made to use the double-exponential formula devised by Marshall and Hoar [336]. To calculate the constant z of this formula, the method requires that differences between rectal and environmental temperatures should be calculated at successive hourly intervals after death up to more than 12 hours post-mortem. Natural logarithms of these differences are then found and subtracted from each other. Results of this procedure are plotted versus time. The curve thus obtained is supposed to be rising in the beginning and to stabilize to a horizontal line after 12 hours post-mortem. The value obtained at this stage is equal to the value of z. Values of other constants are then derived depending on the z value. When this method was tried it was not possible to find the value of z as the horizontal curve was not achieved even after 12 hours post-mortem. In fact the curve was not stable even after a longer period.

The possible errors in time estimates inherent in the method described in this research and the errors

obtained using methods of other authors [78,205,242, 265,329] were also compared for rectal measurement in the naked body group. This was because methods of other authors used the rectum as the site of temperature measurement in naked bodies and therefore data for covered bodies and for other body sites were not available. Also, the comparison was limited to a period of 23 hours after death because there were no data from other authors beyond this point. The results of this comparison are given in Table 3.36a. Tests of significance (zM and Student's t tests) were carried out for successive post-mortem intervals and values of z, t and P are shown in Table 3.36b. Note that the zM test was used when only one datum was present for comparison. Also, because data were not available the comparison with some other methods such as that of Fiddes and Patten[166] was not performed.

It was found that the accuracy of the estimation of the time of death was significantly improved when the method devised in this study was used. This was true for most post-mortem intervals. At other points, the accuracy of time estimation obtained by this method was similar to that found by other methods. In fact there was no result which was significantly worse than those of other methods. The confidence limit was also enhanced by this study. In addition to that this method was the easiest to use in practice compared to other methods presently available.

Table 3.36a: Mean and standard deviation values of errors in time estimate using the method developed in this research and methods of other authors.

POST-MORTEM (hours)	This Method (N = 74)		TDZE Method Ref.No.[205]			METHODS USING DOUBLE-EXPONENTIAL FORMULA DATA FROM REF. NO. AS INDICATED											Extended Linear Method, Ref.No [265]	
						Method 1 * Rf.No.[329]			Method 1 * Rf.No.[205]			Method 2 ** Rf.No.[205]			Method 1 * Rf.No.[78]			
	Mean Error	S.D.	Mean Error	S.D.	N	Mean Error	S.D.	N	Mean Error	S.D.	N	Mean Error	S.D.	N	P.M. Interval	Error	P.M. Interval	Error
1	0.09	0.4															1	1.1
1.5	0.05	1.1															1.5	1.5
2	0.0	1.8	0.13	0.3	3	1	–	1	0.6	0.24	3	0.6	1	3				
4	0.24	1.3	0.7	1.6	34	0.7	–	1	0.14	1.9	34	0.5	2.1	34				
6	0.34	1.6	1.1	1.9	54	1	0.5	5	0.13	2.5	54	1	2.8	54			6.75	6
7	0.38	1.7	1.2	2	62	–	–	–	0.14	2.7	62	1	3	62	6.6	4.9	7	4
8	0.43	1.9	1.3	1.8	64				0.3	2.73	64	1	2.9	64				
9	0.45	2.2	1.1	2	66	1	0.7	16	0.44	2.8	66	1	3	66				
12	0.51	2.45	0.3	2.1	66	1.4	1	14	0.7	3.1	66	1.2	3.3	66	13.5	6.2		
18	0.62	3.4	1	2.5	44				2.3	3.1	44	0.2	3.6	44			20	6
23	0.32	4.0	2	4	5				2.9	3.1	5	1.1	1.6	5				

* Method 1 is that devised by Marshall and Hoar [329]

** Method 2 is that devised by Henssge [242]

Table 3.36b: Values of t, z and P for the comparison of the estimate errors resulting from the method devised in this research and from the use of other methods. Methods 1 and 2 are as explained in Table 3.36a.

POST-MORTEM (hours)	TDZE Method Ref No [205]		METHODS OF DOUBLE-EXPONENTIAL FORMULA.DATA FROM REF.NO.AS INDICATED:									Extended Linear Method Reference No.[265]		
			Method(1) Ref.No.[329]		Method(1) Ref.No.[205]		Method(2) Ref.No.[205]		Method(1) Ref.No.[78]					
	t	P%	z or t	P%	t	P%	5	P%	Point at	z	P%	Point at	z	P%
1												1	2.53	~1
2	0.6	>10	0.6	<10	2.9	<1	2.9	<1				2	1.32	<10
4	3.0	<1	0.4	<10	0.7	>10	1.7	<5						
6	4.1	<0.2	3.5	<0.2	1.1	>10	3.6	<0.2	6.6	2.85	<1	6.75	3.5	<0.2
7	4.2	<0.2			1.2	>10	3.1	<0.2				7.0	1.9	~5
8	3.9	<0.2			0.5	>10	2.6	~1						
9	2.5	~1	2.5	~1	0.04	<10	2.2	<5						
12	0.7	>10	3.1	<1	0.7	>10	2.42	<5	13.5	2.3	<5			
18	1.0	>10			4.2	<0.2	1.1	>10				20	1.6	>10
23	3.6	<0.2			5.6	<0.2	1.7	>5						

CHAPTER 4 : DISCUSSION

4.1 Introduction

The time of death is often of vital importance in a medico-legal investigation [326,400]. It is now well accepted and documented that the cooling rate offers, so far, one of the most reliable and easiest methods of estimating the post-mortem interval during the first 24 hours after death [12,325,326,332,400,408, 410,412,413,415,461,463]. Other methods for time of death estimation have, so far, failed to find empirically useful parameters; in other words, they are unable to provide better estimates of the time of death than those given by the temperature method. Nevertheless, this does not mean that the rate of cooling is absolutely reliable and accurate as it has been shown that even the best of the cooling models may give significant errors in the estimation of the time since death when it is applied in the field [78,205, 279,337,391].

In the literature, many factors affecting the post-mortem drop in body temperature are discussed [78, 188,193,276,326,337,380,400,420,435,474]. As will now be apparent, the overall picture is rather complicated. Accordingly, simple models are intrinsically insufficient and the mathematical complexity of more sophisticated models or formulae should not be a restriction on their use in practice, whenever such a

model or formula can offer even a slight improvement in the reliability of the estimation of the time of death. This is particularly true today when almost every discipline has been partially or even totally affected by the computer. Using the computer, it is now easy, in practice, to apply even the most complicated formulae.

The present study represents a novel approach to the use of cooling models, in that:

1. It used microwave thermography to measure the temperatures of internal body organs by non-invasive techniques.
2. It also used computer-based methods extensively for data acquisition and processing.
3. A useful practical version of the triple-exponential formula was developed and evaluated for the first time.
4. The shape of the cooling curve in three body sites was extensively evaluated both mathematically and statistically and the concept of the temperature plateau was tested.
5. The temperatures of each body site at the moment of death were studied and calculated by extrapolation from empirical data.

4.2 Microwave Thermography System

4.2.1 The Instrument

The microwave thermography device used in this

study was recently developed in the Department of Natural Philosophy at Glasgow University[505]. Apart from its application to forensic medicine in this research for the first time, it has also been used for the diagnosis of disease in rheumatology, clinical oncology and gastro-enterology [47,48,295]. Details of the parts forming the microwave thermography system were explained earlier (Paragraph 2.1). In summary the system consists of four main components. These are a microwave radio receiver, two microwave probes, two thermocouples connected to digital pyrometers and a BBC model B microcomputer. The latter has an inbuilt Analogue to Digital Converter (ADC). Thus analogue to digital (AD) conversion of the signals from the receiver and the pyrometers was performed as explained in paragraph 2.6.1.

This technique is able to detect and measure the microwave emission of internal organs using detectors placed on the skin and thereby the temperature of tissues several centimetres below the body surface can be obtained. It should be noted that the microwave component of the natural thermal radiation of body tissues is ordinarily very small, therefore the receiver is designed to magnify the microwave signal to a usable level.

4.2.2 Mode Of Operation

The mode of operation of the microwave thermography technique is based on the knowledge that

body tissues naturally emit thermal radiation which varies in intensity with their temperature [47,505]. This radiation consists of three types of waves according to the wavelength. These are: the centimetric (microwave), the millimetric and the infra-red parts of the electro-magnetic spectrum [47,48,295-298,505]. In general, the longer the wave the greater is its ability to penetrate body tissues[47,48,297]. Properties of electromagnetic waves detected by the microwave thermography system in biological media are given in Table 4.1.

The penetration distance of a wave is defined as the distance over which the radiation intensity is reduced by the factor e^{-1} (i.e. 0.37) [47,48,273,295-298,505]. In other words it is the distance over which tissue absorption attenuates the radiation intensity by 63% of its original value. Accordingly, the penetration distance of microwaves is more than that of millimetric or infra-red waves. In fact body tissues are relatively transparent to electromagnetic radiation in the microwave region [47,48,296,505]. However, the penetration distance is also dependent on the nature of the tissue, particularly, on the amount of water contained in the tissue. Generally speaking, the less the water content the more is the penetration distance [47,48]. Therefore the distances of penetration through fat and bone are more than those through muscle and skin [297].

Table 4.1: Properties of electromagnetic waves, detected by the microwave thermography System, in Biological Media*. (Waves are of $3GH_z$ frequency and 10 cm length in air)

PROPERTY	High Water content tissues (skin, muscle)	Low Water content tissues (fat, bone)
Dielectric Constant	43 - 48	5.5 - 7
Conductivity	2 - 26 (mho/m)	110 - 234 (mho/m)
Wavelength in tissue (cm)	1.45	4.25
Depth of Penetration (cm) (Penetration Distance)	1.61	9.74
Tissue Wave Impedence (ohms)	55	150
Reflection Coefficients:		
at air boundary	0.56	0.19
at air-muscle interface	0.751	-
at air-fat interface	-	0.406
at fat-muscle interface	0.495	0.495

Compiled from Reference Numbers 47, 48 and 295-298

The equipment used in this study operates at radiation frequencies from 3.0 to 3.5 GHz corresponding to wavelengths in air from 10 to 8.6 cm. As a result, the penetration distances obtained are 1.6 and 10cm in high and low water content tissues, respectively. It was experimentally shown that readings from the microwave probes represented temperatures of body structures 5-9cm deep from the body surface (See Paragraph 3.1.3, point 1 and Figure 3.5). Accordingly, direct observation of thermal radiation emitted from internal body organs can be made at the skin surface. In this respect, this technique is fundamentally different from infra-red thermography, in which the observed radiation comes only from the skin surface [47,48,295,505].

Because body tissues have relatively high values of refractive index in the microwave part of the electromagnetic-wave spectrum [47,48,505], a large fraction of microwaves radiated from tissues is reflected back into the body when they reach the skin/air boundary. To overcome this problem the microwave probes are designed to simulate the tissue refractive index of microwaves and, also, the probes should be placed in contact with the skin during the temperature measurement.

4.2.3 Calibration Of Microwave Thermograph

Since the microwave theromography system is a recently developed device, and since it was applied to

this field for the first time, it was necessary to perform calibration procedures during the initial stage of its use. This was to check the accuracy of temperature measurements made by the microwave probes. Thus the instrument was calibrated with liquid and solid media at temperatures ranging from 10-37°C as described in paragraph 2.2. A mercury thermometer or a thermocouple was used as a control for this purpose.

A linear relationship was found to exist between the response of the microwave thermograph and the temperature of the calibrating media (Figure 3.1). This means that temperature readings obtained by the microwave device represent the actual measured temperatures of the calibrating media correctly (bearing in mind the problem of microwave attenuation by body tissues). These media contained glycerol in water (1:3 v/v) to simulate the microwave refraction of body tissues. Both microwave probes were compared and found to give similar temperature readings, varying by less than 1°C. Nevertheless, each probe was always used to measure the temperature of the same site i.e. the brain or the liver respectively. This was to ensure that temperature readings of body sites obtained from the microwave probes were not affected by variations in the measurement characteristics of the probes. It was necessary to repeat these calibration procedures at regular successive intervals during the period of the study. Thus discrepancies in temperature

data of the cases studied resulting from technical or calibration faults were avoided.

The main technical fault observed was the occurrence of background electrical noise ("spikes") in the brain traces. This resulted from a faulty connection between the cable of microwave probe 2 (usually used for monitoring the brain temperature) and the main unit. However these spikes could often be corrected by editing the file to remove the aberrant data unless the noise problem was irremediable, when the case was abandoned. This type of fault occurred very rarely in the liver traces and was treated in the same way.

4.2.4 The Problem Of Microwave Attenuation

It is now clear that the temperature reading given by the microwave equipment is dependent on the actual temperature of the tissue to be measured and, also, on the degree of radiation loss or microwave attenuation during the passage of the wave through that tissue[296]. To examine this problem two sets of experiments were performed to assess microwave attenuation by body tissues as described earlier (Paragraphs 2.3 and 3.1.3).

The first set was conducted in living subjects and the second one was performed in dead bodies. In this way the problem was examined in living and dead tissues. It was concluded that attenuation of microwaves in the temporal region was significantly

less than in the right hypochondrium. This is explained by the fact that the microwave penetration distance of low water content tissues e.g. bone (skull) and high fatty content brain material, is greater than that of high water content tissues such as muscle and liver. Attenuation of microwaves in the temporal and the liver regions was found to lower the temperature reading by 0.6 ±0.9°C and 2.9 ±1.8°C, respectively.

It was also shown that attenuation observed at the mid-frontal region was greater than that noted at the temple. This is due to the fact that the temporal bone is usually thinner than the frontal bone and is perhaps also due to more vascularity and hence more blood in the temporal region than in the forehead, which also contains air sinuses. For this reason and to avoid variations in brain temperature readings due to positional factors one of the temples was usually chosen as a site of temperature measurements. The forehead was not used for this purpose although it was technically easier to place the probe on the forehead than on the temporal region.

In general, there was no significant difference in microwave attenuation between living and dead tissues. It was noted, however, that in dead bodies there was a wide range of individual variation in microwave attenuation. Also the magnitude of attenuation varied with the post-mortem interval, being

the least at 4.5-7 hours after death. These variations might be attributed to changes of physical properties of body tissues and alterations in the patterns of electrolyte and water distribution which usually take place in the body after death. Further investigations are required before definite causes can be given.

An important part of these investigations is to find the actual thicknesses of the layers which form the abdominal wall at the right hypochondrium. The first attempt in this direction was performed in this study and relevant data were given in Table 3.4. It was anticipated that such data would be useful for future work to estimate the exact contribution of each of the layers of the abdominal wall to microwave attenuation. Also of importance was the conclusion that temperature differences between various regions in the body existed not only immediately after death but also throughout a long post-mortem interval. In all experiments conducted with dead bodies, the temperature of the skin was lower than those of the deeper layers. This is because the skin is on the boundary with the environment and therefore it is the first to cool after death. In addition the normal temperature of the skin in life is usually lower than those of the deeper layers.

4.2.5 Are Microwave Probes Affected By Heat?

To answer this question two types of experiments were carried out as described in paragraphs 2.4 and

3.1.2. The conclusion was that the microwave probes were significantly affected by heat (See Table 3.2). This was true for unusually high temperatures of the order of more than 50°C. Changes in the temperature of the environment within a narrow range (as was the situation during monitoring of the cases studied in this research) had no significant effect on the readings of the probes. This was shown in the monitored cases, for example, in an environmental temperature as low as 8°C, temperature readings by the microwave probes were similar to those obtained at higher environmental temperatures e.g. 15°C (Compare liver traces in Figure 3.11b and e). However the effect of very low temperatures was not studied due to technical difficulties in establishing such conditions. Also in regard to this problem probe 1, which was usually used for the measurement of the liver temperature, was left uncovered when temperatures of the covered bodies were monitored. This was achieved by cutting small holes in the blankets so that the probe was put in contact with the skin of the right hypochondrium without being covered by the blankets. This was described in detail in paragraph 3.1.2.

In summary, the Microwave Thermography System, which acts as a thermal stethoscope 'listening' to the temperature-dependent microwaves, offers an accurate technique to measure the temperature of internal body organs or tissues by a non-invasive and ethically acceptable method.

4.3 Mechanism Of Temperature Regulation And Cooling

Temperature regulation is one of the most complicated phenomena which take place in a living subject. Many physical and physiological factors act and interact to influence the overall picture. This regulation is mediated through physical, chemical, sensory, hormonal and behavioural mechanisms. There is increasing evidence that the anterior hypothalamus is the site in the body where thermoregulation is initiated[58-61]. With regard to the thermoregulation mechanisms and aspects of 'body temperature' in life under different conditions, a great number of investigations and reviews have been published [18, 42-45, 49-54,56-61,74,75,84,85,88-91,95-98,111,114,116, 123,126,134-138,140,143,144,171,177,179,184,185,198-200, 204,207,218,221-228,230,231,233,235,236,240,244-246,256, 263,308,320,321,342,343,345,348,375,376,378,386,394,397, 398,418,427,428,431,442,481,486,489,492,501,507,512,517-520,523,526-528]. Discussion of these is outside the scope of this thesis and it is sufficient to discuss briefly some of the aspects which are of direct relevance to the main subject and perhaps necessary for a better understanding of post-mortem cooling.

Heat is lost from the body by four principal channels, namely, radiation, convection, vaporization and to some extent conduction [18,44,75,91,97,98,116, 134,135,138,140,171,178,180,218,221,223-228,230,236,240, 276,321,336,518]. The essential influencing factor is

the temperature difference between the body and its surrounding medium [376] in addition to other factors such as the movement of air, the humidity and the surface area of the body [44,91,221,223,518]. Calorimetric experiments in living subjects have shown that under basal conditions, when the environmental temperature is 25-26°C, heat loss from a nude subject is 66-68% by radiation, 21% by vaporization and 11-13% by convection [228]. The contribution of conduction is low [44].

In a living subject, when the environmental temperature is gradually changed from 22°C to 35°C, it is found that heat loss is 100% by radiation at the lowest environmental temperature but this diminishes uniformly until radiation disappears completely when the skin and surrounding temperatures become equal [44, 226,228]. The loss by convection remains about the same between 22 and 35°C but vanishes when air and body temperatures are equal [226,228]. The loss by vaporization is minimal at the lower environmental temperature, increasing gradually until it amounts to 100% of the heat loss at an environmental temperature near or equal to that of the body [136,226,228]. The effects of air movement and humidity have been discussed by many authors [44,91,134,223,227,276,518, 519].

As far as post-mortem cooling is concerned, there is some controversy in the literature concerning

vaporization as a mechanism of heat loss. In life, vaporization is mediated through sweating, panting and the passive diffusion of moisture through the skin [44, 74,180]. While there is certainly no post-mortem sweating, passive diffusion has been proved to continue after death. For example, the occurrence of mummification is definite evidence of excessive post-mortem vaporization under certain conditions [326,420]. In fact, it is known that a certain degree of vaporization from the dead body exists under all conditions [74,276,435].

Joseph and Schickele have proved that drying of body tissues (vaporization) continues until the vapour pressure at the body surface falls to that of the air. This takes place when the temperature of the surface becomes equal to, or a little cooler than, that of the air [276]. Obviously this condition can not be satisfied unless heat transfer from the "core" of the body to the surface stops. This means that the temperature of the body interior approaches that of the skin and hence it is equal to, or cooler than, that of the environment. It is, therefore, not surprising that for many cases in the present study it was found that the temperature of the body site did not drop to that of the environment only, but also it reached a limit of about 1.5-3°C lower than the environmental temperature. This was visible in cases for which the monitoring period was long enough to permit the final

stage of the cooling to be traced. It is worth mentioning that this observation has also been noted in cases reported by some other authors [276,317,335,336].

4.4 Site Of Temperature Measurements

It is generally accepted that in any study of the post-mortem rate of cooling, the temperature to be measured is that of the "inner core" of the body [330]. However, several investigators have shown that, in life and even under resting conditions, temperature differences exist between central regions of the body [346]. In other words temperatures of the inner parts of the body are not homogeneous. This is evident in Table 4.2. Therefore concepts such as body "core", "critical tissue" and a "single representative measurement of body temperature" have been questioned [143, 346]. Nevertheless, there is a common belief now that the anterior hypothalamus is the "critical tissue", to use the term employed by Eichna et al. [58-61], which contains thermo-regulatory receptors which, on stimulation, activate the mechanisms of temperature regulation.

Several studies, conducted in living subjects, have demonstrated regional differences in the rate of warming or cooling during transient heating or cooling of the body [56,58,59,96,111,114,184,185,342,343,481, 512]. In general the rectum has been found to be the slowest to respond to heating or cooling

Table 4.2: Internal temperature differences in resting human subject related to rectal temperature*.

BODY REGION	Number of Subjects	Temperature Difference ^{o}C	REF. NO.
Mouth	46	-0.45	489
	40	-0.35	114
Oesophagus	40	-0.24	114
	6	-0.6	96
	4	-0.26	346
Gastric	75	-0.07	198
Tympanic Membrane	4	-0.18	346
Sphenoid Sinus	1	-0.45	59
Rosenmuller's fossa	1	-0.45	59
Jugular vein:			
Jugular vein (low)	15	-0.22	143
Jugular vein (high)	4	-0.01	143
Superior vena cava	19	-0.35	143
Right heart:	6	-0.6	96
Atrium	24	-0.26	143
Ventricle	17	-0.23	143
Inferior vena cava:			
low	7	-0.26	143
high	10	-0.22	143
5-8 cm below diaphragm		3	343
Liver	75	-0.21	198
Hepatic Vein:	75	-0.125	198
near inferior vena cava		6	143
deep in liver	6	-0.03	143
Femoral artery	22	-0.22	143
Subclavian artery	3	+0.3	343

* Modified from Reference No 346

processes [56,198,226,228,342,346,481] and there is evidence that the brain (more correctly, central parts of the brain) responds most quickly [226,256]. On the other hand, the liver responds more quickly than the rectum but still slower than central parts of the body [198-200,346].

Graf, for example has shown that in states of increased heat elimination from the body surface (i.e. body cooling) the liver cools faster than the rectum, so that the rectal-liver temperature gradient after 10 minutes of cooling was greater than at 15 minutes, by which time the rectum had started to respond to cooling [198]. Other researchers have shown that, under induced hypothermia for surgery and subsequent rewarming, the rectal temperature in man may lag by as much as 2-4°C behind that of the central parts of the body [111,481].

As a result of these studies, increasing numbers of authors have doubted the reliability of the rectal temperature in reflecting the actual temperature of the body and have suggested that if the main concern is the rate of change of body temperature, rectal temperature will be less suitable than most other sites [44,60,342, 346,481]. There are some forensic authors who have also expressed similar opinions [250,276].

It has been repeatedly noted by investigators that in life, even under resting and equilibrium states, the rectal temperature in man is one of the

highest temperatures in the "body core" (Table 4.2), being higher than temperatures in central arteries and veins [51,54,95,143,346] and also higher than the liver temperature [198]. The exact reason for this is not known, but it is believed to be anatomical [256]. A further difficulty associated with the rectal temperature is the variation in temperature measurements with depth of penetration or position of the sensing element in the rectum [342,346]. Mead et al. have found that the rectal temperature, measured 8 inches from the external anal sphincter, was almost always lower than temperatures recorded at intermediate points along the rectum. Also, they encountered some technical difficulties in positioning the sensing element in the rectum and suggested the use of a rigid thermocouple rather than the catheter type so that depth of penetration and position could be adjusted more easily [342].

Many researchers have pointed out that the rectum is not positioned in the centre of the body, and Hiraiwa et al. have shown by computer tomography (CT) scanning that the rectum is placed near to the junction of the anterior three-quarters and the posterior quarter inside the body [250,276,317]. In summary, measurements of other regions such as the brain and the liver are considered preferable to those recorded in the rectum.

Nevertheless, most methods using post-mortem

cooling, so far, have been based on temperature measurements in the rectum and few studies have been carried out on the post-mortem temperature drop of the brain or liver. This is mainly because there is no easy way to access these organs. A thermometer or electrical thermocouple has to be introduced through a puncture wound in the abdomen [326,336] or one of the eyes [461] unless a hole is drilled in the skull. This may not only be ethically unacceptable but also may affect the actual cooling process. Using the microwave thermography technique, it is now possible to take the temperature of these and other organs by a non-invasive and ethically acceptable method. Since microwave thermography was used for the first time in post-mortem temperature measurements in this study, it was necessary to include a traditional method of temperature measurement after death as a control. The obvious choice was to monitor the rectal temperature using a thermocouple.

In this study a rigid thermocouple was used of a sufficient length so that its depth and position could be adjusted in each case. However, it happened sometimes, in a few cases, that the thermocouple accidently slipped back out or was pushed further into the rectum than required, so that some artificially lower readings were found - as mentioned earlier, the temperature at 8 inches was lower than intermediate position [342].

Several authors have anticipated that temperature measurements of the brain would yield valuable information [317,365,461]. The theoretical basis for this expectation may be summarized thus: first of all, the shape of the head and hence the brain is approximately globular; secondly, there are only slight individual variations in the size of the head between people; thirdly, the clothing of the body, apart from a head-covering, if any, plays no role; fourthly, the amount of hair, however, varies, but in area covered as opposed to length; lastly, the centre of the brain is rather uniformly insulated by brain tissue and cerebrospinal fluid [461]. Lyle and Cleveland found that the temperature fall in the brain showed the least individual variation [317] and Naeve and Apel concluded that the measurement of the brain temperature was the most useful of all temperature measurements [365].

4.5 Conditions of Studying the Problem

4.5.1 Controlled or Uncontrolled Conditions?

There has been some controversy in the literature concerning the conditions under which studies of the post-mortem cooling should be conducted. Some authors have suggested that controlled conditions are not relevant to the actual situation in the field. However, our view is that if the problem is to be approached systematically, the post-mortem drop

in body temperature should only be studied under controlled conditions. It is impossible to interpret results from studies of randomly selected cases, whereas it is more practicable to study the problem under different sets of controlled conditions which are likely to be met in practice.

It was also noted from the literature that, although several factors influencing post-mortem cooling have been discussed by many authors, nevertheless, many of these discussions were based on hypothetical postulations or sporadic individual observations rather than being inferred from scientifically planned experimental work. These sporadic observations and hypotheses, although of importance, are not sufficient to draw generally meaningful scientific conclusions. This is particularly true in complicated phenomena such as the post-mortem cooling of a human body. Some researchers, on the contrary, concentrated on purely non-biological experimental models which were constructed to simulate the human body [276]. Such theoretical studies and experimental models are obviously scientifically planned and undoubtedly of great importance, however their practical usefulness is limited.

4.5.2 Post-mortem Cooling in this Study

The cases used in the present study were chosen to satisfy the criteria given earlier (Paragraph 2.5.1). Firstly, the death should be due to

natural causes: this had the twin advantages that the body was not urgently needed for necropsy nor was it involved in medico-legal investigations normally required in suspicious deaths. Most of the cases used died as a result of heart diseases, reflecting their high incidence in the Glasgow area. Also, apart from two cases of bronchopneumonia, deaths involving hyper- or hypothermia were excluded to avoid wide variation of the body temperature at the moment of death. Secondly, the cases investigated in this research were demographically typical of the population norm. However there were no children amongst them. Despite that, the age groups studied covered those most likely to be involved in real practice.

The initial movement of the body from the locus of death to the experimental room in the mortuary was inevitable not only because it was difficult to monitor body temperatures at the locus, but also because our plan was to study the problem under controlled conditions. It is obvious that the room conditions could more easily be controlled. Apart from this initial movement and the movement during divestment of clothes, the body was kept undisturbed, in most cases, throughout the whole course of the monitoring period. All bodies were laid in a supine position in the manner described earlier, so that the effect of posture on cooling was uniform. The bodies were monitored either naked or covered because these two situations are the

most likely to be met in practice. In all covered bodies the amount of covering was kept the same, therefore the effect of this factor was also controlled.

Monitoring was started as soon as possible after death, mostly within 45 minutes post-mortem so that the initial stage of cooling was examined. This is of great importance because this stage is the most critical and controversial of all stages of post-mortem cooling. However it was quite difficult to find cases which satisfied this condition and this was a restriction on the number of the cases collected. It is also important to study the problem of post-mortem cooling for a long time after death. This is because most suspicious cases in forensic practice, where timing of death is likely to be required, are not discovered shortly after death. In this study monitoring was continued for as long as possible, preferably up to 60 hours after death. Where possible, monitoring was continued for at least 30 hours, at which point the temperature difference between the body and the environment was less than a few degrees and a practical limit was imposed by the precision of the device used (±0.6°C).

In each case, additional body measurements were collected such as the head circumference and the width of the hip as well as the weight and the height. This was to establish which factors have significant effects on the cooling rate, particularly because some models,

for example, the Infinite Cylinder Model, assume a knowledge of the cylinder radius.

This study was conducted under a wide range of environmental temperatures, so that the effects of cold and moderately warm weather were studied. However it was not possible to study the effect of air movement on cooling and all cases were studied under practically stagnant air.

4.6 Body Temperature at the Moment of Death

4.6.1 A Factor Affecting the Accuracy of Estimates

The need to know the body temperature at the moment of death is a prominent drawback in all methods that depend on the rate of cooling for estimating the post-mortem interval [11,12,276,326,400,420,461]. There is, in fact, some controversy as to what extent this drawback affects the accuracy of the estimates. While it is right to draw attention to the possible errors which may be introduced due to the lack of precise knowledge of the temperature at the moment of death [276], it is unjustifiable to go further and conclude that, because of this short-coming, efforts to achieve accuracy are of no value. First of all, accuracy is a relative term. It is well known that in most disciplines of knowledge, particularly those which deal with biological matters, perfect or 100% accuracy, from the practical point of view, is impossible. Secondly, it is always possible, in practice, to find

normal ranges within which most variations may fall and therefore in many cases, the temperature at death can be assumed with a good deal of certainty.

However, in some fatalities, particularly those which are most likely to be encountered in forensic practice, the body temperature is expected to be outside its normal range. This fact should be taken as a stimulus to provoke more studies to find the probable ranges of body temperature at the moment of death under various conditions, including various causes and manners of death. There has been, so far, no serious and systematic study of this subject under normal conditions and most information is derived from sporadic observations during routine pathological practice [276,436]. An exception of this was the work of Saram et al. [435,436], in which the rectal temperature of legally executed individuals was studied, however this situation is, obviously, abnormal.

4.6.2 Factors Affecting the Body Temperature at Death

The temperature at the moment of death is probably determined by interactions between physiological and pathological elements involved in the process of death. In life, there are individual and diurnal variations in temperature due to sex, age and season. In addition there are many physiological factors which affect body temperature such as muscular exercise and emotion. Moreover, the temperature also varies from one organ, or body site,

to another (Table 4.2). On these subjects, and others which concern the body temperature, there exists a vast amount of published work [44,48,51,54,56-61,95,96,111, 123,136,143,177,180,184,198-200,233,342,343,346,376,481, 507,517,526,528].

When life ceases, this situation becomes more complicated and obscure. The temperature may then be affected by factors such as the manner and the cause of death and other extrinsic factors such as the temperature and nature of the environment. For example, death may occur in the bath when the body is surrounded by warm water, or in bed when an electric blanket is used, or a hot water bottle is placed beside the body. Some workers have even reported that the body temperature rises by about 0.5°C after death, irrespective of the above factors [258].

Deaths which are due to systemic hyperthermia, or associated with elevated body temperature are well discussed by many authors [25,120,121,170,183,259,278, 300,303,356,357,394,429,459,492]. Of particular importance are those cases of death in which hyperthermia is expected and are likely to be encountered in forensic medicine, such as drug fever. Pyrogens associated with this type of fever may increase heat production as with dinitrophenol or may decrease the rate of heat loss as with epinephrine [300,492]. There are many drugs or substances which may induce fever such as barbiturates, amphetamines, antihistamines,

anticholinergics (e.g. Atropine), penicillin, sulfonamides, salicylates, diphenylhydantoin, cocaine, methyldopa, DDT and dinitro-ortho-cresol [183,300,492]. Fever may be seen after blood transfusion and in conjunction with splenectomy [492]. Recently an association between anaesthesia and cases of fatal hyperpyrexia has been reported. This occurrence, which is of considerable medico-legal importance, is sometimes called malignant hyperthermia. It has been studied and reviewed by many authors [120,121,183,259, 492]. Conflagration hyperthermia is also of medico-legal importance and has been reviewed in many published works [179,183,238,356,357,492]. Fatalities associated with elevated body temperature seen in forensic practice are: pontine haemorrhage [326,400, 420], severe infections such as septic wounds or abortions [400,420], fat or air embolism and other vascular conditions affecting the thermal control centres of the brain [326].

Asphyxial deaths are said to be associated with elevated body temperaure, but this has no convincing foundation [400]. In legally executed subjects studied by Saram et al. the rectal temperature ranged from 36.6°C to 38.2°C, only 27% being 37.8°C or above [276,436].

Deaths due to hypothermia are well discussed in several publications [17,76,141,180,183,208,237,251, 278,299,302,394,404]. Endogenous factors which may

aggravate, precipitate or associate with hypothermia are disease processes and drugs. Examples of these diseases are: lesions of the heat-regulating centres in the brain, hypopituitarism, hypothyroidism, exfoliative dermatitis and some debilitating diseases. Drugs which may be associated with hypothermia are barbiturates, phenothiazines, benzodiazepines (e.g. diazepam) and other hypnotics, sedatives, narcotics and, of course, alcohol. These drugs, in fact, may cause deep coma which in turn may lead to hypothermia, especially if associated with cold exposure.

It has been said that severe agonal bleeding lowers body temperature but this is probably without foundation [400].

4.6.3 Effect of Temperature at Death on Cooling Rate

If the temperature of the environment is kept constant the 'body temperature' at the moment of death will determine the temperature gradient between the body and its surrounding medium. Therefore, a higher temperature at the moment of death will mean a higher gradient and a greater quantity of heat which is to be lost to bring the body to equilibrium. The heat to be lost is given in Equation 4.1.

$$Q = M.S. (B-E) \quad \text{.....Equation 4.1}$$

where Q is the quantity of heat,

M is the mass,

S is the specific heat, and,

(B-E) is the temperature difference between the body B and the environment E.

If factors M, S and E are kept constant, B will determine Q. According to the definition of the cooling rate:

$$B - E = t.R \qquad \text{.....Equation 4.2}$$

where t is the time required to reach equilibrium, and R is the rate of cooling.

From these equations it follows that if R and Q vary by the same proportion as a result of changes in B, the time required to bring the body to equilibrium with the environment is independent of B. This means that if the same body is allowed to cool twice, starting from different body temperatures and if the conditions of cooling are kept exactly the same, one can expect the time required to reach the equilibrium state with the environment in both cases to be the same.

This assumption is impossible to verify in practice. Also, the post-mortem rate of cooling of a human body is not constant at all stages after death and therefore R and Q may not vary by the same proportion. Accordingly, the time required for two bodies to reach the temperature of the surroundings when they cool from different temperatures at death, may be prolonged, the same or shortened, according to the factors which determine the relation between R and Q for each of the bodies at any time after death and particularly at the initial cooling stage.

In this study temperatures of the brain, liver and rectum at the moment of death were estimated by

using the raw data collected during the first 3 hours of the monitoring period and extrapolating backwards as described earlier (Paragraph 2.6.4). Problems due to variation between one measuring device and another were also avoided by this approach, particularly with respect to the microwave attenuation by tissues which result in artificially low temperature readings. Neverthless, the problem still exists in the field where an assumption must be made with respect to the temperature of the chosen body site at the time of death. In this event and when using the same device employed in this study or one similar to it, the temperatures at the moment of death for the brain, liver and rectum to be used should be the average values calculated in this research and given in Table 3.13. In effect, this means that the formulae devised in this study contain in addition to the parameters P_1-P_6 which are constants, another constant which is the temperature of the chosen body site at the moment of death T_{b0}.

If for any reason another assumption is used and the temperature at the moment of death is expected to be higher or lower than the average values given in Table 3.13, the following correction should be made:

Let T_{bt} = the temperature of the body measured at time (t).

Let T_{et} = the temperature of the environment at time (t).

Let T_{bO1}= the average value from Table 3.13.

Let T_{bO2}= the new assumed value for body temperature at the moment of death.

Let R_1 and R_2 be the temperature difference ratios in both cases. According to Equation 2.2

$$R_1 = \frac{T_{bt} - T_{et}}{T_{bO1} - T_{et}} \qquad \text{.....Equation 4.3.}$$

$$R_2 = \frac{T_{bt} - T_{et}}{T_{bO2} - T_{et}} \qquad \text{.....Equation 4.4.}$$

By division of Equation 4.4 by Equation 4.3 it follows that the correction factor is:

$$F = \frac{R_2}{R_1} = \frac{T_{bO1} - T_{et}}{T_{bO2} - T_{et}} \qquad \text{.....Equation 4.5}$$

where F is the correction factor. Therefore,

$$R_2 = F(P_1.e^{P_2.t} + P_3.e^{P_4.t} + P_5.e^{P_6.t}) \qquad \text{.....Equation 4.6}$$

This type of correction assumes that all other factors influencing body cooling are constant in both cases. In other words, to use this correction in practice temperature measurements should ideally be made under the same conditions as used in this study.

4.7 Acquisition and Processing of the Data

4.7.1 Acquisition and Analogue recording

In this study, analogue signals from the microwave receiver and the pyrometers were converted to

digital forms (AD conversion) by two methods. In the first method, which was used for a few cases in the beginning of the study, the conversion was performed manually by a BBC-compatible digitizer as mentioned in Paragraph 2.6.2. For digitization purposes a suitable programme was made (Appendix I; Programme 2). Secondly, for most cases, AD conversion was carried out using a 12-bit ADC contained in the BBC model B microcomputer. An important feature of this ADC is its ability to handle up to four channels of input [535]. In this research four types of temperature data were recorded, namely, temperatures of the brain, liver, rectum and the environment. The ADC procedure was given earlier (Paragraph 2.6.1).

With any ADC there is a possibility that the returned value may be unacceptably inaccurate because of manufacturing tolerances in the resistors of the voltage divider [535]. This problem can easily be overcome by implementing an adjustment or a correction factor into the programme used for the conversion. One way for performing this is to apply known voltages and calculate the factor for correcting the output values on the assumption that these voltages are accurately known. For the purpose of this work, a similar test was carried out and the returned digital output value was found to be in a good linear relationship with the converted voltages (Figure 2.6). This meant that there was no need to implement a correction factor into the

AD conversion programme.

Another problem of the ADC is the possibility of small random errors in the ADC readings [535]. For this reason it is advisable that a given voltage, which is to be converted, should be read several times and an average of these readings should be taken. In this study each signal, i.e. each datum, was read 300 times before an average returned digital value was stored. This was possible because each input channel took only 10 milliseconds to convert an input voltage to a digital value. Although manual digitization was easy and accurate, AD conversion by an ADC was far easier and more accurate. Temperature data for the four sites mentioned above were recorded on magnetic tapes and a logical timing scale was provided by the computer.

4.7.2 Data Processing

The procedures used for primary processing of the data were described earlier (Paragraph 2.6.3 and 3.3.2). A trace was considered irremediable either because it was interrupted or because it contained too many electrical "spikes" to be corrected by editing the file,. It was emphasized that the procedure used for the correction of the monitoring traces should not change the shape of the traces and should not alter the actual data. Several types of data reduction were performed. These involved, for example, plots of temperature versus time, which were usually carried out to check that the correction of the data file had been

performed accurately. Other examples are plots of temperature difference versus time, site temperature ratio versus time and temperature difference ratio versus time.

Temperature difference ratios (Equation 2.2) are used in preference to simple temperature measurements in all stages of data analysis. This is because firstly, these ratios are required for the Infinite Cylinder Model equation and secondly, the temperature difference between the body and the environment is the most important of all factors that influence post-mortem body cooling. Thirdly, using these ratios can probably minimize the possible errors which may result from fluctuations of environmental temperature during the period of body cooling. It is important to note that in the version of the Infinite Cylinder Equation used in this work it is assumed that the environmental temperature at the moment of death is equal to the temperature of the environment at the time when monitoring is started. However, in most cases in this study, there were, in fact, small differences between the temperature of the locus of death and that of the experimental room. These small changes in environmental temperatures may have either very small or no effect on the cooling behaviour. This has been shown to be true by some authors [249,250].

Inferences made from the primary analysis of the data were given earlier (Paragraph 3.3.2, points 1-4)

and were illustrated in Figures 3.10b and 3.11a-j. In summary it was concluded that; firstly, there were major differences between body sites in respect of the rate of post-mortem cooling. Secondly, cooling of covered and naked bodies were also different for most body sites, particularly the liver and the rectum. Thirdly, there were considerable individual variations in the rate of cooling of a given body site. Fourthly, despite these variations it was observed that there were general features of post-mortem cooling of each body site for the covered and the naked groups. The aim of this study should therefore be to find these common features and put them in a manner which could be used practically in the field. It should also be noted that the primary processing of the data indicated that post-mortem cooling was a complicated phenomenon which could not be described by simple procedures.

4.8 Evaluation of Cooling Models

4.8.1 Mathematical Expression of Cooling Models

The aim of this study, in principle, was to find the equation which best described the data regardless of complexity. Obviously, each cooling model is represented by a mathematical equation. Therefore, the linear model (i.e. the rule of thumb) is described by a first order polynomial equation; Newtonian cooling is given by a single-exponential formula and the Infinite Cylinder Model is represented by exponential equations

containing two or more terms. It should now be known that a term of an exponential equation consists of an intercept and an exponent coefficient or parameter (constants) and the time interval which is to be estimated. This is better explained by an example as follows:

$$\text{Let } R = P_1.e^{P_2.t} + P_3.e^{P_4.t} + P_5.e^{P_6.t} \quad \text{Equation 4.7}$$

Therefore, in this example the intercept constants are P_1, P_3 and P_5 and the exponent constants are P_2, P_4 and P_6. Also this equation contains three exponential terms. t is the time to be estimated and R is the temperature difference ratio.

4.8.2 Criteria of Best Fit

Values of the temperature difference ratio for the three body sites, namely the brain, liver and rectum, were fitted to different mathematical expressions as described earlier (Paragraphs 2.7.1, 3.3.3 and 3.3.4). For the purpose of comparing the results of curve-fitting procedures, certain criteria were established and followed. Therefore, a good fit of the data by a function was considered to exist when it had a small value of the residual mean square (RMS) of the deviation between the observed (actual) data and those predicted by that function. Also, both sets of data were plotted versus time and a fit was said to be good if both curves were close to each other throughout the whole monitoring period. In addition to that, the

fitted curve should behave in the logically expected manner for a period of 60 hours after death. For example, although the curves shown in Figure 3.17a & b had low values of the RMS, they were not considered to be good fits because both deviated from the logical expectation outside the monitoring period. The "logical expectation" means that the body (in fact, a body site) should cool until its temperature becomes equal to, or a little lower than, that of the environment. It is, therefore, illogical to find, under the same environmental conditions, that the body starts to heat up after many hours of post-mortem cooling as shown in Figure 3.17a. In the same way, the example shown in Figure 3.17b indicates an illogical behaviour of the fitted curve because, outside the monitoring period, the curve decreased steeply so that after 60 hours of cooling the body temperature was many degrees below that of the environment. In fact, it was below the freezing point of water

4.8.3 Curve-fitting of Data

Curve-fitting procedures were classified into Class 1 and Class 2 as described in Paragraph 2.7.1. In the first class the linear and Newtonian models of cooling were examined. Thus data from 30 temperature traces (i.e. 10 cases each with 3 body sites) were fitted to first order polynomial and single-exponential equations. Both models were found to be insufficient to describe the data. This was indicated by high

values of the RMS, 1.9±0.3 and 1.3±0.5 for the linear and the Newtonian models respectively. This insufficiency was also confirmed in Figures 3.12 and 3.13.

It is worth noting that in 13% of the tested traces, the Newtonian model of cooling was sufficient to fit the data (Figure 3.14); the RMS was 0.8±0.4. Accordingly, it seems unjustified to say that Newton's law of cooling is completely invalid for describing the post-mortem cooling of a human body. In fact, this law merely points out the fact that the most important factor influencing the cooling of an object is the temperature difference between that object and its surroundings. This is absolutely true for the cooling of the human body after death.

However, there are other factors which should be considered in regard to the cooling of the human body. This is better expressed by the Infinite Cylinder Model which is a modification of Newton's law of cooling under certain conditions as will be discussed below. The ability of the Newtonian model of cooling to describe the data was also tested by another method. Thus, data of a site temperature trace were classified into three stages. Then, each stage was fitted to a single-exponential equation. Curve-fitting was improved as indicated by a low RMS (0.5±0.3) and by satisfying the other criteria of the best fit (Figure 3.15). This meant that Newton's model was reasonably

sufficient to describe the data for each stage . The more stages fitted by single-exponential equations, the better was the curve-fitting. This obviously indicated that the cooling curve could be best described by a series of exponential functions or multi-exponential formulae i.e. the Infinite Cylinder Model.

In curve-fitting Class 2 the ability of multiple exponential functions to fit the data was tested. Thus the data were fitted to exponential functions containing 2-4 terms. It was clear that the curve-fitting process was improved by the use of a double-exponential formula; values of RMS were 0.076 ±0.75, 0.106 ±0.139 and 0.153 ±0.105 for the rectum, liver and the brain respectively (Table 3.15a, Figure 3.19a). However, those cooling curves which were only poorly or moderately fitted by single or double-exponential formulae were almost exactly fitted by a triple-exponential formula thoughout the whole monitoring period (Figures 3.18a and b, 3.19a and b and 3.20-3.22). The four-exponential equation was also tried with some cases but the fit was not improved. In fact, the triple-exponential formula was found to yield a significantly better fit of the data than both the double and the quadri-exponential equations (Table 3.15a and b).

The technique of the curve-fitting Class 2 was a least squares estimates (LSE) method [125]. The data were not weighted because their error variance was

already homogeneous [392]. It was not mandatory to use the CONSTRAINT paragraph (See Table 3.14a) during the curve-fitting technique. However, it was advisable to use this option to obtain more accurate results from the curve-fitting. This can only be employed when there is a known constant relationship between the data of the X and Y axes and therefore the function of the constraint option is to restrain the curve-fitting process within a certain limit supplied by this relation. This is better explained discussing the constraints used in the study of post-mortem cooling.

The programme control file given in Table 3.14a contains two types of constraints. Both concern the body temperature at the moment of death. In other words they describe the relation between the heat loss and the heat production in life. Similar constraints have been discussed by Brown and Marshall [79]. The first constraint satisfies the condition that, at the moment of death, there is ordinarily no cooling and therefore the temperature difference ratio (Equation 2.2) should equal unity. Considering Equations 2.2 and 3.1, this constraint can be expressed mathematically as follows:

$$R = \frac{T_{bt} - T_{et}}{T_{b0} - T_{et}} = (P_1.e^{P_2 t} + P_3.e^{P_4 t} + P_5.e^{P_6 t})$$

.....Equation 4.8

At the moment of death $T_{bt} = T_{b0}$, and t = 0(zero)

Therefore,

$P_1 + P_3 + P_5 = 1$ .....Equation 4.9

This is a linear constraint.

The second constraint also assumes that there is no cooling at the moment of death and therefore the rate of cooling (i.e. the slope which is the first order derivitave of Equation 3.1 with respect to time) should be equal to zero.

Hence,

$$P_1.P_2 + P_3.P_4 + P_5.P_6 = 0 \quad \text{.....Equation 4.10}$$

This constraint could not be used because BMDP, P3R programme does not accept a non-linear constraint.

The triple-exponential equation is not only the best mathematical description of the post-mortem cooling curve found in this study but also a useful representation of the actual biological processes which take place in the body after death and which are involved in the cooling process. In this equation the first term always has a negative value. In fact, it has a negative intercept parameter, and therefore the slope of this term is always positive. It represents a "heating up" process which may be explained by the fact that some heat production continues after death. It is well known that many metabolic processes and activities do not stop immediately after death in a clinical sense. It may also be interpreted in terms of the manner of heat loss according to the Infinite Cylinder

Model as will be explained later.

The second and the third terms always have negative slopes. This means that these terms express cooling and heat consumption processes. The second term, which is the most important and dominant of all three terms, may represent the actual cooling process initiated by the temperature difference between the body and its surrounding medium. The third term, which is a small one, may represent heat consumption mediated by different metabolic, muscular and perhaps microbial activities that occur in the body after death. It is important to observe that the magnitudes of the three terms diminish as the post-mortem interval increases. The rate of diminution depends on the rate of cooling of the body or more correctly the body site concerned. Therefore when the cooling is slow the terms require a longer time to approach zero i.e to approach the temperature of the environment (compare Figures 3.20a and 3.22a,b and c with Figures 3.18b, 3.19b, 3.20b & c and 3.21a, b & c).

The triple-exponential equation used and developed in this study is very similar to the three-term formula of Wisler which was developed to explain the thermoregulatory process in life [520], except that, in this study, the proportions between the terms are quantitatively different from the Wisler formula and also the equation itself is simpler.

4.8.4 Effect of Body Variables on Cooling

Ideally, there are two methods of employing the triple-exponential formulae in the field. The first method is to use the average values of the parameters derived from curve-fitting Class 2 as will be discussed below. In the second method the parameters or the coefficients (i.e. intercept and exponent constants) should be calculated specifically for each body site in each case. It was noted that curve-fitting Class 2 yielded site-specific parameters which fitted the actual data exactly, so that errors in the estimated time resulting from these operations were within very narrow limits (in most instances they were only a few minutes). When average parameters were used such precision could not be maintained and the limit of the probable errors broadened. This indicated that the second method is more useful in practice, but this is not possible unless a procedure is developed to enable site-specific parameters to be calculated precisely.

Thus, each of the cooling parameters resulting from curve-fitting Class 2 were correlated with body variables of the cases studied. These variables, which represented the body build or physique, involved the 'cooling size factor'(Z), weight, surface area and hip and head diameters. The cooling parameters (the exponent constants P_2, P_4 and P_6) were notably variable, reflecting individual and/or site variations in post-mortem cooling. The intercept parameters were

not found to change within groups of related cases. The surface area was calculated according to the formula devised by DuBois and DuBois (Equation 2.3) which is widely accepted for this purpose [139,336, 444]. The 'cooling size factor'(Z) was used as first described by Saram et al. [435]. This term was also explained by Marshall and Hoar who used it extensively in their estimation of the time since death [336].

As far as post-mortem cooling is concerned, the build of the body is best represented by the relationship between the surface area exposed to cooling (i.e. the effective surface area) and the weight [332,336,435,436]. This is, in fact, the meaning of the "cooling size factor Z" whose values were calculated according to Equation 2.4 and 2.5. It was correctly considered by some authors that, when a body was laid in a supine position, about two thirds, or approximately 80%, of its surface area contributed to heat radiation and hence to the cooling process. Accordingly Marshall and Hoar suggested using two thirds of the 'cooling size factor'. However this was not followed in the present study because the aim was to correlate each of the cooling parameters with the 'cooling size factor Z' and therefore dividing or multiplying the value of Z of each case by a certain factor would not change the correlation.

According to Equations 2.3-2.5 an increase in the body build may affect heat loss in two ways; an

increase in the surface area may lead to faster cooling, or, on the other hand, a greater weight means a greater heat capacity (i.e. the amount of heat possessed by the body) and more heat to be lost. Also, it means a greater body radius which has been shown by many researchers to have an important role in the cooling process [97,98,250,276]. From another point of view, a greater weight may imply either a greater amount of fat and hence a greater insulation, or an increase in the bulk of muscles, in which case, the amount of insulation may decrease. Equations 2.3 and 2.5 indicate three points: Firstly, the 'cooling size factor Z' is inversely proportional to the weight W and directly proportional to the height H. Secondly, from a quantitative point of view, the effect of the height on both the surface area and the 'cooling size factor Z' is greater than that of the weight. Thirdly, the effect of the height on Z is less than its effect on the surface area. Accordingly, the proportional relation between the cooling rate and the "cooling size factor", which is claimed by some investigators cannot be demonstrated [326,336].

In this study it was found that there was no good correlation between the cooling parameters and any one of the body variables mentioned above. This meant that these variables alone could not be used for the precise prediction of the parameters. These results are in agreement not only with the theoretical basis

described above but also with results shown by some other authors such as Molnar and Green and Wright [205,353]. It has been correctly noted that while remote parts of the body such as the limbs would not be expected to affect the cooling, they do contribute to the weight of the body [353].

4.8.5 Average Cooling Curves

Cases were classified initially into either naked or covered groups. These, in turn, were subdivided into two categories, fat and thin bodies. This subdivision was based on arbitrary values of the weight, height and the 'cooling size factor'(Z), given in Table 3.16. Methods used to average the parameters of related cases for each group and category were explained earlier (Paragraphs 2.7.4 and 3.4.3). The first method was a simple numerical averaging of the parameters and the second method essentially averaged the temperature difference ratios prior to curve fitting in the usual way. The ability of the formulae derived by each method of averaging to predict the post-mortem interval accurately was then assessed.

Average values used in the first method were either arithmetic or geometric means, depending on the type of distribution curve of the parameters. For instance, when the distribution was logarithmic, geometric means were used. Both means were calculated initially for brain traces in the naked body group. This was an attempt to reduce the scatter of individual

variations in time estimates, but it was found that errors of time estimation resulting from the application of arithmetic means were not encouraging.

The second method yielded more useful results than either the geometric or arithmetic mean formulae, as indicated by the more accurate time estimates resulting from it. As a result, the simple numerical averaging method was not used for the covered group or the fat and thin body categories.

In the study of naked bodies it was found that, on average, the liver and rectum had similar cooling curves. However the rectum cooled slightly slower than the liver in the first few hours after death. The brain temperature dropped rapidly and therefore the average brain curve was usually rather steep. On average, the brain temperature in the naked group reached that of the environment in about 30 hours (in many cases it approached the enviromental temperature in about 15 hours). In fact, it sometimes dropped to levels below the environmental temperature, probably as a result of vaporization as discussed earlier. The applicability of brain cooling, therefore, is confined to the first few hours after death while the liver and rectum cooled more slowly and could be used for much longer periods. These findings agree well with those of other authors [317,461].

As expected, the brain was found to be only very slightly affected by covering of the torso. However,

cooling of the brain in the covered group was slightly slower, at the end stage of monitoring, than in the naked group. By contrast both the liver and the rectum cooled more slowly in covered bodies (Figures 3.24, 3.27 and 3.28). This was apparent as a "flattening" of the cooling curves for these organs. The liver was generally more affected than the rectum. In a few covered cases, the liver showed a slight temperature elevation in the first stage of monitoring. This was most probably an artifact resulting from the delay in the covering of the body, after divestment on arrival at the mortuary, so that the temperature of the liver appeared to increase relatively.

For some rectal temperature traces in naked bodies, it was possible to distinguish between fat and thin bodies. It was also noted that the average cooling curve for the fat body category was flatter than that for the thin category, but this was by no means the rule when individual cases were studied. It was actually found that in about 42% of the rectal traces in the naked group, the behaviour of the cooling curve was independent of the body weight, i.e. there were thin bodies which behaved as if they were fat and vice versa. In summary, there was no single body parameter which could be used to predict the cooling behaviour.

4.9 Assessment of the Shape of the Cooling-Curve

4.9.1 Theoretical Bases

The importance of determining the shape of the post-mortem cooling curve is self-evident. In forensic literature, the manner by which the body cools after death has been a matter of great controversy. The debate has usually concentrated on the initial stage of cooling which involves the first few hours post-mortem [380]. Although both fast and slow initial cooling patterns have been reported equally [8-10,78,79, 86,87, 117,129-131,133,139,188,192,205,206,241-243,284,292,293, 317,328-330,333,336,338,340,351-353,358,359,362,365,379, 380,388,416,419,435,436,440,441,443,444,456,457,461,474, 500,522], the latter, namely slow initial cooling, has been emphasized and, in fact, over-emphasized, particularly in the last 30 years or so [336,456,457]. In the literature, slow cooling, the sustention or even the elevation of the body temperature in the first hours after death is called the "initial temperature plateau" [188,336,456]. For the plateau to exist there should be either negligible cooling or none at all.

The current opinion is that the post-mortem cooling curve has a sigmoid or S-shape, comprising of the plateau stage, followed by a second stage of rapid cooling and a third stage where the temperature drops slowly. Many theories have been proposed to explain the sigmoid shape, particularly the initial temperature plateau. According to these theories the cause of the

plateau is either the continuation of heat production for some time after death or the manner of radial transmission of heat from the centre to the periphery, according to the Infinite Cylinder Model. Both explanations may be accepted.

In life, heat is produced in the body by metabolic activity and muscular contractions. There is no doubt that some heat is produced after the clinical diagnosis of death [79,193,316,380]. This is due to the continuation of many anaerobic or even aerobic metabolic processes for some time after somatic death [193,277,316]. Rigor mortis, for example, constitutes muscular contraction and hence heat production. The amount of heat produced after death depends on the energy store that is possessed by the body at death and the speed of the chemical reactions which make up the metabolic processes. Heat production by microbial activity is probably insignificant, particularly in the early stage when the plateau may occur. Lundquist has quantitatively estimated the amount of heat produced post-mortem from the breakdown of muscle glycogen [78,316,332,380]. Many other authors have discussed this matter [79,276,380,435,436]. Nevertheless, there are a few points to be stressed in this respect:

1. Metabolic processes do not produce heat only but they also consume heat to maintain their reactions (i.e., to use chemical terms many reactions are exothermic - produce heat and others are endothermic - require heat).

2. Both the amount and the rate of heat production after death is, to a great extent, affected by extrinsic factors such as the environmental temperature and the presence or absence of insulation (ie. clothes and the like). Accordingly, heat production may extend over a short or long period depending on whether the rate of production is increased or decreased respectively.

Extrinsic factors also affect the rate of cooling but perhaps in opposite directions; for instance, if the weather is warm one may expect faster heat production and a slower rate of cooling and hence, the plateau is more likely. But of course, there are other factors which may affect contrarily.

The other important explanations of the sigmoid cooling curve are the shape and the physical properties of the human body. Essentially, the body is not a mass of uniform substance but constitutes tissues of different thermal properties [193,457]. This means in physics that the body is not thermally "thin". In general, body tissues are relatively poor conductors of heat (Table 4.3). Also the shape of the human body is similar to that of a cylinder (Figure 4.1).

4.9.2 The Infinite Cylinder Model

The Infinite Cylinder Model supposes a cylinder of infinite length placed in an environment of lower temperature (Figure 4.1). According to this model, heat flows from the surface to the surrounding medium

Table 4.3: Thermal conductivity of some substances and materials (compiled from many authors)

SUBSTANCE	Temperature (^{o}C)	Specific Thermal Conductivity (cal/sec/cm/°C)	Reference
Air	0	0.0000568	44
		0.000058	98
Water	17	0.00131	44
	15	0.00144	98
Ice		0.0053	98
Snow (fresh		0.00025	98
Soil (average)	ordinary	0.0023	98
Wood (spruce,with grain)	"	0.00055	98
Wool	ordinary	0.000054	44
Leather (cow hide)	"	0.0004	228
Cork	"	0.0007	228
Paper	"	0.0003	228
Skin (in situ)	"	0.0013 - 0.0023	223 and 240
Muscle (wet)	"	0.00092	223 and 236
Muscle (wet)	"	0.001	75
Muscle (wet)	"	0.0011	238
Muscle (dry,beef)	"	0.00047	228
Fat	"	0.0003	75
Fat	"	0.00049	236
Fat	"	0.0004	238
Fat (beef tissue)	"	0.00049	228
Fat (human 1-2 days after death)	"	0.000489	236
Muscle (human 1- 2 days after death)	"	0.00067	236
Fat (human)	"	0.000488	236
Muscle (human)	"	0.00092	236

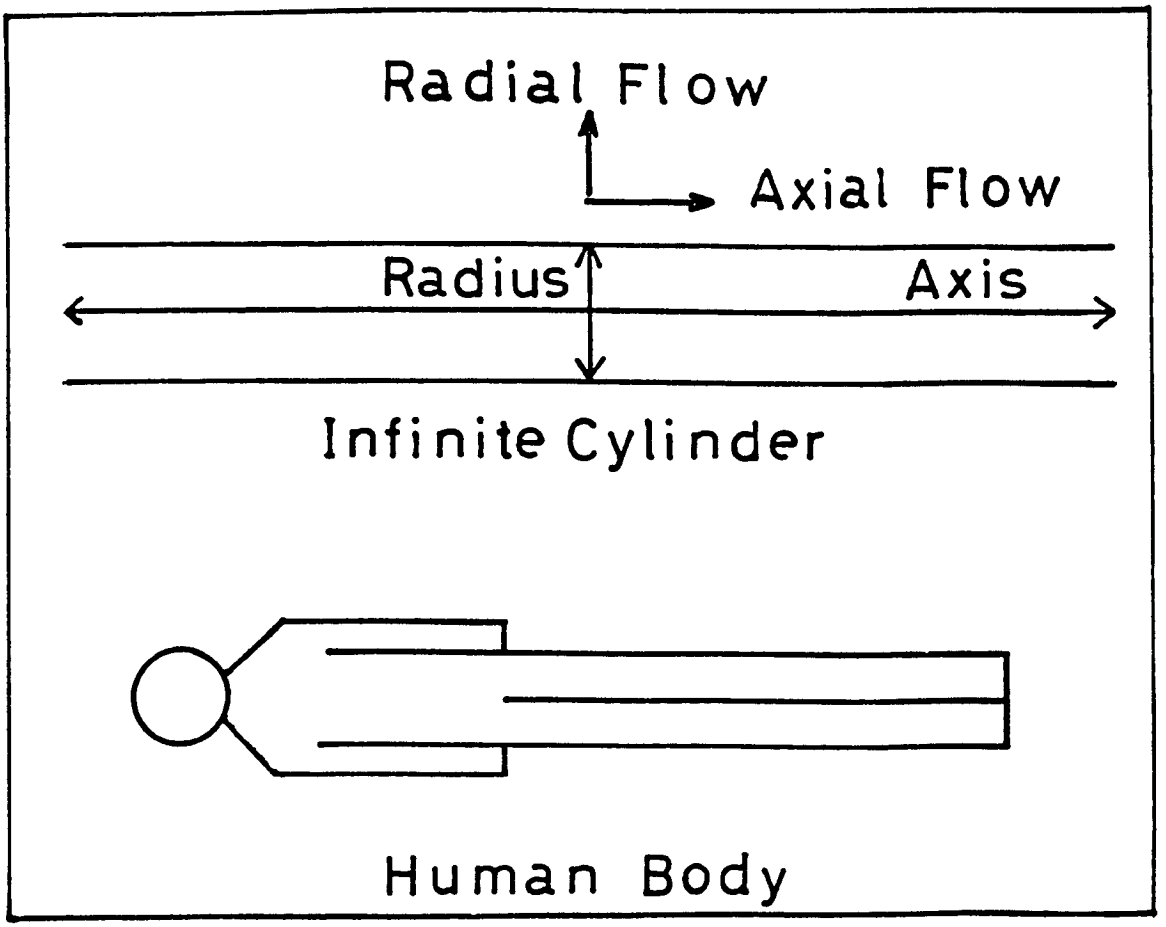


Figure 4.1: An infinite cylinder model of post-mortem cooling as applied to the human body.

and all or most of the heat of the cylinder is lost radially and not axially. This establishes a temperature difference between the surface and the region immediately central to it. Thus a gradient is set up between the surface and the centre. Since the gradient at the centre is small at the beginning, cooling will proceed, slowly at first, build up to a maximum rate and then slow down again as the temperature of the centre approaches that of the surrounding medium. This differs from the Newtonian model which assumes that the body is of a uniform temperature, i.e. thermally "thin".

It is obvious, therefore, that cooling of the centre, according to the infinite cylinder model, will start almost immediately but may take time to be apparent. The length of this time depends on the environmental temperature, the cylinder radius and the thermal properties of the interposed substances. If this theory is applied to the human being the rectal curves should exhibit the most pronounced plateau of all body sites. This is due to the rectum being closest to the body core and therefore having the maximum radius, bearing in mind the limitations of this approximation noted earlier. Several points should be considered with respect to the human body. These are:

1. There are many variations between individuals in respect of the radius of their body and the thermal properties of their tissues.

2. There is no uniform temperature for all central regions in the body and therefore concepts such as "body core", "one representative body temperature" and "body centre" are now questioned.
3. The precise position of the rectum is different from one individual to another and it has been shown not to be in the centre.
4. In life, the temperature of the skin and other superficial regions may be much less than that of the rectum and other internal regions and a temperature gradient normally exists between these parts of the body. This gradient may be built up even before death, particularly when the dying process is prolonged and it is of the type involving slowing down of body activities or if death occurs outdoors in a cold environment.

According to the discussion above, the temperature plateau may or may not occur depending on many intrinsic and extrinsic factors. In other words, the properties of the Infinite Cylinder are augmented or prevented by these factors. Thus the sigmoid shape is predicted by a more sophisticated theory than that of the Newtonian model and the appearance of a plateau is not a paradox. Rather the absence of a plateau requires explanation in terms of the intrinsic and extrinsic factors discussed above.

4.9.3 The Shape of the Cooling Curve in This Study

For any curve, the rate of change is best

expressed by its slope at any time: when the slope is negative, the curve is said to be falling, for example, in the cooling curve the temperature drops down and hence the slope is negative. On the contrary, a positive slope indicates a rising curve, for example, when heat is gained. If the slope is nil this means that there is no change. Therefore, from a mathematical point of view, for the plateau to exist, the slope should either be of a small absolute value (positive or negative) or nil.

As described earlier, the formulae used in this study contained three exponential functions. The slope of the first term was always positive and the slopes of the other two terms were always negative. The interpretation of these functions in terms of the actual processes that were involved in the cooling, was given earlier (Paragraph 4.8.3). The slope ratio indicated the role played by each of these functions in enhancing or preventing cooling. Therefore, when the value of the slope ratio at a given time is big, this means that the first term has, at that time, a greater effect in modifing the cooling curve.

It is confirmed by this study, that the post-mortem cooling curve of the human body is of the compound type. This means that the rate of cooling is not uniform at all intervals post-mortem. In fact, it is of the type predicted by the Infinite Cylinder Model. However, the sigmoid shape has not been found

in all cases (the plateau was seen in 21% of the cases studied). This conforms well with the theoretical basis discussed above. When the plateau was found, it was a smooth curve or slow cooling rate rather than a sustention of the temperature. This may be attributed to the great sensitivity of the temperature measuring elements used in this study (the absolute accuracy of temperature measurements was ±0.6°C and the response time was 2 seconds).

It is clear from this study that in most individual and average cooling curves the rate of cooling in the beginning was greater than later on. However, a study of the slope ratios indicates that, in most curves, cooling is modified in the beginning by the effect of the first exponential term. If cooling proceeded without this modifying effect, the rate of temperature drop would be very much faster than observed. But this effect is not sufficient to prevent cooling. In the late stage of cooling, the modifying effect of the first term decreases. However, cooling is already slower because the temperature difference between the body and the environment is reduced, i.e the temperature of the body site approaches that of the environment. This also explains the observation that the cooling rate of the brain at 12 hours post-mortem was, on average, less than those of the liver and the rectum at the same interval after death.

It was found in this study that the occurrence

of the plateau is significantly affected by factors such as the site of temperature measurements and the covering of the body. For instance, the incidence of the plateau in the rectal cooling curves was statistically significant irrespective of other factors such as the weight and the covering of the body. In other words, the finding of similar incidences of the plateau in the rectal curves for fat and thin categories and covered and naked groups indicates that the plateau is inherent in the rectum as a site of temperature measurements. This does not indicate that the weight has no effect on the plateau because this factor was not studied in all body sites. The high incidence of the plateau noted in the liver curves for the covered group means that this site of the body is greatly affected by the covering. This may result from the relative wide surface area of the liver and its position near to the body surface.

4.10 Accuracy of Estimating the Post-mortem Interval

4.10.1 Assessment of Average Formulae

One of the principal aims of this work was to find a more accurate and reliable method for the estimation of the post-mortem interval. It was, therefore, necessary to examine the extent to which the method developed in this study would be useful in actual forensic practice. This was carried out by assessing the ability of the average formulae to

predict the time after death. The procedures used for this purpose were described earlier but principally used average formulae derived from the second, or two-step averaging method (Paragraphs 2.7.4, 3.4.3 and 4.8.5). These average formulae are denoted by (Cf) in Table 3.21. In the assessment procedures estimated intervals (i.e. those predicted by the average formulae) were compared with actual post-mortem intervals which were recorded experimentally.

Thus differences between both the actual and the estimated intervals were calculated throughout the whole monitoring period. This was performed for each body site in all cases, but for practical use only values at selected interval were averaged (Tables 3.22 and 3.23). These values represent the deviation of the estimated time from the actual time and therefore they indicate the possible errors in time estimates when the average formulae are used.

It was noted in the literature that many words such as the "accuracy", the "precision", the "reliability" and the "confidence limit" of the method of estimating the time of death, were used to express the same meaning. Although these terms are closely related to each other, they actually indicate different concepts. An estimation of the post-mortem interval is said to be good when the estimated time and the actual time are the same. Similarly, a formula is considered precise in the prediction of the time after death if

the standard error of estimation is small or near to zero (i.e. precision is good if it gets the same answer every time the measurements are carried out - this is not meaningful here but is more useful in chemistry etc.). Because there are ordinarily many variations in the rate of cooling for bodies under different conditions, a cooling formula is accurate if it takes these variations into consideration and is able to calculate them. It should estimate not only the post-mortem interval but also the possible differences in time estimates which may occur due to various factors. This is not concerned with whether the range of these differences is narrow or wide. When one speaks about the precision of the method the magnitude of the range of the variations is considered.

It follows that a cooling formula is both accurate and precise when it combines both meanings mentioned above: that is, the average deviation of the estimated time from the actual time, for a group of related cases, is small and the range of the scatter around the average is narrow. A method should be considered as reliable only if it is statistically tested with a reasonable number of cases so that the confidence limit of the probable error in time estimates is scientifically calculated. This is of great importance particularly when the estimation of the time since death is given as investigational evidence in a court of law.

In this study it was noted that the mean error in time estimates was small (Figures 3.30-3.35). It was also observed that these errors increased at the end stage of monitoring. This is explained by the fact that at the end of the monitoring period, when the body temperature approaches that of the environment, we are attempting to measure a small temperature difference using the same equipment which has a constant degree of error in its use. Therefore the same relative accuracy of temperature measurements as that obtained at the beginning of monitoring can not be maintained due to the precision limit of the sensory devices (microwave probes and thermocouples). This observation was noted by many authors. For example, Fiddes and Patten, who proposed that the rate of cooling method should not be used when the difference between the body temperature and that of the environment was about 15% of its value at the moment of death and called the time required for the body to cool to this point as "the Virtual Cooling Time" [166] . Obviously the value of 15% was suitable for the precision limit of the device used by them and should not necessarily be applied to all measurement devices.

It is known that the standard deviation represents the scatter around the average. In all groups and categories the scatter was least during the first 5 hours or so. This may be due to changes in the physical and the chemical properties of body tissues

which may occur at different rates in individual cases, particularly as the post-mortem interval increases. Of interest in this respect are the results reported recently that the velocity of the passage of ultrasound waves through body tissues changes with time since death [536]. It should be mentioned, however, that a definite explanation can not be proposed unless further investigations are carried out.

It was also noted in the present study that individual variations were marked and increased with time exept for rectal cooling in the thin body category where the scatter at 2 hours after death was greater than at 4 hours post-mortem. There is no explanation for this but probably it is not significant. One standard deviation is quoted in our results: therefore the probability of encountering this range of errors in time estimates in the field is 68%. It was found that the average cooling curves for brain and liver, obtained by microwave thermography, gave a similar range of probable error to that of the average rectal curve which was based on temperature measurements made by a thermocouple. Nevertheless, the microwave probes were easier and more convenient to use in practice.

4.10.2 Practical use of the Method in the Field

The solution of the average formulae developed in this study can be carried out manually using a reference graph or, more accurately, using a micro or even a pocket computer. It is therefore, entirely

possible to use this method in the field if the portable version of the microwave system, which has recently become available, is used. For this purpose reference graphs were prepared for each body site in the naked and the covered body groups (Figure 3.44). Each graph contains the average cooling curve for a body site in either the covered or the naked group. In addition to that each graph contains a sheded area around the average curve. This area represents values of the standard deviation and indicates the possible errors in time estimates which are likely to be encountered in the field. It is important to note that one standard deviation is used and therefore these errors can, from a statistical point of view, be encountered in 68% of the cases in practice. In other words, the confidence limit of estimating the post-mortem interval according to these graphs is 68%.

For the reference graph to be used, the temperature of a body site and the temperature of the environment are first measured in degrees centigrade. The second step is to calculate the temperature difference ratio R from Equation 2.2. The temperature of the chosen body site at the moment of death T_{b0} should be obtained from Table 3.13. The resulting average time after death and the probable range of error in the time estimate can then easily be measured as explained in Figure 3.45.

To enable practising pathologists and police

surgeons to use the triple-exponential formulae developed in this study for more accurate estimation of the time since death a simple computer programme was written (Table 3.25). This programme is compatible with the BBC microcomputer which is widely used in this country but it can easily be modified for other computers. Following the instructions given by the programme, the investigator is asked to input the temperature of a body site and the temperature of the environment and to indicate which body site is used (rectum, brain or liver). Other data required for the prediction of the post-mortem interval are implemented in the programme itself. It should be observed that whenever the temperature of the environment is required this should be measured at about 2 metres distance from the body to avoid artifacts due to the heat of the body.

4.11 Relative Cooling Rates of Body Sites

When this study was started it was thought that the ratios between temperature measurements made at different body sites might offer a better approach to the problem of estimating the time of death. Temperature ratios between measurements of the rectum, brain and liver were calculated for all cases of the naked body groups at several successive post-mortem intervals. The values were averaged and plotted versus time (Figure 3.62). The usefulness of these curves in estimating the time of death, in practice, was shown to

be very limited. This is attributed to two reasons: the first is the wide range of individual variation involved, and the second is due to the fact that these curves, in particular that of the liver/brain ratio, are very flat, almost approximating a horizontal line. Therefore, if they were applied in the field to real cases, the expected range of error in time estimates would be very wide. Nevertheless, they could be used in association with the methods described earlier.

4.12 Comparative Study

For comparative purposes, the double-exponential formula devised by Marshall and Hoar [336] was used for estimating the post-mortem interval in five cases, but it was not possible to calculate the constant z although temperature measurements were studied over a long period of at least 12 hours. These difficulties in obtaining the constants required by the double-exponential formula were described earlier (Paragraph 3.8). Similar difficulties were also reported by some other authors such as Green and Wright [205] and Molnar [205,353].

The possible errors in time estimates resulting from application of the triple-exponential formulae developed in this research were compared with some of the most widely used existing methods of other authors (Tables 3.36a and b). Thus errors in post-mortem estimations at several successive intervals were

selected according to the availability of data. The method developed in this work was found to give significant improvements in the precision and accuracy of estimating the time after death (Table 3.36b). In addition, this method is very easily applied in practice: only one temperature measurement is required and the post-mortem interval as well as the probable range of error can be calculated simply from reference graphs or by using a microcomputer as described earlier (Paragraph 4.10.2).

4.13 Suggestions for Future Work

The problem of estimating the time after death is of great importance to forensic practice. There still exists the requirement to develop a very accurate and precise method, which should have a high limit of confidence, to be used as an evidence in a court of law. Therefore, this problem should continue to attract researchers in further investigations.

The main advantage of the microwave thermography system is its ability to measure the temperature of an internal tissue by the application of the sensory element on the skin. Thus invasive and ethically unacceptable methods for the measurement of the temperature of an internal organ are avoided. In future further studies are required to improve this system; in the first instance, the problem of microwave attenuation by body tissues particularly after death

should be completely assessed. This should be performed over a long post-mortem period.

The effects of the environmental conditions on the temperature readings from the microwave probes should be tested extensively. According to the results of these studies the device should be redesigned and calibrated again so that discrepancies between actual and recorded temperatures no longer exist. Also the machine should be supplied with suitable handles to hold the microwave aerials when they are placed in contact with the skin. The problem of the electrical noise "spikes" should be overcome also. If the microwave thermography system is thus improved future studies may better concentrate on the post-mortem rate of cooling of body sites such as the liver and the brain rather than the rectum.

The temperature of the body (or more correctly of a body site) at the moment of death should be the subject of extensive experimental investigation so that ranges of normal values can be established. This is, obviously, the best alternative to the method of extrapolation which is employed in this research. Calorimetric studies are anticipated to be of importance to improve the current understanding of the processes involved in heat production and loss after death. Of great importance and particular interest is the investigation of the changes that may possibly occur in the thermal properties of body tissues after

death. These must be considered in connection with the study of the effects of different environmental conditions on post-mortem cooling. Of importance amongst these are the movement of the surrounding medium i.e. air or water, cooling in river and sea water, cooling after burial in the earth, the state of radiation and variable microwave reflection from the room walls. This is, of course, in addition to the state of the clothes or covering of the body which has been studied in many works including the present one but needs further extensive investigation. The technique of curve-fitting is well established in this research, however further improvements and simplifications can be achieved. Expressions containing more than three exponential terms may be examined extensively although the triple-exponential equation has been shown to be adequate.

PART TWO

STEROID HORMONES AFTER DEATH IN RELATION TO THE ESTIMATION OF THE POST-MORTEM INTERVAL: A Study of Degradation of Some Steroid Hormones After Death in Rats

CHAPTER 5

5.1 Biochemical Methods of Estimating the Post-mortem Interval: A literature review

Studies of the changes that occur after death, in the chemical constituents of body fluids and tissues are useful in a variety of situations: they may help in the establishment of the cause of death when no necropsy is performed or when no pathology can be revealed on necropsy; they are also helpful in the evaluation of the physiological effects of a lesion found at the necropsy; more importantly, they may assist in the estimation of the time that has elapsed since death [108,314]. Coe in his recent review estimates that post-mortem chemistry is of value in about 10% of forensic cases [108,239].

For the purpose of estimating the post-mortem interval considerable efforts have been directed towards the analysis of body fluids such as blood, cerebrospinal fluid, intra-ocular fluids, synovial fluid and pericardial fluid to find regularly and constantly occurring post-mortem changes to be used as a 'post-mortem clock' [1,3,4,19,21,40,41,66,68,69,72, 73,99-108,112,113,119,124,127,147-150,158,163,164,167, 176,181,189,209,217,220,239,252,257,260,264,270,271,277, 288,301,305-307,309,310,314,315,319,339,341,354,363,364, 366-372,403,422,423,426,437,439,466,479,480,482,483,485, 499,502,509,511,534]. Many substances have been studied. Examples of these are: carbohydrates such as

glucose, inositol, lactic acid, pyruvic acid and ascorbic acid; nitrogenous compounds such as urea, creatinine, ammonia, uric acid, xanthine, allantoin, blood urea nitrogen (BUN), non-protein nitrogen (NPN), amino acid nitrogen, creatine and glutamine; enzymes such as acid and alkaline phosphatases, amylase, transaminases, lactic dehydrogenase, phosphoglucomutase and esterases including cholinesterase; some electrolytes such as sodium, potassium, calcium, phosphorus, sulphide, magnesium, chloride, iodide and selenium; other organic compounds such as cholesterol, cholesterol esters, bilirubin, urobilinogen, protein, ptomains, amino acids, fatty acids and vitamin E. Post-mortem blood levels of very few hormones have been studied as will be explained later on. Hydrogen ion concentration (pH) in blood, carbon dioxide content of the blood and vitreous humour have been also examined by many investigators [260,271,479]. Studies of post-mortem changes in chemical constituents of body tissues have been very few in number [341,534]. Several reviews of the biochemical methods of estimating the time of death have been published [104,106,108,158,332,368,387,400].

In general, there are great discrepancies between results obtained from different authors and wide variations between people and studies under different conditions. It is now well recognised that there are many factors which contribute to the

limitations of biochemical methods of estimating the time of death. These factors will be discussed later. It is sufficient to emphasize here the fact that the velocity under which these biochemical changes occur after death is greatly influenced by the cause and the manner of death, length of agony, ante- and post-mortem enzyme activities, ante-mortem state of health, post-mortem microbial activities and most importantly of all, the intrinsic and the extrinsic temperatures and other physical conditions of the body and environment at death and post-mortem [72,108,288,316, 400,438]. It is therefore now a common idea amongst pathologists and other forensic practitioners that biochemical methods of estimating the time of death are both cumbersome and inaccurate [78,326,332,387,400].

The work on post-mortem levels of hormones is just beginning [237]. Done et al. have studied 17-hydroxy-corticosteroids on blood samples obtained from bodies after death and Murray has studied cortisol binding to plasma proteins in man in health, under stress and at death. However, neither of these studies were directed towards time estimation after death [127,363]. Finlayson has studied blood cortisol both pre- and post-mortem in 15 infants and 20 adults [167]. He found that post-mortem cortisol concentrations were the same as those during life and remained stable for at least 18 hours after death. He also found that cortisol levels for children were comparable to those

of normal adults and that femoral vein and right atrium blood gave similar results. It may be of value to mention that the original object of Finlayson's study was not related to the time of death problem, but was to evaluate the role of adrenal failure in unexpected infant death syndrome and other fatalities which might be related to this endocrinal gland. Coe has found that thyroxin values tend to fall after death, but the rate of fall is individual and erratic, while thyroid stimulating hormone is less variable and more stable for one to two days post-mortem [105,108]. Other hormones such as catecholamines, insulin, growth hormone and chorionic gonadotropin have been studied for purposes other than time estimates after death [108,309,310,314,480].

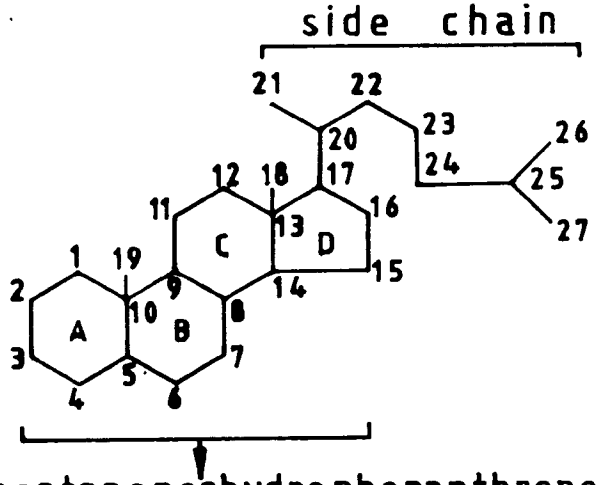
In this research we have studied some of the steroid hormones in biological samples of the rat after death in an initial attempt to find whether they can be used as indicators of the post-mortem interval. The reasons why steroid hormones were chosen for study will be discussed later.

5.2 Notes on Steroid Chemistry and Biochemistry

The parent compound of all C_{27} steroids is cholestane ($C_{27}H_{48}$) which consists of a cyclopentanoperhydrophenanthrene nucleus and a side-chain eight carbon atoms long, attached at C-17 in ring D [279]. This structure and the numbering

sequence, which is common to all steroids, is shown in Figure 5.1. The cyclohexane rings A, B and C are in the 'chair' form rather than in the 'bed' or 'boat' form, because the latter is less stable [195,279]. Valencies which are at right angles to the plane of the carbon rings are called axial valencies ('a' bonds) and the remainder which are approximately in the plane of the rings are called equatorial valencies ('e' bonds) [195,279]. It is conventional that the side chain and the angular methyl groups C-18 and C-19 define the upper side of the steroid plane; this is referred to as the ß-side of the molecule. The opposite is the lower side which is referred to as the α-side.

Rings A and B can be joined either in '<u>cis</u>' or '<u>trans</u>' forms. If the union is <u>cis</u>, both the angular methyl group (C-19) and the C-5 hydrogen atom, are on the same side of the molecule; this is therefore referred to as the 5ß-structure [195,279]. If the union is trans, the C-5(H) and the C-10(methyl) groups are on the opposite sides of the molecule and this is the 5α-structure [195,279]. This is illustrated in Figure 5.2. Obviously this isomerism cannot occur if there is unsaturation at the C-5 position or if the A ring is aromatic. Other isomers can occur such as the isomerism of the substituent group, according to the concept of the 'axial' and 'equatorial' bonds described above, and also isomerism in the side chain at C-17 [195,279]. When a steroid is represented in a



cyclopentanoperhydrophenanthrene ring system

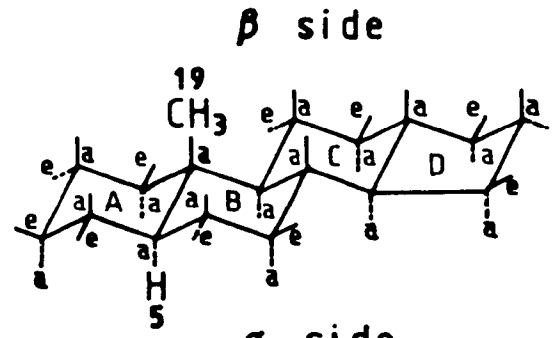
chair form boat form

Figure 5.1: Numbering sequence and structure of cholestane and forms of cyclohexane ring

e = equatorial

a = axial

trans A:B ring junction



cis A:B ring junction

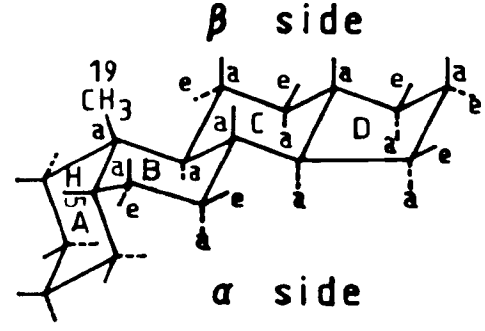


Figure 5.2: Cis-trans isomerism about A:B junction with positions of equatorial & axial valency bonds

two-dimensional way, it is conventional that a full line is employed to indicate a ß-group lying above the plane of the paper, and a broken line to indicate an α-group lying below that plane.

Carbon-carbon bonds at different sites of the cholestane molecule may be broken by desmolase (or lyase) enzymes to produce various other hydrocarbons by which all steroids can virtually be defined as shown in Figure 5.3 [279].

As there are a great number of naturally occurring and manufactured steroids, the use of trivial names is no longer possible and systematic names are now universally used [279]. Systematic rules for the nomenclature of steroids have been approved by many international bodies [195,261,262,279]. Accordingly, in a systematic name of a steroid there may be any number of prefixes but only one suffix. The choice of suffix is governed by the group concerned and the order, in decreasing preference, is acid, lactone, ester, aldehyde, ketone, alcohol, amine and ether [195].

The series of reactions involved in the conversion of cholesterol to corticosteroids, androgens and oestrogens are shown in Figure 5.4, which also shows the sites of production of each of these steroids in man. In general, many of the reactions involved require cytochrome P-450 [173,195-197], NADPH and molecular oxygen. Details of the biosynthesis of steroids are given by many authors [63-65,122,173,195-

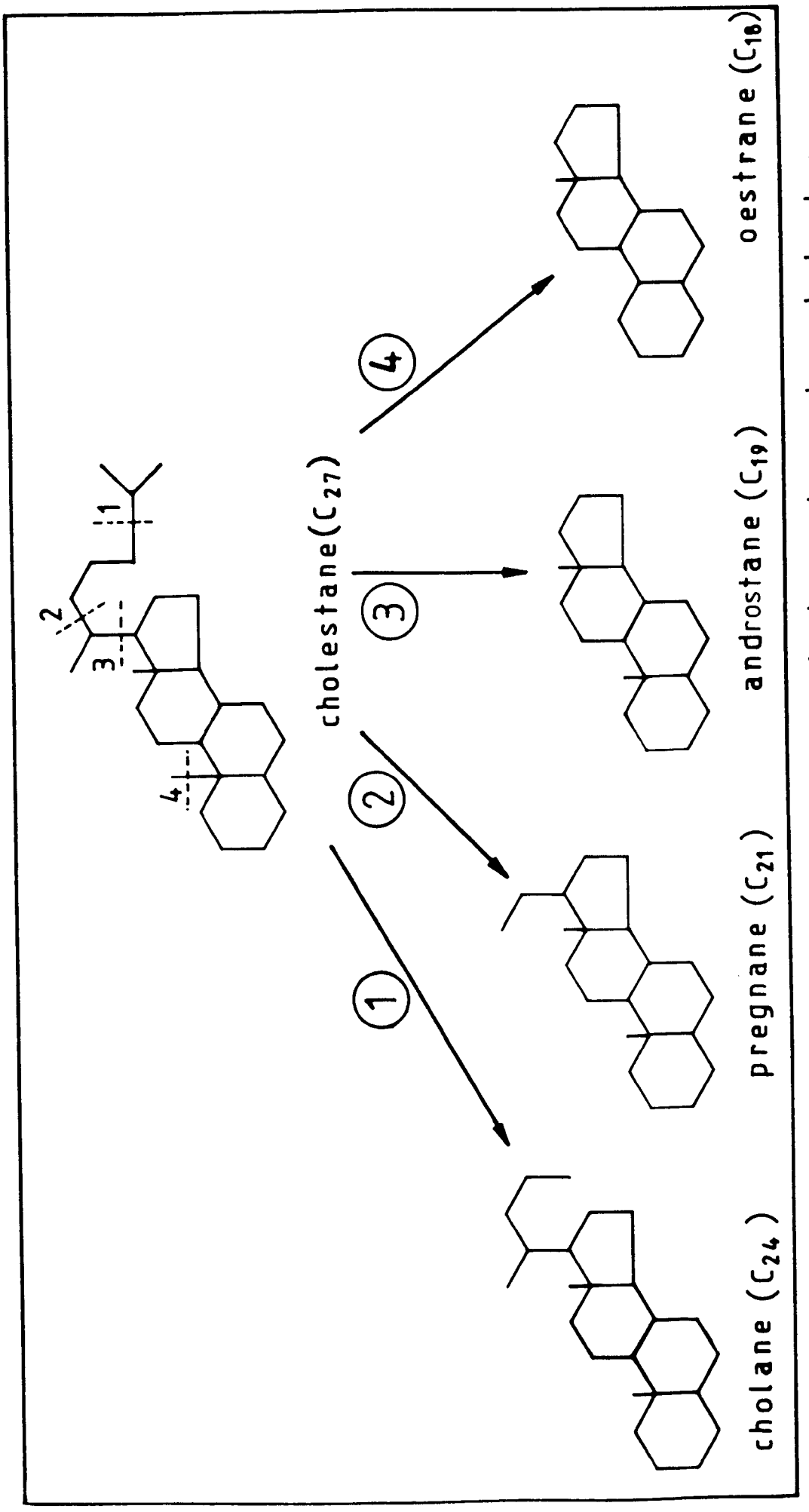


Figure 5.3 : Relationship of parent steroid hydrocarbons to cholestane

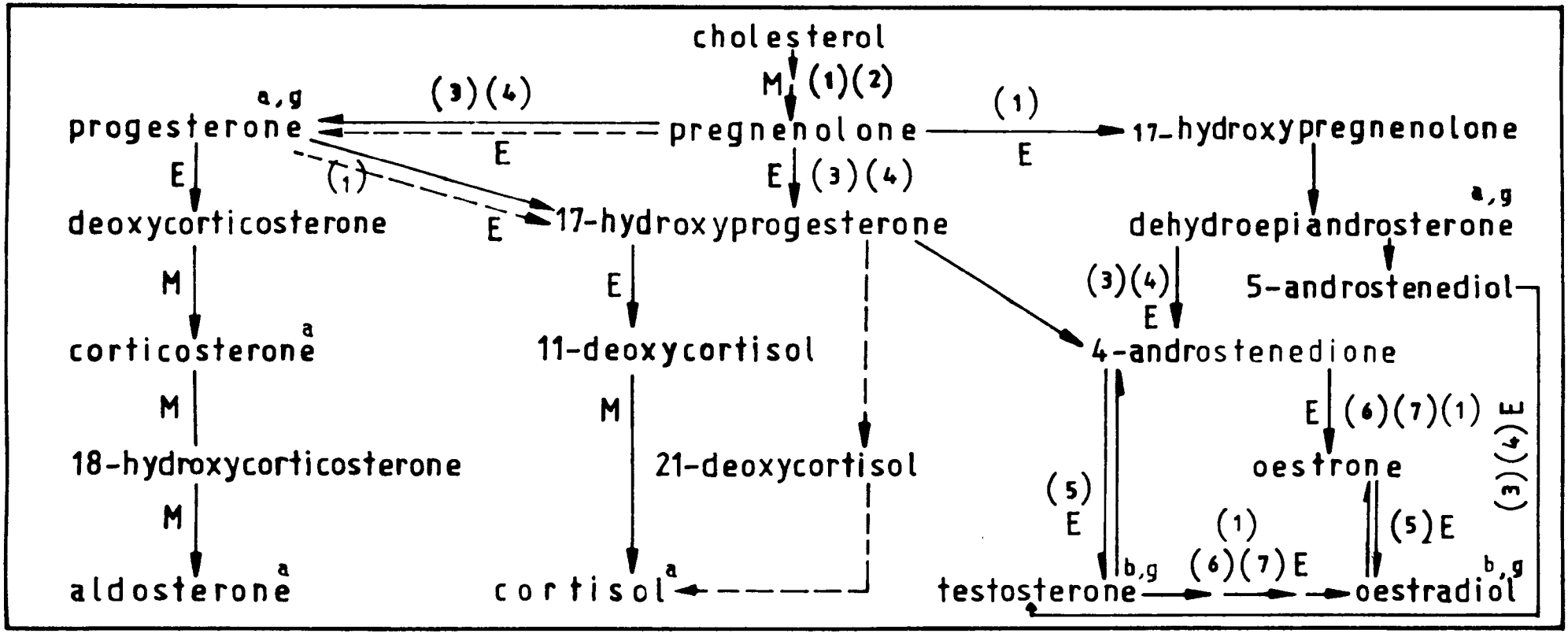


Figure 5.4 : Steroid Biosynthesis.

a	Adrenocortical hormones.	g	Gonadal hormones.	b	Minor adrenocortical hormones.
E	Endoplasmic Reticulum.	M	Mitochondria.	↣	Indicates more than one reaction.
---→	Indicates a minor pathway in the human adrenal cortex.			1	Hydroxylases.
2	Lyase	3	5-ene-3ß-hydroxysteroid dehydrogenase		
4	Isomerase	5	17ß-hydroxysteroid dehydrogenase		
6	19-hydroxysteroid dehydrogenase.	7	C-10,19 lyase.		

197,203,210,213,214,232,311,312,374,432,445,478,532]. Micro-organisms, particularly the intestinal micro-flora, are known to play an important role in the biosynthesis of some of the post-hepatic steroids [7,67,146,151-154,211,212,215,247,266,280,373]. Most steroid hormones present in plasma are bound to plasma protein: specifically to globulin and non-specifically to albumin [173,195-197,203]. Steroids are catabolised by the reduction of the double bond at C_4, the reduction of the ketones at C_3 and C_{20} to secondary alcohols, by the oxidation of the 17ß-hydroxyl group and by other oxidations at various points in the steroid nucleus [195]. Most steroids are excreted as glucoronide and sulphate conjugates [173,195-197,203].

5.3 A Summary of the Methods of Steroid Analysis

Steroids can be extracted from biological samples into either organic solvents or solids [194, 322,468]. Numerous solvents are used. However chloroform/methanol 1:1 v/v is most commonly used for extraction of a wide variety of lipids[468]. Examples of solid extractions are: the use of neutral resins (ie. Amberlite XAD-2) [28,29,70,71,162, 454,493]; Sephadex gel [142]; ion-exchangers (e.g. DEAE-Sephadex) [16,28-30,33,34,36,145,174,289,468,469, 472,493] and Sep-Pak C_{18} cartridges [16,29,30,450, 455,469,513,514,529]. Solid extraction has sometimes been used at 64°C by the use of jacketed columns and

a hot-water circulating bath to overcome the problem of protein binding to steroids in plasma [29,28,34,468]. Old methods of steroid analysis such as gravimetric, titrimetric, colorimetric and spectrophotometric methods have lost their popularity because their sensitivity and/or selectivity are usually poor [194,322]. Chromatographic techniques have been adequately used for the isolation, separation and fractionation of steroid mixtures into their constituents and for purification of a steroid from a complex biological sample [194,322].

All forms of chromatography have been used at one time or another for these purposes: partition, adsorption, ion-exchange and gel-filtration using columns, paper and thin-layer plates in liquid chromatography and packed and capillary columns in gas-liquid chromatography. If the mobile phase is less polar than the stationary phase the system will be referred to as a straight phase system whereas in a reversed phase system the mobile phase is more polar than the stationary phase. The ability of column-chromatography to resolve steroid mixtures is greater than that of thin-layer or paper chromatographic systems [322]. Column chromatography is considered an important step in complex analytical procedures for steroid hormones preceeding quantitation by spectrophotometry [80,194], colourimetry [201,503,504], fluorimetry [229], gas chromatography [27,32,290,381,

452-454,471,472,491], high-performance liquid chromatography [194,430], radio-immunoassay [55,194,203,216, 390], and by other radioactive [159], enzymatic [229] and gas chromatography-mass spectrometric methods [15, 26,28,31,33-35,38,39,267,451,468,470,476,493]. Several adsorbents have been used with liquid-solid (adsorption) chromatography [16,28,29,30,33,34,36,142, 145,174,289,468,469,472,493]. Neutral resins as Amberlite XAD-2 [16,29,30,450, 455,469,513,514] and ion-exchangers as Amberlyst A-26 [381,454] have been used. The most important development in column chromatography has been the synthesis of different dextran gels to be used in liquid-gel permeation techniques (GPC) [14,194). Examples of these gels are Sephadex G-25 [14,15,35, 145], Sephadex LH-20 [38,39, 267,381,448,451-453,471], Lipidex-5000 (hydroxy-alkoxy derivative of Sephadex LH-20) [28,31,32,290,493], Sulphoethyl-LH-20 [446], triethylamino-Sephadex LH-20 (TEAP) [27,30,34] and the diethyl-aminoethyl derivative (DEAE-Sephadex) [33,174,447,491]. The combined use of neutral and ion-exchange Sepahadex columns has also been demonstrated [174].

Gas liquid chromatography (GLC) is a partition system in which the solute is in partition between a gaseous mobile phase (usually an inert gas) and a liquid stationary phase, normally thermostable polysiloxanes like SE30 or QF1, a polyester (such as NGS or Hieff HBP) or others [194,202,322]. In GLC a

steroid is identified by its retention time which is the time taken for a steroid to emerge from the column (measured at the peak maximum), or by its relative retention time which is the retention time measured in relation to that of an internal standard. A steroid can also be identified by the steroid number, which is calculated by reference to the retention times of a number of saturated steroid hydrocarbons, and by the steroid index in which the methylene unit value is calculated in relation to the retention times of n-alkanes (ie. C_{16}-C_{34}).

A detection device is used to monitor the steroids in the GLC carrier gas emerging from the end of the column. Many detectors are used such as the flame-ionization detector (FID), the electron-capture detector (ECD) and the nitrogen detector. A 'splitting device' may be used to divide the effluent gas: some to pass to the detector and the remainder to be collected. Alternatively the column eluent may be subjected to mass spectrometry and combined gas chromatography-mass spectrometry (GC-MS) is obtained. GC-MS is considered to be the most powerful and versatile method of steroid analysis. Mass spectrometry and combined GC-MS are discussed in many published works and text books [6,77,81-83,194,203,322,468,506,533]. Gas chromatography with or without mass spectrometry has been widely used for steroid analysis in urine [16,27,30,142,162,174,255,268,290,

381,421,433,446,447,450,452-455], blood and plasma [15, 28,29,33-35,37,254,318,320,448,472,508], faeces [20,64, 67,151-153,211,212,215,266,280,290,515] and tissues [26,31,32,65,210,213,214,232,311,312,445,468,491,493].

In this way either steroid profiles or individual steroids are determined. Mass fragmentography, also known as selected-ion detection or monitoring (SID or SIM), is increasingly used as a highly specific and sensitive means for quantitative mass spectrometry of steroids in biological samples [5,182,194,322,344,394]. Many methods have been published for cell cultures, urine and plasma.

In most cases, steroids of biological origin are derivatized before gas chromatographic analysis. There are many options of derivatization methods and types. However, the most frequently employed derivatization reactions are the formation of trimethylsilyl (TMSi) ethers on hydroxyl groups and O-methyl oximes on the carbonyl groups (MO-TMSi) [194,283,322]. The purposes of derivatization are to increase the volatility and thermal stability of steroids, improve their separation on the column and to permit the use of sensitive detectors. The greatest chromatographic efficiency can be achieved with capillary columns which can be wall-coated (WCOT) or support-coated (SCOT) open tubular or micro-packed (MP) columns. WCOT capillary columns are considered to have the best efficiency and therefore are the most widely used. There are many injectors for

the application of the sample onto the column: split injector, splitless injector, cold on-column injector and solid injector. The latter is considered to be the most useful and to result in the good accuracy during quantitative analyses.

CHAPTER 6: EXPERIMENTAL

6.1 Materials

6.1.1 Reagents

Solvents were of either HPLC or glass-distilled grade. Pyridine (Analar grade, BDH, Poole, England, U.K.) and hexamethyldisilazane (HMDS, Applied Science Laboratories, State College PA, or Pierce Chemical Company, Rockford, IL, USA) were both refluxed over calcium hydride (Sigma Chemical Company, St. Louis, USA) and anti-bumping granules for 3-4 hours and were then redistilled before use. Small quantities were processed in this way every few months. Trimethylchlorosilane (TMCS, Sigma Chemical Company) and trimethylsylilimidazole (TSIM, Pierce Chemical Company) were used as supplied. The latter was obtained in 1 ml glass ampoule. Methoxyamine hydrochloride was from Eastman Organic Chemicals, (Rochester, N.Y.) or from Pierce Chemical Company. 2,2-Dimethoxypropane (BDH Chemical Ltd., Poole, England, U.K.) was redistilled before use. Helix Pomatia juice, in one millilitre ampoule containing 100,000 FU of ß-glucuronidase and 1,000,000 RU of sulphatase was from Reactifs-IBF (Villeneuve-La-Gorenne, France). Amberlite XAD-2 was from BDH and Amberlyst A-26 was from Rohn and Hass (Philadelphia, PA). Hydroxyalkoxypropyl Sephadex was obtained as Lipidex-5000 from Packard Instrument Company (Downers Grove, IL, USA). Sep-Pak C_{18}

cartridges and packing material were obtained from Waters Associates Inc. (Milford M.A,. USA).

6.1.2 Standard Steroids

(a) Unlabelled steroids: These were either kindly supplied by Professor D.N. Kirk (Steroid Reference Collection, Westfield College, London, U.K.) or obtained from Sigma Chemical Company or BDH Limited Poole, England, U.K.

(b) Radioactive Steroids: Tritium-labelled (7-^{3}H(N1)-dehydroepiandrosterone ammonium sulphate, 35.0 Curies per millimole, (6,7-^{3}H(N))-estradiol 17ß-D-glucuronide, 50.0 Curies per millimole and (1,2,6,7-^{3}H(N))-corticosterone, 105.0 Curies per millimole were obtained from New England Nuclear (NEN, Boston, MA, USA). Radioactivity measurements were obtained using a Philips Scintillation Spectrometer. Scintillator 299 (Packard Instrument Company) was used as the scintillation liquid. In some of the earlier experiments the Packard Tri-Carb Liquid Scintillation Spectrometer 2000 was used. Quenching correction was made with tissue samples in separate experiments.

6.1.3 Glassware

Prior to use, all glassware was rinsed three times with hot water and once with distilled water, ethanol and acetone, and was dried in air at room temperature or in an oven. Cleansing was carried out in an ultrasonic bath. Glass columns for the adsorption and liquid-gel chromatography steps, were

specially constructed with the co-operation of the glass-blowing workshops at the Chemistry and Natural Philosophy Departments of the University of Glasgow (Figure 6.1).

6.1.4 Preparation of Chromatography Materials

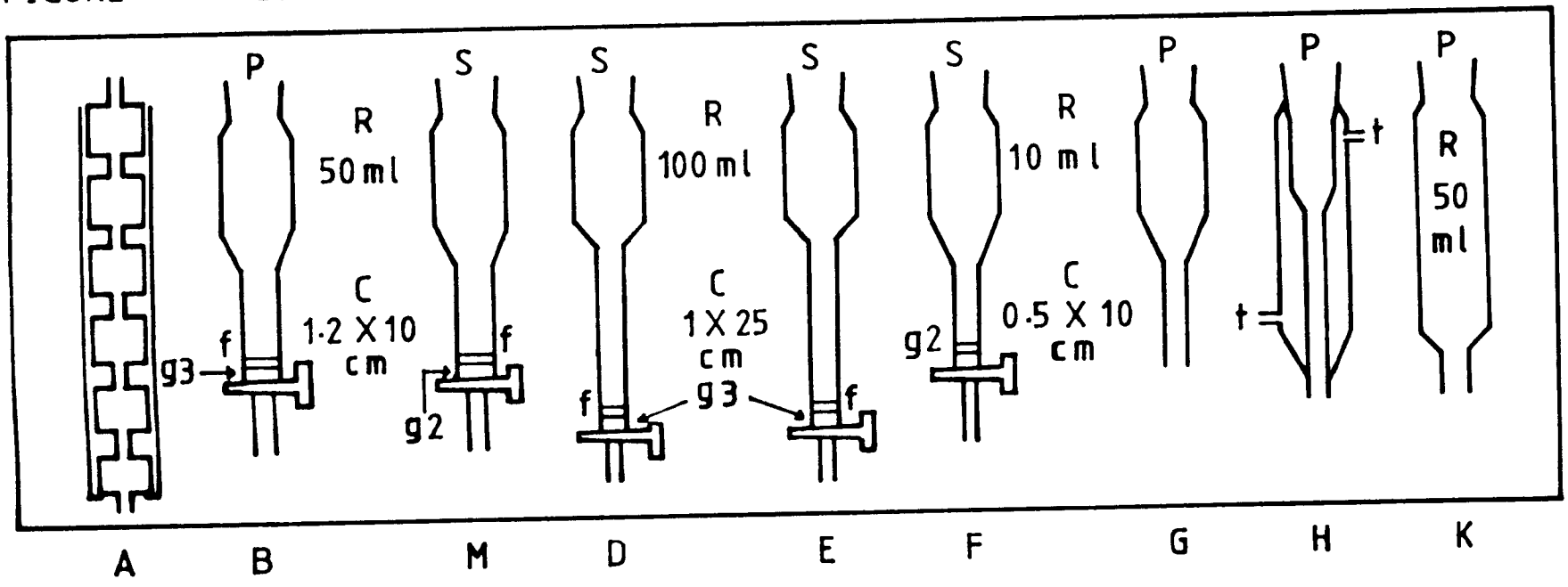
(a) Lipidex-5000 for purification step

New Lipidex-5000 was washed with 10 volumes of the eluant solvent mixture used in the reversed phase system (Paragraph 6.2.4 (a)). Old Lipidex, which had already been used, was extensively washed with at least 5 volumes of the following solvents, in a Buchner funnel under water pump vacuum: methanol/chloroform (1:1 v/v), ethyl acetate, acetone, further methanol/chloroform and lastly 10 volumes of the reversed phase system, i.e. methanol/water/chloroform (90:10:20 v/v/v). In both cases, the Sephadex was allowed to swell in the system for at least 3 hours or, alternatively and preferably, it was stored at $4^{o}C$ suspended in the system in a dark bottle.

(b) Amberlyst A-26

The anion exchange resin was washed with 5 volumes of ethanol, hexane, ethanol again, distilled water, hydrochloric acid (4M) and distilled water until neutral. It was then converted into bicarbonate form with 10 volumes aqueous sodium bicarbonate (8% w/v), washed until neutral and stored in a dark bottle at $4^{o}C$.

FIGURE 6·1 : GLASS COLUMNS USED IN STEROID EXTRACTION & PURIFICATION



C = column
f = filter paper
g = glass sinter grade 2 or 3
P = pressure
R = reservoir size 10, 50 or 100 ml

S = stopper
t = circulatig water

(c) Lipidex-5000 for derivatization step

Only new Lipidex was used for this purpose. It was washed with 10 volumes of hexane and hexane/pyridine/hexamethyldisilazane/dimethoxypropane (97:1:2:10 v/v/v/v) mixture and stored suspended in the mixture in a dark bottle at 4°C.

(d) Sephadex LH-20

This material, whether used or unused, was washed with at least 5-10 volumes of each of cyclohexane/ethanol (4:1 v/v), ethanol, methanol, chloroform/methanol (1:1 v/v), ethyl acetate, acetone, ethanol and cyclohexane/ethanol (4:1 v/v). The gel was then stored suspended in the last solvent in a dark bottle at 4°C.

(e) Sodium acetate buffer solution (pH 4.6): glacial acetic acid (Analar Grade, 5.7 ml) was diluted with distilled water (200 ml). Sodium acetate (anhydrous, Analar Grade, 16.4 g) was dissolved in distilled water (400 ml). Thus 0.5M solutions of the acid and sodium acetate were obtained. 200 ml of the former were mixed with 300 ml of the latter and the pH was adjusted to 4.6 with concentrated hydrochloric acid (Analar Grade).

6.1.5 Biological Material

For the prelimary and radioactive experiments, human materials were employed. Blood, faecal, liver and urinary samples were collected from cadavers dissected at the City Mortuary of Glasgow. Only bodies that died due to natural causes, particularly heart

diseases, were used. In the animal study rat samples were used as will be described later (Paragraph 6.4). Specimens, whether human or animal, were immediately cooled after collection and stored at -20°C until required for analysis. 10 ml blood and 2-3 g liver and faeces were used for each analysis.

6.2 Assessment of Analytical Procedures

More than thirty experiments were carried out to assess the usefulness and practicability of some existing analytical procedures published by other investigators for the extraction and purification of steroids in different biological samples. These are described in the following paragraphs.

6.2.1 Homogenization of Biological Samples

For the purpose of homogenization of the blood, faeces and tissues, organic solvents, sodium acetate buffer and TE-buffer solution (Tris hydrochloride-EDTA, 0.01M, pH 7.9 at 0°C) were evaluated as homogenizing media. Organic solvents used for homogenization were either methanol/chloroform (1:1 v/v) or ethanol/acetone (1:1 v/v). The method used for preparing the TE-Buffer was described in Reference 26. Three sets of human samples comprising blood (10 ml), liver tissue (3 g) and faeces (2 g) were prepared. Samples of one set were homogenized separately with one of the above homogenizing media using a teflon-glass grinder for a period of 10 minutes. When the TE-buffer or the

organic solvents were used, tissue and blood samples were fixed and homogenization was difficult. This was overcome by the use of a stainless steel bladed homogenizer (MSE Scintific Instrument, Manor Royal Crawely, Sussex, U.K.). Steroids in the aqueous homogenates (in sodium acetate and TE-buffers) were extracted by organic solvents. Steroid extracts of all samples were purified, fractionated, derivatized and analysed by GC-MS as usual (See analytical procedures below). Evaluation of the usefulness of the above homogenizing media was made depending on the results of the GC-MS analysis.

6.2.2 Solvent Extraction (Purification)

The classical method of steroid purification was initially attempted in six experiments. In these experiments human specimens, i.e. liver, blood and faeces were homogenized as above and the extracts were taken to dryness under water pump vacuum. The residues were dissolved in sodium acetate buffer (pH 4.6, 50 ml) under ultrasonic agitation. One ampoule of Helix Pomatia juice and a quantity of the radio-labelled steroids were added to each sample and the mixtures were incubated at 37°C for 36-48 hours. Aliquots were taken for radioactivity determination and each sample was then put in a separating funnel and methylene chloride (20 ml) was added to it and the mixture was shaken gently and continuously for a few minutes. The flask was left to stand for 30 minutes so

that the aqueous and the organic phases were separated. The aqueous phase (i.e. the upper portion in the flask) was trasfered to another separating flask and rewashed with another portion of methylene chloride (20 ml). The upper phase was transferred to a third separating flask and washed twice with ethyl acetate (20 ml). The bottom portions in the three flasks were collected together. The pooled extracts were purified by washing in separating funnels with sodium hydroxide (0.1 M, 3 X 20 ml) and distilled water (3 X 20 ml). After standing for 30 minutes, the bottom phases were collected and the pool was dried over magnesium sulphate and was taken to dryness under water pump vacuum.

The residue was dissolved in 50 ml acidic ethyl acetate (100 ml ethyl acetate were mixed with 10 ml sulphuric acid (2M) and allowed to separate for 2 hours: the ethyl acetate portion was used). An aliquot was taken for radioactivity measurements. The mixture was incubated at 39°C for 16 hours to complete the slow acidic solvolysis and another aliquot was taken for radioactivity determination. The sample was then washed with 6 ml portions of an aqueous solution of sodium bicarbonate (8.4% w/v) until alkaline (pH was tested by litmus paper). The top phases were collected and washed with distilled water until neutral. The sample was dried over magnesium sulphate and was taken to dryness under <u>vacuo</u>. Other

steps of fractionation by Sephadex LH-20 and Silicic acid columns were carried out as described below. GC-MS analysis was carried out (see below) to evaluate the recovery of steroids. The disadvantages of the above procedure were the occurence of emulsions when the aqueous steroid solution was washed with the organic solvents (methylene chloride and ethyl acetate). These caused losses of steroids, therefore results of GC-MS were not encouraging. Also the procedure was tedious and time consuming. Accordingly this method of purification was not used in all subsequent experiments.

6.2.3 Solid-Phase Extraction

(a) Amberlite XAD-2 and Amberlyst A-26 Columns: In 5 experiments the solid extraction and purification of steroids by Amberlite XAD-2 and Amberlyst-A26 were performed. The method used was described in References 70, 71 and 454. Five grammes of Amberlite XAD-2 were washed prior to use with 5 volumes of methanol, acetone and distilled water. The resin was packed into a column (200 x 10 mm i.d., see Figure 6.1 D). After enzymatic hydrolysis for 36-48 hours (see above 6.2.2), the sodium acetate buffer solution was allowed to pass through the resin column. The flow rate was 0.2 ml per minute. The resin was then washed with distilled water (20 ml) and the steroids adsorbed by the resin were eluted with ethanol (50 ml).

The effluent from the Amberlite XAD-2 column was

passed through a column of 0.5-1 g Amberlyst A-26 (Figure 6.1 M, prepared as described in 6.1.4). This column was then further washed with ethanol (30 ml). All effluent was collected in a 100 ml flask and taken to dryness. The sample was derivatized as usual (described below) and analysed by GC-MS. Steroid recoveries by this procedure were better than those resulting from the procedures depending on the solvent purification described above. However, the flow rate through the Amberlite XAD-2 was very slow and therefore the procedure was not practical. Also, this column was easily blocked particularly when blood or tissue was analysed. To overcome this problem a special jacketed column was made (Figure 6.1 H), so that the Amberlite XAD-2 column was surrounded by circulating hot water and the elution was carried out under circulating hot water (at 64 °C).

(b) Sep-Pak C_{18} and Amberlyst A-26: Sep-Pak C_{18} cartridges were used instead of the Amberlite XAD-2 columns in all subsequent experiments as will be described later (Paragraph 6.4). In some experiments the sample was warmed by a circulating hot water jacket (64 °C, Figure 6.1 A and H). A single Sep-Pak C_{18} cartridge and a chain of four, five and six cartridges were assessed. This was performed by adding a known quantity of one of the radio-labelled steroids to the hydrolysed mixture and allowing this to pass through different numbers of the cartridges. Radioactivities

of the effluent, the sediment and the water phase were then measured. Thus, recoveries and losses of steroid with different numbers of the cartridges were assessed. In a few initial experiments, the Sep-Pak C_{18} cartridge step was followed by the Amberlyst A-26 column as above. This procedure was easier in practice, but results of the GC-MS analysis showed that the extracts obtained contained some impurities and therefore another step should be employed which was the straight phase chromatography described below.

6.2.4 Fractionation of Extracts

Several methods of fractionation and separation of steroids were tried. Initially, fractionation was carried out by liquid-gel permeation chromatography (GPC) over a column of Sephadex LH-20 packed in methanol/chloroform (1:1 v/v) and separation of steroids from cholesterol was performed over silicic acid columns. These procedures are well described by Novotny et al.[381]. In subsequent work, straight phase chromatography was used for fractionation, as described by Shackleton et al. [451,452]. Non-polar lipids were removed by the procedure developed by Axelson et al. using reversed phase chromatography [31]. The efficiencies of three reversed phase systems for steroid recoveries were compared: methanol/heptane (95:5 v/v), methanol/water/heptane (95:10:5, v/v/v) and methanol/water/chloroform (90:10:20 v/v/v).

The volume of solvent required for the elution

of steroids from silicic acid columns and reversed and straight phase gel columns were established using thin layer chromatography (TLC) as the detection technique. In each case, steroid standards, chosen to represent a wide range of polarity, were used. Examples of these steroids are given in Table 7.7. After a quantity of each steroid standard (1 mg) was applied to the top of the column in question, 5 ml fractions of the eluant were collected, concentrated by evaporation in a stream of nitrogen to 10-20 microlitres each and applied to a silica gel TLC plate.

As a result of all these experiments, a multi-component analytical method for the extraction, purification, separation and the fractionation of steroids in biological samples was established as described below (6.4).

6.3. Assessment of Extraction Efficiency

The tritum-labelled standard steroids used in this work have been mentioned earlier. Using these radioactive steroids, several experiments were conducted to assess the effiecency of the method used to extract and purify steroids.

6.3.1 Quenching Correction

Twenty three experiments were performed to establish the quenching correction required for the calculation of the efficiency of radio-activity measurements obtained by the Tri-Carb Packard 2000

Scintillation Spectrometer. For blood, faeces, tissue and urine and each step of the analytical method, a quenching experiment was performed as follows:

To 40 ml of the scintillation liquid, 0.5 μCi of a radio-labelled steroid was added and well mixed. The pool was divided into 10 vials each containing 4 ml of the unquenched liquid and having an activity of 111,000 disintegrations per minute (dpm). Radioactivity of these vials was measured. One vial was left unquenched and the other nine vials were quenched with 20,40,60,80,100, 250,500,750 and 1000 l of the sample obtained from the step in question. The radioactivity of each of the ten vials was measured again. Efficiency was calculated by the following formula:-

$$\text{Efficiency percentage} = \frac{Q}{A} \times 100 \quad \text{.....Equation 6.1}$$

where Q is the radioactivity reading of the quenched sample obtained from the machine, and,

A is the actual radioactivity added to the sample which is equal, in this case, to 111,000 dpm.

Efficiencies obtained were plotted versus Automatic External Standard (A.E.S.) ratios and thus the quenching correction graphs were obtained (Figure 7.2).

6.3.2 Assessment of Steroid Recoveries

Twenty five experiments were carried out to assess steroid recoveries of the established analytical method step by step. Thus recoveries of free and

conjugated steroids (glucuronide and sulphate) were evaluated for samples representing blood, faeces and tissue. In each experiment of these, a known quantity (usually 111,000 dpm) of one of the radio-lablled steroids (6.1.2 (a)) was added during the homogenization step. An aliquot was taken for the radioactivity dtermination at the end of each step of the analytical method. Recoveries of steroids were calculated according to Equation 7.1.

6.3.3 Assessment of Elution Volume

A few experiments were conducted to establish and evaluate the volume of solvent required for the elution of steroids from the reversed phase Lipidex-5000 column and Sep-Pak C_{18} cartridge. For the former, the three radio-labelled steroids were separately applied to the tops of three Lipidex-5000 columns, prepared and eluted as described below (Paragraph 6.4). Ten fractions (10 ml each) were collected from each column and the radioactivity of every fraction was measured. Plots of radioactivity versus volume of the eluting solvent were drawn (Figures 7.3-7.5). A similar procedure was conducted regarding the use of a single or multiple Sep-Pak cartridge as explained later (Paragraph 6.4).

6.4 Animal Study

6.4.1 Sample Collection

In this study, thirty male and female rats were

used. Each rat, of about 50 gm weight and 1-3 months of age, was rapidly killed either by cervical dislocation or by asphyxiation in a nitrogen atmosphere. After dissection, the following quantities of animal specimens were utilized: whole liver from each rat (about 9.3±2 g), 5 pairs of adrenal glands (all male or all female rats) were pooled together and used as one sample, and blood or faeces from two rats were also pooled and used as a single sample. This constituted 3±1.6 ml blood and 1.66±0.64 g faeces respectively.

The rats were divided into two groups: in the first group, samples were collected immediately after killing the animals. Therefore, steroid levels of tissues at the moment of death could be obtained to be used for comparison with levels at subsequent periods. The rats of the second group were similarly killed but left at room temperature (about 17-18^{o}C) for 24 hours. They were then dissected and samples were collected as described above.

6.4.2 Extraction of Steroids

An amount of the biological material, as specified above, was mixed with 75 ml of chloroform /methanol (1:1 v/v). For steroid recovery assessment one μCi of the tritium-labelled steroids was added. The mixture was homogenized using the stainless steel bladed homogenizer for 10-15 minutes. The homogenizer was washed with 10 ml of the same solvent. The

collected slurry and the wash were kept in an ultrasonic bath for 30 minutes. The extract was filtered into a round-bottomed flask in a Buchner funnel under water pump vacuum, using double filter papers or two fibre glass filter papers. A further 15 ml of the same solvent were used to wash the tissue container under ultrasonic agitation and also the Buchner funnel. The total volume of the combined extract was measured and an aliquot was taken for radioactivity determination. The extract was then taken to dryness in a rotary evaporator under vacuum and the residue weighed.

6.4.3 Purification of extract

A glass column (10 mm x 250 mm) with a 100 ml capacity reservoir was used (Figure 6.1 E). About 15 g of the prepared Lipidex-5000 were slowly packed under gravity to make a bed volume of 10 x 230 mm. After packing and immediately before use, the column was washed with 5 volumes of the mobile phase, namely, methanol/water/chloroform (90:10:20 v/v/v) to remove possible contaminants. Other systems: methanol /heptane (95:5 v/v) and methanol/water/heptane (95:10:5 v/v/v) were also evaluated. For this purpose, nine experiments were performed. Therefore, the ability of the above solvent systems to elute and recover the three labelled steroids was assessed. The procedure used was similar to that described in Paragraph 6.3.3. The sample residue was dissolved, under extensive

ultrasonic agitation in at least 5 x 1 ml of the mobile phase and applied gently to the top of the column. Alternatively, it was dissolved in 2 x 1 ml chloroform/ methanol (1:1 v/v) and then 5 x 1 ml mobile phase. The column was eluted with 60-80 ml of the mobile phase according to the system used. The flow rate was about 0.5 ml per minute. All the effluent was collected in a round-bottomed flask. Most of the steroids were eluted in the first 20 ml of the effluent or so. An aliquot was taken for radioactivity determination then the solvents were removed under vacuo and the residue was weighed.

6.4.4 Enzymatic Hydrolysis

The residue was dissovled in acetate buffer (0.1M, pH 4.6, 10 ml) with ultrasonic agitation. Helix Pomatia juice (0.3 ml) and a further 10 ml of the buffer solution were added and the mixture was resonicated. An aliquot was taken for radioactivity determination. Steroids were either rapidly hydrolysed by the incubation of the sample for 3-4 hours at 53°C or slowly at 37°C for 48 hours. After the hydrolysis was accomplished an aliquot was also taken for the radioactivity measurement.

6.4.5 Sep-Pak C_{18} Cartridges

Sep-Pak C_{18} packing material equivalent to 4-6 cartridges was used, namely 1-1.5 g weight of the dried material, was used. A special glass column was made for this purpose (Figure 6.1 B) in which the packing

material was poured dry and the top was evened by gentle shaking of the column. Alternatively, small narrow glass tubes were used to connect 4-6 cartridges to each other and the whole chain was inserted into a glass tube whose internal diameter was approximately the same as the external diameter of the catridge. In this way, the cartridge chain was fixed in place during use. The chain or the Sep-Pak C_{18} column was conditioned by washing with methanol (5-10 ml) and distilled water (5-10 ml). The buffer solution containing the liberated steroids (20 ml) was applied to the column through the reservoir of the column or to the cartridge chain by using a funnel-shaped tube specially made for this purpose (Figure 6.1 K). The solution was forced to pass through the Sep-Pak C_{18} material by the application of pressure from a nitrogen cylinder (about 0.5 p.s.i was required). The flow rate was kept at about 1-2 ml per minute. After this step, the column or the chain was washed with distilled water (5-10 ml). Steroids were eluted with methanol (10-15 ml). Aliquots were taken from the effluent, water phase and the sediment for radioactivity determinations.

6.4.6 Acidic Solvolysis

The effluent from the last step was taken to dryness as usual. The residue was weighed and dissolved in 5 ml tetrahydrofuran. Aqueous sulphuric acid (4M, 5-10 μl) was added and the solution was sonicated. After incubation for 1-2 hours at 53^{o}C,

methanol (1 ml) was added with ultrasonic agitation. An aliquot was taken for radioactivity determination.

6.4.7 Anion Exchange Chromatography

Prepared Amberlyst A-26 (500-600 mg) were packed into a glass column (100 x 7 mm i.d.) with a 50 ml capacity reservoir. The resin was then washed with methanol (50 ml) and tetrahydrofuran/methanol (5:1 v/v, 15 ml). The sample was applied carefully to the column and allowed to pass through. The column was eluted with methanol (8 ml). An aliquot was taken for radioactivity measurement. The residue was taken to dryness under vacuum and weighed.

6.4.8 Straight Phase Chromatography

This step was required for purification and fractionation of the steroids as only a certain group of steroids was required. Prepared Sephadex LH-20 (14 g) was poured into a glass column (250 x 10 mm i.d., Figure 6.1 E) which had a 100 ml capacity reservoir. The gel was allowed to sediment under the effect of gravity. Thus a column of bed volume 10 x 100 mm was used. The residue from anion exchange chromatography was dissolved in ethanol (o.5 ml) with ultrasonic agitation. Cyclohexane (2 ml) was added and sonication was repeated. The sample was applied to the top of the column. This process was repeated twice. After the sample had passed through the column the latter was further eluted with further cyclohexane/ethanol (4:1 v/v, 24 ml). The first 12-15 ml of the effluent

was discarded and the second 16 ml were collected. This portion contained the steroids required. If all or most steroids were required the column was eluted with 150-160 ml eluant of which the first 15 ml were discarded and the remainder was collected. The eluant was taken to dryness under vacuum and the residue was redissolved in ethanol (5-10 ml) and stored in labelled vials at -20° until required for derivatization and GC-MS.

6.5. Derivatization

6.5.1 Trimethylsilyl Ether (TMSi) Derivatives

These were used in all animal study analyses. The derivatization reagent consisted of dry redistilled pyridine, hexamethyldisilazane and trimethylchlorosilane (3:2:1 v/v/v). Before use, the reagent was centrifuged or filtered over a filter paper and stored in a dark bottle. The steroid sample was taken to dryness in a stream of nitrogen. The derivatization reagent (0.33-0.5 ml) was added and mixed well. The mixture was kept at 60° for about 10-15 minutes. The solvent was evaporated under nitrogen and the residue was dissolved in hexane (20-50 µl) to be used for GC-MS.

6.5.2 O-Methyloxime-Trimethylsilyl Ether (MO-TMSi)

Methoxymine hydrochloride (5 mg) and pyridine (50 µl, prepared as above) were added to the steroid residue. The mixture was warmed in a heating block at 60°C for 30-60 minutes. Then, trimethylsilimidazole

(TSIM, 50 µl) was added. The mixture was heated in an oven at 100°C for 2.5-3 hours.

Lipidex-5000 prepared as described in 6.1.4 above, was poured into a small column of about 4 mm internal diameter and 80 mm length which had a 10 ml capacity reservoir (Figure 6.1 F). Thus a bed volume of 70 x 4 mm was used. The column was washed with 10 ml of the equilibrium solvent system (i.e. hexane/pyridine/hexamethyl disilazane/dimethoxy-propane (97:1:2:10 v/v/v/v)). The steroid sample was diluted with 1 ml of the equilibrium system (above) and rapidly filtered through the column. The flow rate was about 3 ml/minute using a nitrogen pressure aid. This was repeated 3 times. The eluent was collected and used for GC-MS.

6.6 Gas Chromatography-Mass Spectrometry (GC-MS)

6.6.1 Instrumentation

GC-MS was carried out using a Perkin Elmer Sigma 3B gas chromatograph interfaced to a VG 16F single focussing magnetic sector mass spectrometer. The chromatograph was fitted with a Groß split/splitless injector and a 25 m x 0.3 mm i.d. glass capillary column coated with BP1 (Chrompack, Netherlands), a non-polar methyl silicone stationary phase similar to SE-30 and OV-1. The carrier gas was helium at a flow rate of 2 ml/min. Samples were injected into the flash heater zone at 300°C and, after 1 minute, the

splitter valve on the injector was opened to purge the inlet system and reduce tailing of the GC peaks arising from dead-volume effects. The column temperature was initially held at 240°C for 2 minutes then programmed at 3° per minute to 300°C. The final temperature was held for 10 minutes (Table 6.1).

The mass spectrometer was operated in the electron impact ionisation mode with an electron energy of 70 eV and a source temperature of 240°C. The effluent from the gas chromatograph was introduced directly to the mass spectrometer source using an interface temperature of 250°C. The spectrometer was used in two data acquisition modes - repetitive scanning, for recording full mass spectra of standards, and selected ion monitoring, for quantitative measurements. In both cases, data was acquired with an on-line dedicated data system, type 2050 from VG Analytical, and stored on demountable hard discs for processing. All computer programmes used for data manipulation were supplied by VG Analytical and were operated using the standard system protocols.

Repetitive scanning was carried out over the mass range 750 - 20 using an exponential down scan rate of 1.5 seconds per decade and an inter-scan delay of 1.5 seconds, total cycle time 3.5 seconds. Spectra of standards were acquired using samples of approximately 100ng and were plotted following background subtraction.

Table 6.1: Temperature programme used for GC-MS analysis.

Initial temperature	=240 °C
Ramp rate (rate of temperature rise)	= 3 °C
Final temperature	= 300 °C
Final time	= 10 minutes

6.6.2 Selective Ion Monitoring (SIM)

This was carried out by voltage switching with a fixed magnet current. Up to 10 ions could be monitored at one time, although only 2 were normally used routinely. These were m/z 117 and 129. Data were acquired using the Foreground/ Background Selected Ion Recording (FBSIR) system with a normal dwell time on each channel of 100 milliseconds and interchannel delay of 20 milliseconds. The ions used for monitoring were selected by examination of the mass spectra of standards recorded under similar operating conditions: where possible, the base peaks of the steroids were used to obtain maximum sensitivity. Quantitative measurements were obtained based on the area of gas chromatographic peaks under selected ion chromatograms. These were measured manually using the FBSIR programme. The concentrations of steroids in the samples were calculated by comparing the areas of the peaks with those of standards run under similar conditions, preferably within a short time interval of the samples (Equation 7.3), with corrections for the sample volume injected using internal standards (ß-Sitosterol and Campesterol)

6.6.3 Identification of Steroids

Steroids were identified on the basis of their GC retention time and mass spectral characteristics by comparison with those standards. During repetitive scanning mass spectrometry, a steroid was considered to

be identified if its retention time and spectrum matched those of the corresponding standard. In SIM, steroids were identified by the presence of a chromatographic peak in selected ion chromatograms at the same retention time as that of the standard and also if the ratios of peak areas in two or more selected ion chromatograms were the same as for the standard. If the latter criterion could not be confirmed, then tentative identification was made on the basis of the former criterion only. Retention indices of standard steroids were calculated from several experiments in which 33 steroid standards were used (Table 7.12). These standards represented many major steroids which were most likely to be encountered during the animal study. Also used in these experiments, were two plant steroids; campesterol and ß-sitosterol, which were to be used as internal standards in subsequent experiments. These steroid standards were first divided into three groups, each group consisting of steroids which were not likely to have very similar retention times so that good separation was achieved during GC-MS. Steroids standards of each group (1 mg of each standard) were dissolved in methanol/chloroform (1:1 v/v, 3-5 ml) with ultrasonic agitation. The mixture was taken to dryness under nitrogen stream. The residue was derivatised to form MO-TMSi compounds as described above (Paragraph 6.5.2). A standard sample was

prepared containing even numbers of hydrocarbones (alkanes C_{16}-C_{34}, 0.1 mg each). The standard samples of the steroids and the hydrocarbons were analysed by the repetitive scanning mode of GC-MS under exactly the same conditions. Retention times for the hydrocarbons were plotted versus the number of their carbon atoms. Thus the retention indecies for the steroids were calculated according to Equation 7.2.

CHAPTER 7: RESULTS

7.1 Assessment of Extraction Methods

7.1.1 Primary Extraction of Steroids

Before steroids can be adequately extracted from a biological sample, the latter should be homogenized. For this purpose, some organic solvents and aqueous solutions were tested. A mixture of organic solvents such as chloroform/methanol (1:1 v/v) was found to be more useful for homogenization of tissues, faeces and blood samples than aqueous solutions such as sodium acetate or TE-buffers. This was because when the former organic mixture was used both homogenization and primary extraction of steroids were carried out simultaneously in one step. However, tissues and blood samples were more readily subjected to fixation and clot formation when the organic mixture was used. This difficulty was easily overcome using a powerful steel blade homogenizer rather than a Teflon-in-glass grinder.

Solid-phase extraction of steroids was also attempted in a few initial experiments. This method of steroid extraction required that aqueous homogenates of tissues, faeces and blood specimens were prepared and then passed through Amberlite XAD-2 columns or Sep-Pak C_{18} cartridges which were warmed with a hot water jacket. This method was found to be less practicable than extraction with organic solvents. For instance, the flow rate of the homogenate through the columns or the

cartridges was found to be very slow. Also the columns or the cartridges were rapidly overloaded and easily blocked with protein aggregations. Arrangements for the circulating hot water bath and jacketed columns were complex and difficult to maintain. Accordingly extraction into organic solvents was used in all subseqent experiments.

7.1.2 Choice of Purification Procedures

Ordinarily, biological samples contain considerable quantities of non-polar lipids and other materials which contaminate steroid extracts and interfere with their detection by gas chromatography and gas chromatography-mass spectrometry. For this reason an extensive purification procedure is usually required. Two processes of purification were tested.

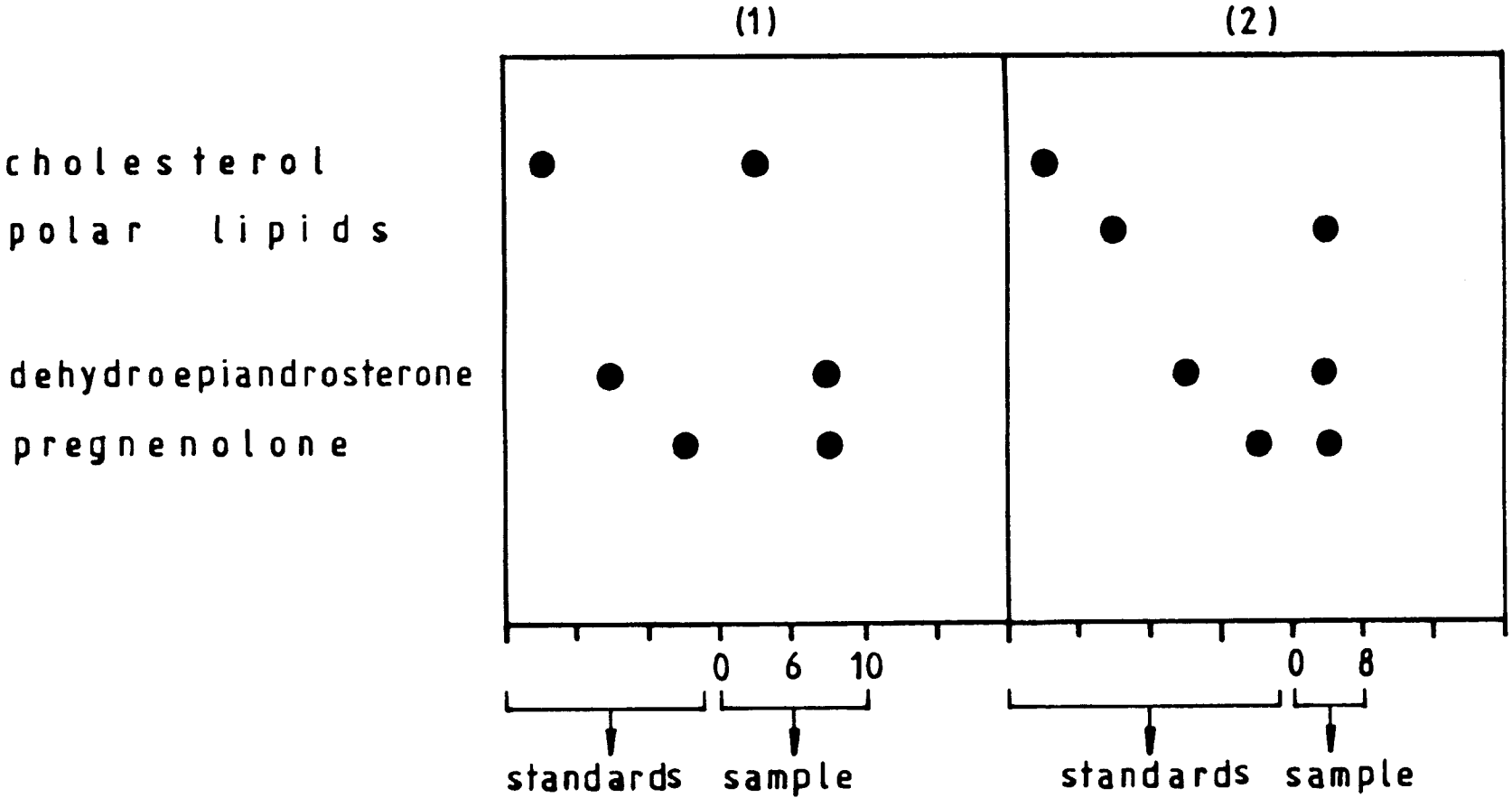
Firstly, the classical method, which depends on washing the sample or the homogenate extract with organic solvents in separating funnels to remove contaminants, was attempted (Paragraph 6.2.2) and found to be tedious and time consuming. It also resulted in unpredictable losses of steroids due to formation of emulsions. Secondly, purification on solid phases was found to be easier and more convenient. In this repect, three types of column chromatography were examined: The first was an adsorption chromatography procedure in which a column of neutral resin Amberlite XAD-2 was used (Paragraph 6.2.3(a)). The main disadvantage of this column was the very slow flow rate during elution (0.2 ml/minute or less). The second

type of column was a straight phase adsorption chromatography process using silicic acid (silica gel). This process was found to be quite efficient in separating cholesterol and other non-polar lipids from the steroids (Figure 7.1, Part 1.). However, it was not sufficient to remove other contaminants such as polar lipids. Also, the flow rate in this procedure was slow and the column was small (Figure 6.1 G) and rapidly overloaded. The third type was a liquid-gel chromatography technique. Thus a column of Lipidex-5000 packed in a suitable reversed phase solution system was used. This technique was found to be the most useful for the purpose of removing non-polar lipids as well as some polar contaminants. This will be discussed later (Paragraph 8.3.2)

7.1.3 Quenching Correction Curves

During the initial stage of the radioactive experiments activities of prepared specimens were counted with the Tri-Carb Pakard 2000 Liquid Scintillation Spectrometer. This machine has no facility for automatic calculation of the actual efficiency of radiation counting. However, it displays reference ratios (Automatic External Standard (AES) ratios) which correspond to radioactivity readings. Using these ratios, percentage efficiency can be measured indirectly. The efficiency of any machine for counting radioactivity varies according to the nature of the sample and the conditions of the analytical method as will be discussed below (Paragraph 8.3.3).

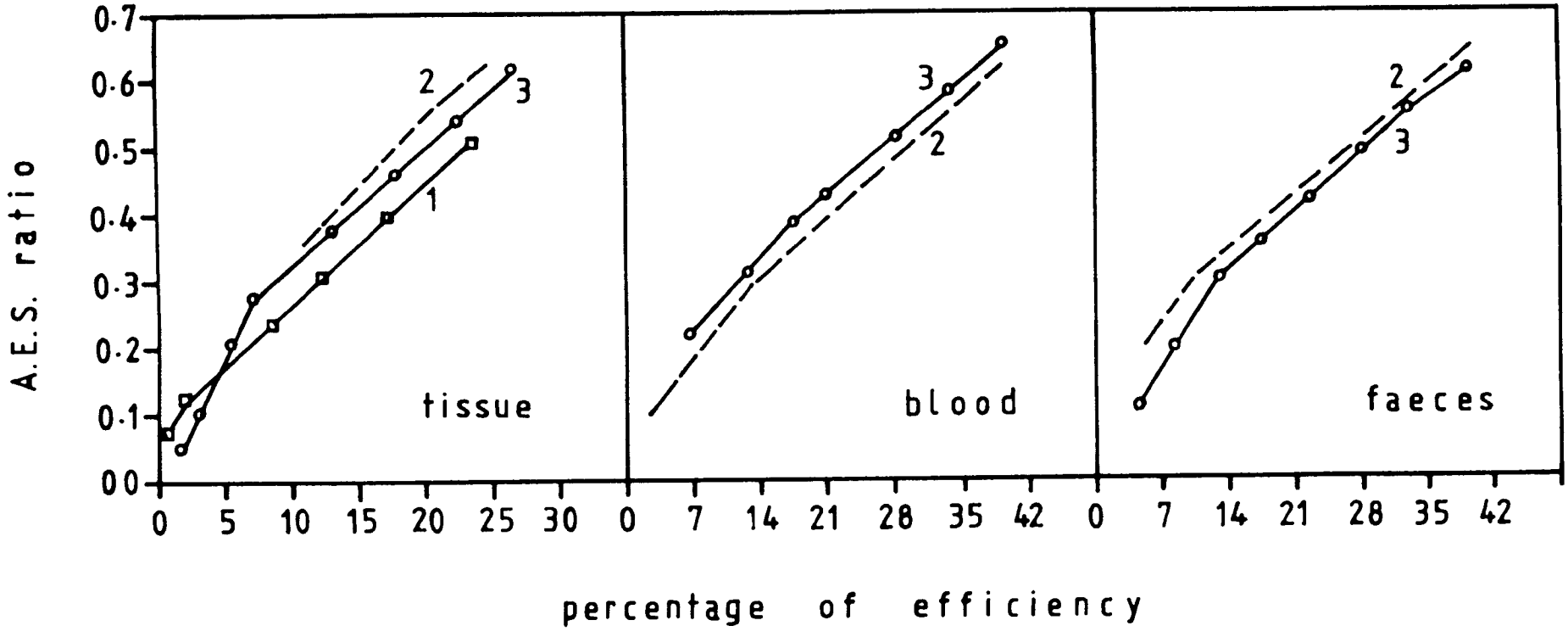
FIGURE 7·1 : THIN LAYER CHROMATOGRAMS OF FRACTIONS FROM COLUMNS USED FOR INITIAL PURIFICATION OF STEROID EXTRACT (1 = Silicic acid, 2 = Sep-pak C_{18})



It was, therefore, necessary to establish the efficiency of radiation counting for extracts of tissue, blood and faeces and for different analytical steps. In other words, this was to establish the quenching correction curves for different types of samples at sequential stages of the analytical method. The procedure used for this purpose was described earlier (Paragaph 6.3.1). In summary, a pool of 40 ml of the unquenched scintillation liquid containing 0.5 μCi of radioactivity (i.e. 1,110,000 dpm) was prepared. This was divided into 10 equal portions. Nine portions were quenched with different dilutions of steroid extracts obtained from a step in the analytical method while one portion was left unquenched as a reference. This was repeated for extracts of tissue, blood and faeces.

Efficiency was calculated according to Equation 6.1. At the same time AES ratios were noted. A quenching correction curve was made by plotting AES ratios versus calculated percent efficiency. Quenching correction curves for tissue, blood and faeces at three important analytical steps are shown in Figure 7.2. According to these curves, the efficiency of radioactivity counting varies slightly with the analytical steps but greatly with the nature of the samples, being the least for tissues. However, when these curves were applied subsequently, their usefulness was found to be limited as wider efficiency variations were noted. Most of these reflected the inevitable slight differences in the

FIGURE 7·2 : QUENCHING CORRECTION CURVES FOR EXTRACTS OF TISSUE, BLOOD & FAECES
1-Initial extract in Methanol / Chloroform 1:1 v/v, 2-After purification on Lipidex -5000 eluted with Methanol / water / Chloroform 9 : 1 : 2 v/v/ v, 3-After hydrolysis (Acetate buffer pH 4·6).



conditions under which an analytical step was performed. Accordingly, this scintillation counter was not used for subsequent measurements. For these, a computerised spectrometer was used. This was equipped with an automatic facility to calculate the efficiency and the absolute radioactivity count.

7.2 Assessment of Analytical Procedures

7.2.1 Choice of Reversed Phase Systems

Reversed phase chromatography with Lipidex-5000 was used as a purification procedure for extracts to remove non-polar contaminants such as cholesterol. Essentially, a reversed phase system was considered satisfactory if it satisfied two criteria. Firstly, it should be able to separate steroids completely from non-polar lipids; secondly, steroid recoveries should be high. Thus three reversed phase solvent systems were tested and compared. These were methanol/water/chloroform (90:10:20 v/v/v), methanol/heptane (95:5 v/v) and methanol /water/heptane (95:10:5 v/v/v). The assessment process was described earlier (Paragraph 6.4.3). In summary, two procedures were used: reversed phase column chromatograpy using radio-labelled steroids and thin layer chromatography.

In the former procedure a column of Lipidex-5000 was prepared as usual using one of the three reversed phase systems mentioned above. A known quantity of the radio-labelled steroid was added to the top of the column and this was then eluted with 100 ml of the solvent (i.e.

the reversed phase system in concern). This effluent was collected in 10 subsequent fractions, each of volume 10 ml. The radioactivity of these fractions was counted and recoveries were calculated in percent according to this Equation:

$$R = \frac{C}{A} \times 100 \quad \text{.....Equation 7.1}$$

where R = steroid recovery

C = counted radioactivity

A = added radioactivity

Steroid recoveries obtained by the three reversed phase systems are given in Tables 7.1-7.3. These tables contain data concerning tritium-labelled dehydroepiandrosterone, œstradiol and corticosterone which differ widely in polarity. Recovery percentages were plotted versus the volume of the effluent and the graphs obtained are shown in Figures 7.3-7.5. From these experiments, the following inferences were drawn:

1. Steroid recoveries for the three reversed phase eluant systems were similar. However the methanol/water/heptane system was less efficient in this respect. The reason for that will be discussed later (Paragraph 8.3.2).
2. For each solvent system, recoveries of different steroids, chosen to represent wide ranges of polarity were similar, being higher for corticosterone in most cases.
3. The ability of the three systems to separate steroids from cholesterol was the same. For all systems and

Table 7.1: Recovery of steroids from a Lipidex-5000 reversed phase column eluted with methanol/ water/chloroform (90:10:20 v/v/v).

Effluent fraction	^{3}H-DHEA		^{3}H-Oestradiol		^{3}H-Corticosterone	
	Radioactivity (dpm)	% Recovery	Radioactivity (dpm)	% Recovery	Radioactivity (dpm)	% Recovery
ml	Mean*	Mean**	Mean*	Mean**	Mean*	Mean**
1 - 10	6167	2.77	8975	4	426	0.19
11 - 20	179577	80	191388	85.3	214946	95.8
21 - 30	7626	3.4	2087	0.93	538.5	0.24
31 - 40	5921	2.6	2692	1.2	561	0.25
41 - 50	5075	2.3	2692	1.2	426	0.19
51 - 60	5176	2.3	2917	1.3	292	0.13
61 - 70	4883	2.2	224.4	1	269	0.12
Total	214425	95.6	210975	94.93	217415	96.9

* Number of Experiments = 3
** Original Radioactivity = 224370 dpm

Table 7.2: Recovery of steroids from a Lipidex-5000 reversed phase column eluted with methanol/ heptane (95:5 v/v).

Effluent fraction ml	^{3}H-DHEA		^{3}H-Oestradiol		^{3}H-Corticosterone	
	Radioactivity (dpm)	% Recovery	Radioactivity (dpm)	% Recovery	Radioactivity (dpm)	% Recovery
	Mean*	Mean**	Mean*	Mean**	Mean*	Mean**
1 - 10	5140	4.6	8436	7.6	4551	4.1
11 - 20	86854	78	87801	79.1	88356	79.6
21 - 30	2630	2.35	2775	2.5	4551	4.1
31 - 40	2916	2.6	2558	2.3	3110	2.8
41 - 50	2087	1.9	1890	1.7	2225	2.0
51 - 60	2785	2.45	1000	0.9	2109	1.9
61 - 70	2324	2.1	1332	1.2	1330	1.2
Total	104736	94	105792	95.3	106232	95.7

* Number of Experiments = 3
** Original Radioactivity = 111,000 dpm

Table 7.3: Recovery of steroids from a Lipidex-5000 reversed phase colum eluted with methanol/water/ heptane (95:10:5 v/v/v).

Effluent fraction ml	^{3}H-DHEA		^{3}H-Oestradid		^{3}H-Corticosterone	
	Radioactivity (dpm)	% Recovery	Radioactivity (dpm)	% Recovery	Radioactivity (dpm)	% Recovery
	Mean*	Mean**	Mean*	Mean**	Mean*	Mean**
1 - 10	3097	7.3	2995	7.1	2953	7.0
11 - 20	30145	71.5	30370	72	30791	73
21 - 30	885.6	2.1	1054.5	2.5	801.4	1.9
31 - 40	896	1.6	506	1.2	632.7	1.5
41 - 50	548.3	1.3	464	1.1	759	1.8
51 - 60	716.8	1.7	379.6	0.9	464	1.1
61 - 70	393.7	0.93	379.6	0.9	253.1	0.6
Total	36682.4	87	36148.7	85.7	36654	86.9

* Number of Experiments = 3.
** Original Radioactivity = 42180 dpm.

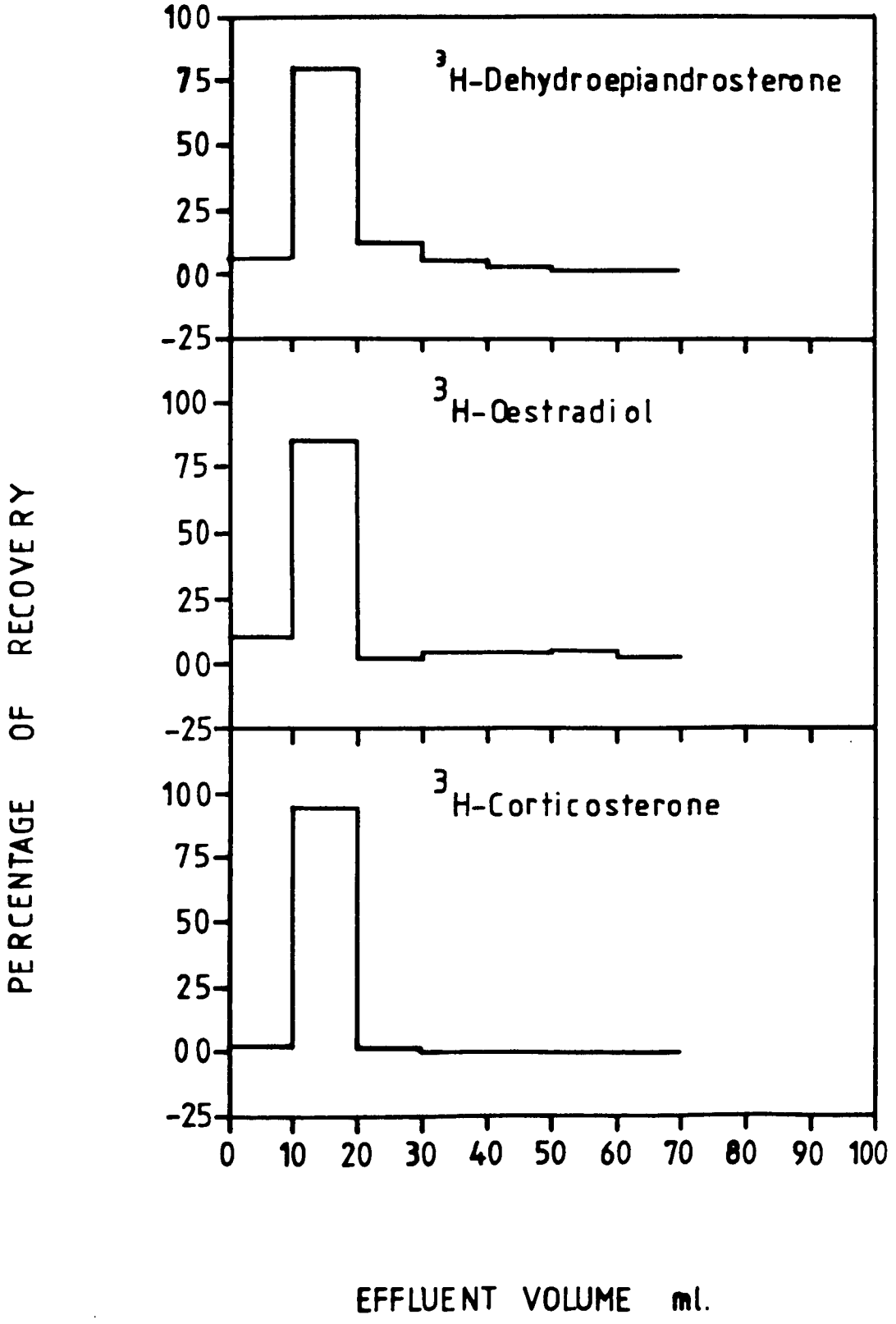


Figure 7.3 : Recovery of labelled steroids from reversed phase Lipidex-5000 columns eluted with Methanol/ Water/ Chloroform 90 : 10 : 20 V/V/V.

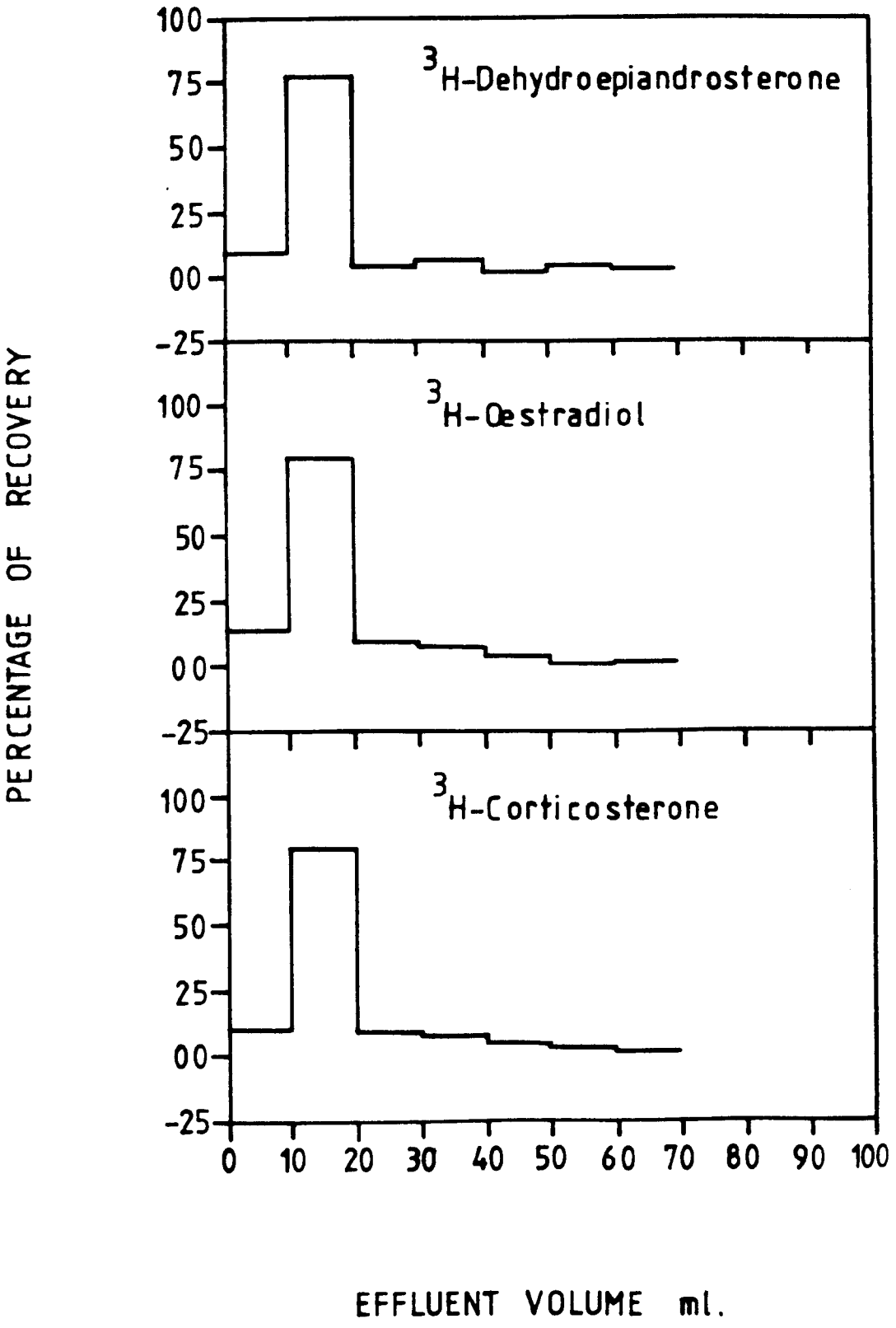


Figure 7.4:
Recovery of labelled steroids from reversed phase Lipidex-5000 columns eluted with Methanol/Heptane

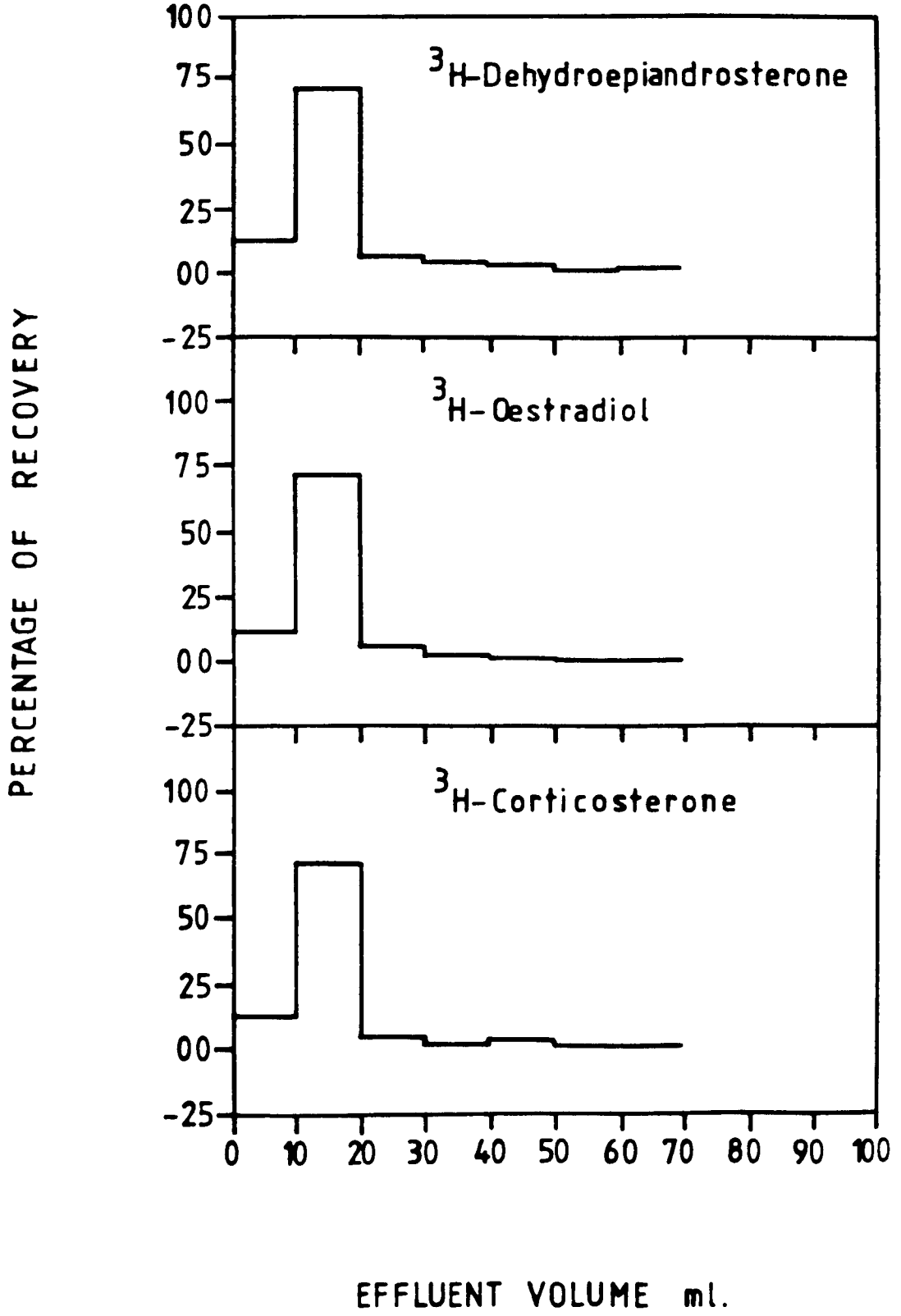
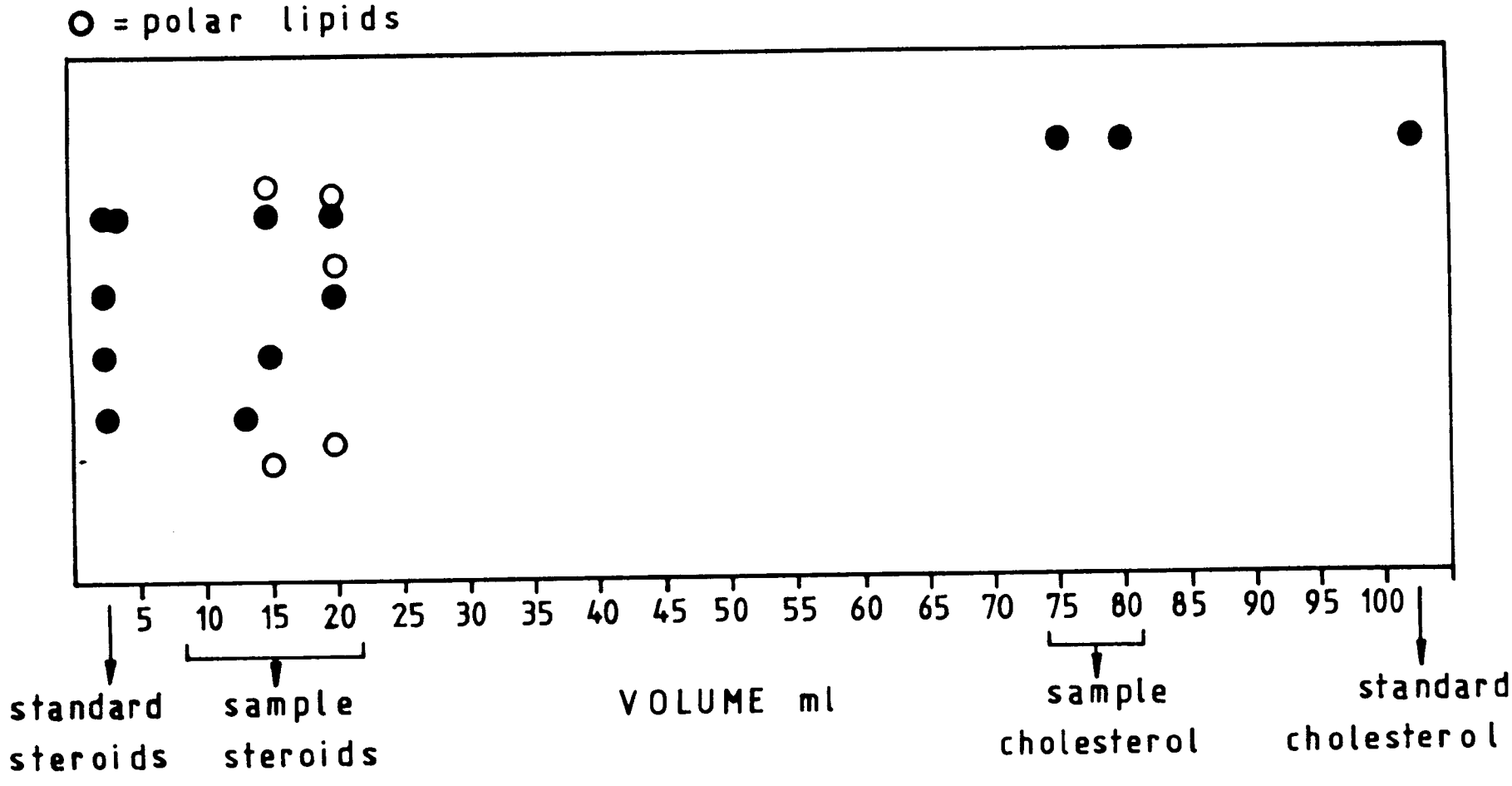


FIGURE 7.5 : Recovery of labelled steroids from reversed phase Lipidex-5000 columns eluted with Methanol/ Water/ Heptane 95/10/5 V/V/V

all steroid standards used, more than 70% of the steroid was recovered in the second 10 ml-fraction of the effluent. Recoveries in the succeeding fractions were relatively low. Although the ability of the three systems to separate and recover steroids was similar, the system consisting of methanol/water/ chloroform was used for all subsequent experiments because similar solvents were used in the homogenization and extraction step and therefore the residue obtained at the end of that step was more readily dissolved in this system. This simplified its application to the Lipidex-5000 column.

The last inference was confirmed by another procedure involving thin layer chromatography (TLC). Only one system (methanol/water/chloroform 90:10:20 v/v/v) was tested by this procedure. Thus, a residue from a tissue sample (human liver), which was prepared as usual (described in Paragraphs 6.1.5 and 6.4.1), was dissolved in 5 ml of the above solvent mixture and applied to the top of the Lipidex-5000 column which was packed in the system. The column was then eluted with 100 ml of the solvent system and 5 ml-fractions were collected subsequently. These fractions were concentrated and applied to a silica-gel TLC plate. On either side of the plate standards of steroids and cholesterol were applied. The plate was eluted with chloroform/ethyl acetate (3:1 v/v). The thin layer chromatogram is shown in Figure 7.6 It was clear that all steroids were eluted in the third and fourth fraction (i.e. between 10-20 ml of

FIGURE 7·6 : THIN LAYER CHROMATOGRAM OF FRACTIONS FROM REVERSED PHASE LIPIDEX-5000 COLUMN ELUTED WITH METHANOL/WATER/CHLOROFORM 9:1:2 v/v/v.



the effluent). Cholesterol appeared in the 15th fraction between 75-80 ml. Accordingly, in all subsequent experiments the reversed phase column was eluted with only 60-70 ml of methanol/water/chloroform (90:10:20 v/v/v). Another important feature shown in Figure 7.6 was the appearance of other contaminants which were the polar lipids. Therefore this column alone was not enough for purification and another step was added which will be described below.

7.2.2 Assessment of Sep-Pak C_{18} Cartridges Chain

Ordinarily, this step succeeded enzymatic hydrolysis in which steroids were present in an aqueous solution (i.e. sodium acetate buffer). Therefore, Sep-Pak C_{18} cartridges were required to remove inorganic salts and also some organic contaminants (for example some proteins and biological pigments). Also they served to transport steroids from the aqueous solution to organic solvents which were used in final steps of the analytical method. The ability of different numbers of the cartridges to perform the above functions was assessed. This was based on the assumption that the more steroid recovered the better was the analytical procedure. Assessment was carried out for tissue, blood and faeces with 11, 8 and 6 experiments respectively. Different numbers of cartridges were used as described in Paragraph 6.2.3(b). Aliquots for radioactivity counting were taken at the beginning and the end of this step. Therefore, steroid recovery percents were calculated

(Equation 7.1) and plotted versus the number of cartridges (Figure 7.7). Similarly, other aliquots were taken from the sediment and the water phase and percentage steroid losses were measured. Recoveries are given in Tables 7.4-7.6, which also show values of z and P for the comparison between results based on 4 and 1 cartridges (zM test was used). Histograms of the recoveries and losses are shown in Figures 7.8-7.10. These figures and tables demonstrate that for all types of samples studied recoveries increased significantly with the number of cartridges used. In contrast, losses decreased with the number of cartridges. However, changes of recoveries and losses were relatively small when five and six cartridges were compared. Accordingly, for all subsequent experiments 4-6 Sep-Pak C_{18} cartridges (or the equivalent packing material) were used.

7.2.3 Straight Phase Column Chromatography

This was used to separate steroids from as much polar contaminant as possible. For this purpose a column of Sephadex LH-20 packed in cyclohexane/ethanol (4:1 v/v) was employed as explained in Paragraph 6.4.8. The ability of this column to remove polar lipid contaminants was examined . The procedure used in this test was similar to that described in Paragraph 7.2.1 above except that a straight phase column was used in this experiment instead of a reversed phase one which was used earlier. The thin layer chromatogram resulting from this experiment is shown in Figure 7.11. Thus, it was found that many steroids

Table 7.4: Recovery and loss of tissue steroids from different numbers of Sep-Pak C_{18} cartridges

Number of Cartridges Used	Recovery in the effluent				Loss in the Sediment				Loss in the Water Phase			
	Radioactivity (dpm)		% Recovery		Radioactivity (dpm)		% Loss		Radioactivity (dpm)		% Loss	
	Mean**	S.D.	Mean	S.D.	Mean**	S.D.	Mean	S.D.	Mean**	S.D.	Mean	S.D.
1*	54834	9945	60.65	11.0	23235	1844	25.7	2.04	1745	497	1.93	0.55
2	64734	3074	71.6	3.4	18534	7052	20.5	7.8	1311	181	1.45	0.2
3	68079	9222	75.3	10.2	5967	1392	6.6	1.54	506	90	0.56	0.1
4*	71424	5153	79	5.7	4611	597	5.1	0.66	425	90	0.47	0.1
5	80917	1175	89.5	1.3	2351	633	2.6	0.7	208	27	0.23	0.03
6	82183	3436	90.9	3.8	1419	316	1.57	0.35	244	99	0.27	0.11

* z and P for comparison of 4 cartridges and 1 cartridge are 5.5 and <0.2, respectively.
** Number of Experiments = 11.

Table 7.5: Recovery and loss of blood steroids from different numbers of Sep-Pak C_{18} cartridges

Number of Cartridges Used	Recovery in the effluent				Loss in the Sediment				Loss in the Water Phase			
	Radioactivity (dpm)		% Recovery		Radioactivity (dpm)		% Loss		Radioactivity (dpm)		% Loss	
	Mean**	S.D.	Mean	S.D.	Mean**	S.D.	Mean	S.D.	Mean**	S.D.	Mean	S.D.
1*	63558	1266	70.3	1.4	16003	4249	17.7	4.7	1672.5	578.6	1.85	0.64
2	60439	6690	66.85	7.4	6057	1085	6.7	1.2	1808	127	2.0	0.14
3	72192	2984	79.85	3.3	6121	1175	6.77	1.3	1356	127	1.5	0.14
4*	74950	7323	82.9	8.1	4430	2441	4.9	2.7	741	117.5	0.82	0.13
5	76758	6057	84.9	6.7	2079	633	2.3	0.7	533	90	0.59	0.1
6	82183	2351	90.9	2.6	1501	741	1.66	0.82	497	99	0.55	0.11

* z and P values for comparison between 4 and 1 cartridges are 25.5 and <0.2%, respectively.
** Number of Experiments = 8

Table 7.6: Recovery and loss of faecal steroids from different numbers of Sep-Pak C_{18} cartridges.

Number of Cartridges Used	Recovery in the effluent				Loss in the Sediment				Loss in the Water Phase			
	Radioactivity (dpm)		% Recovery		Radioactivity (dpm)		% Loss		Radioactivity (dpm)		% Loss	
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
1*	58224	19306	64.4	11.4	18986	5515	21.0	6.1	1763	723.3	1.95	0.8
2	61388	3436	67.9	3.8	9312	3707	10.3	4.1	1808	127	2.0	0.14
3	68079	9222	75.3	10.2	5967	1356	6.6	1.5	1356	127	1.5	0.14
4*	74986	7920	82.94	8.76	4701	768	5.2	0.85	796	99.5	0.88	0.11
5	80917	542.5	89.5	0.6					497.3	90	0.55	0.1
6	82779.4	17.18	91.56	1.9	1582.2	190	1.75	0.21	344	36.2	0.38	0.04

* z and P values for comparison between 4 and 1 cartridges are 4 and <0.2%, respectively.
** Number of Experiments = 6
Original Radioactivity = 90,410 dpm

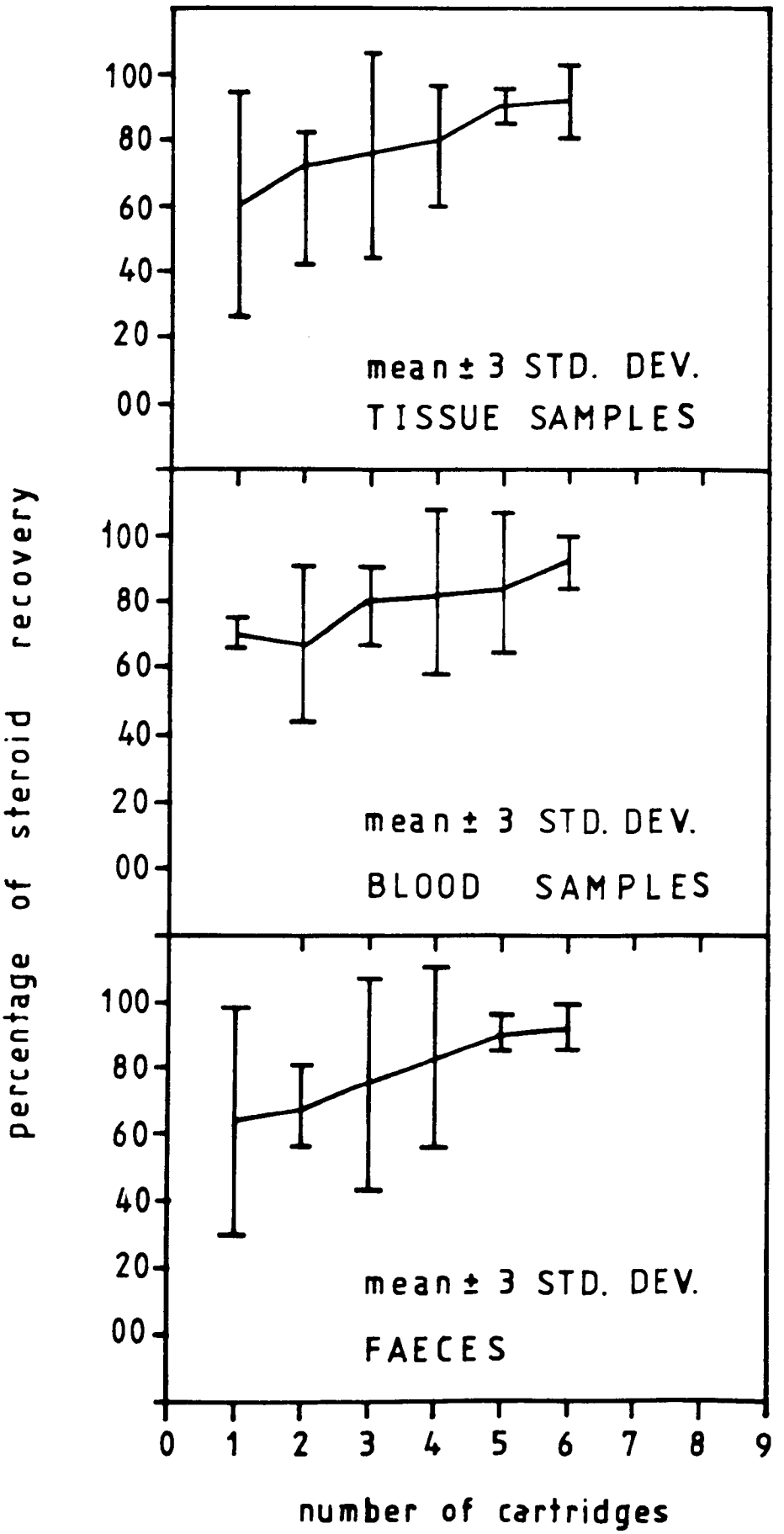


FIGURE 7.7 : STEROID RECOVERIES FROM TISSUE, BLOOD AND FAECES VERSUS NUMBER OF SEP-PAK C_{18} CARTRIDGE.

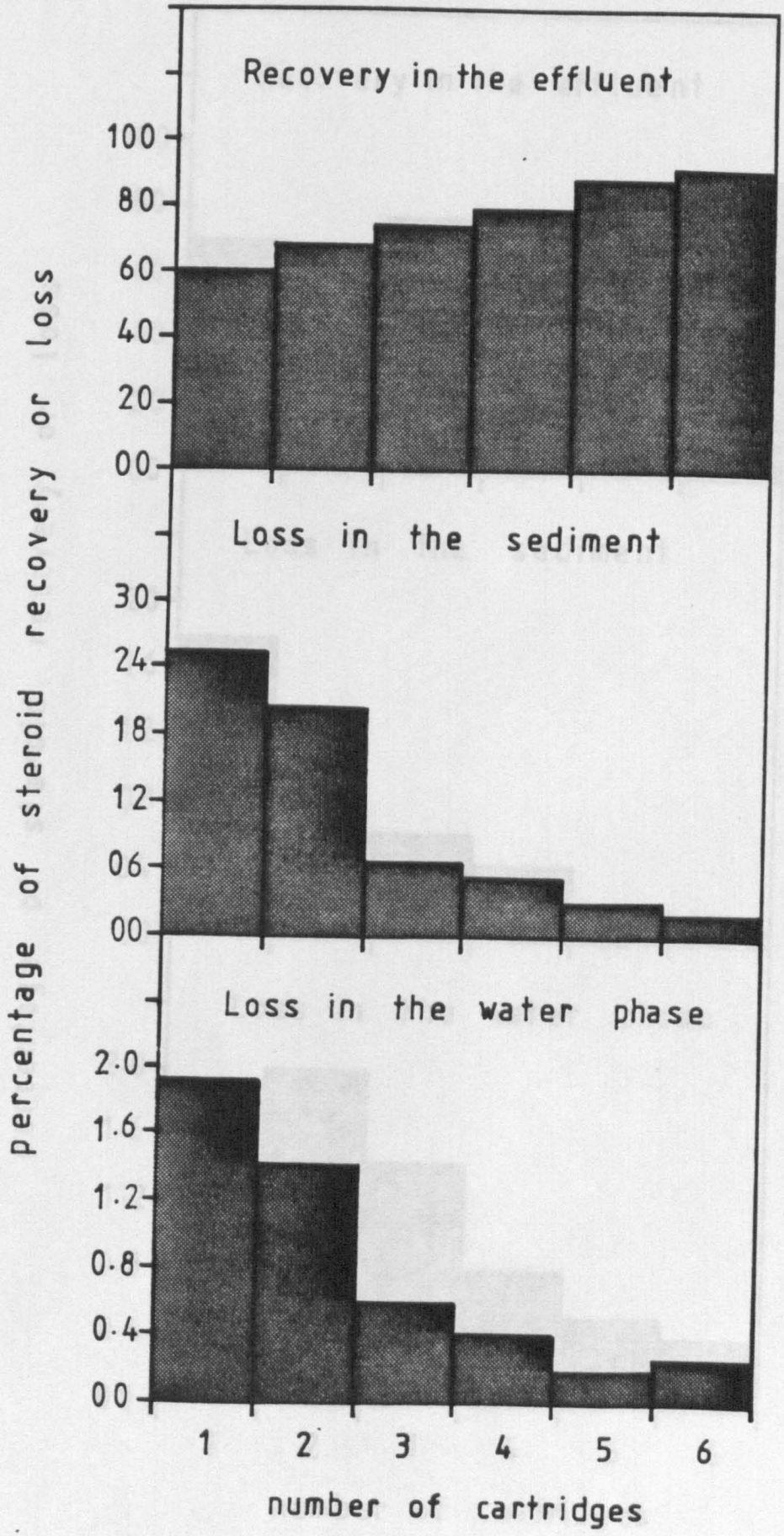


Figure 7·8 : Effect of using different numbers of Sep-pak C_{18} Cartridges on recovery of steroids from tissue samples

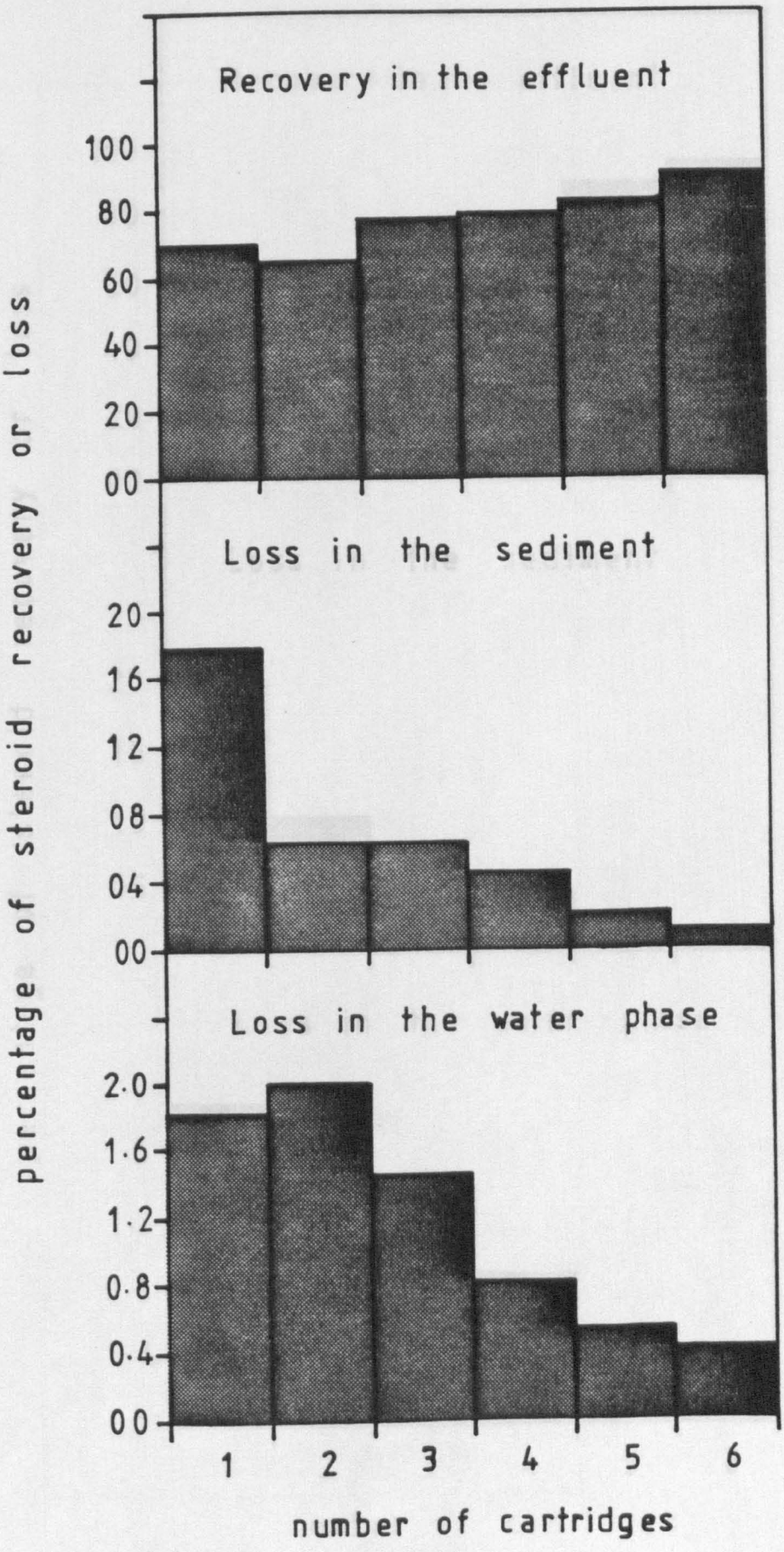


Figure 7·9 : Effect of using different numbers of Sep-pak C_{18} Cartridges on recovery of steroids from blood samples.

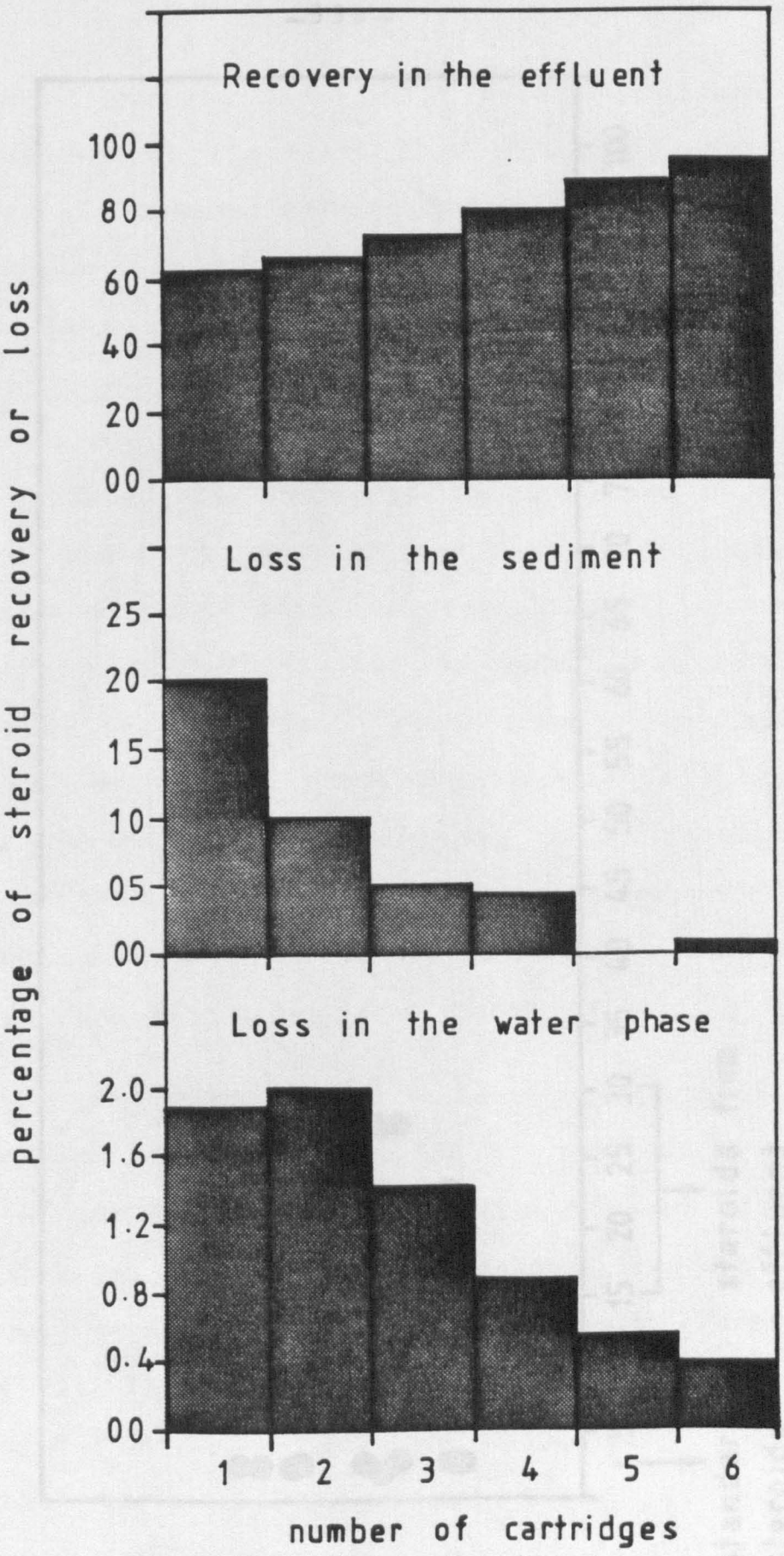
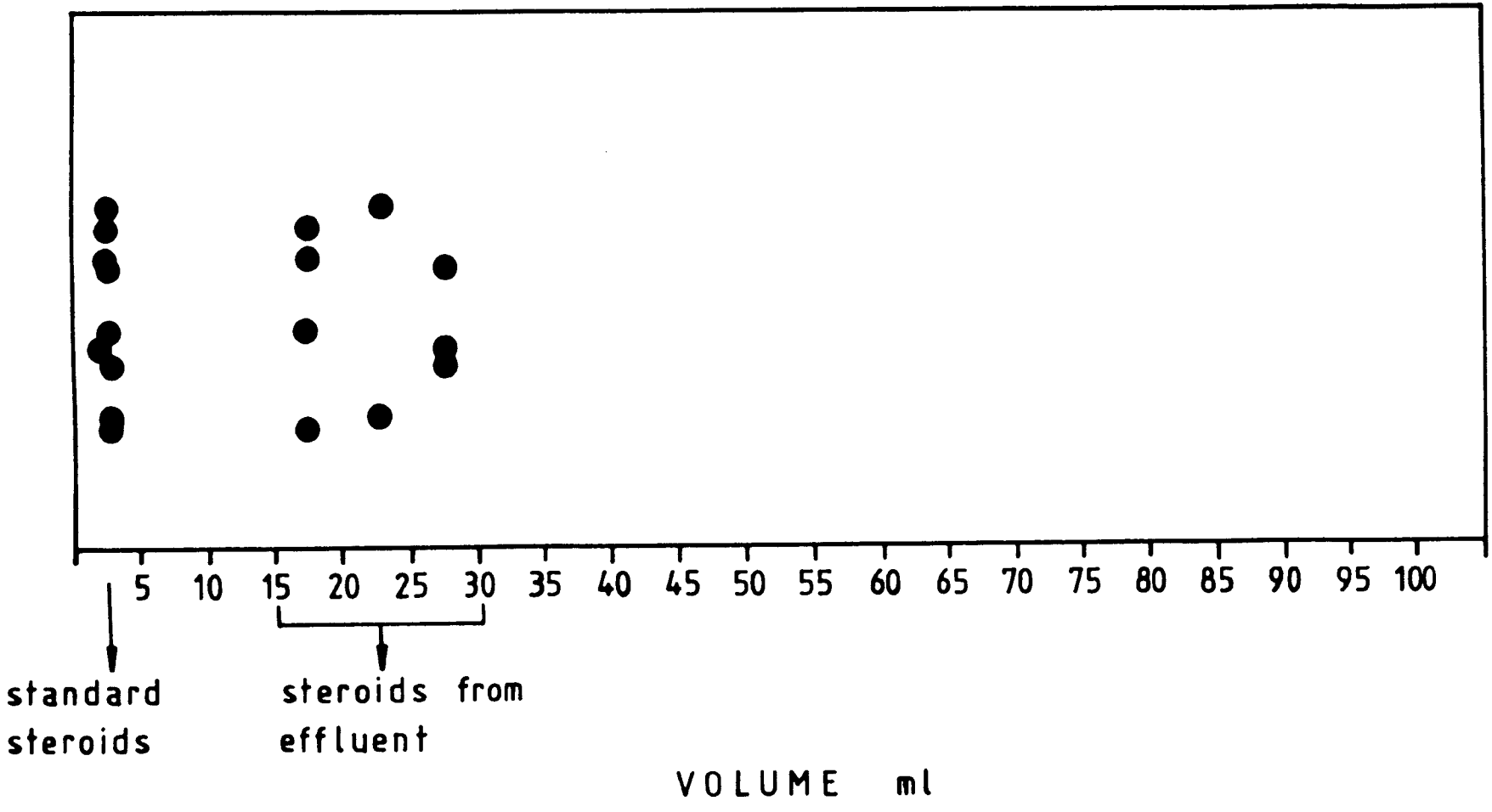


Figure 7·10 : Effect of using different numbers of Sep-pak C_{18} Cartridges on recovery of steroids from faeces

FIGURE 7.11 : THIN LAYER CHROMATOGRAM OF FRACTIONS FROM STRAIGHT PHASE LH-20 COLUMN ELUTED WITH CYCLOHEXANE / ETHANOL 4 : 1 V / V .



were eluted between 16-31 ml. Most contaminants were either removed in the first 12-15 ml of the effluent or stayed in the column because they require larger volumes for elution. However, very polar steroids such as corticosterone could not be recovered unless a large volume of the effluent was collected or the polarity of the solvent was increased, in which case the sample would be less purified and, therefore, the detection of steroids by GC-MS would be more difficult or even impossible. Accordingly only the 16-31 ml fraction was collected and the study was confined to the major steroids which appeared in this fraction. These steroids are shown in Table 7.7, which also shows the systematic names, the molecular weight and five largest peaks in the mass spectra of these steroids. These represented many of the major steroids present in biological samples.

7.2.4 Steroid Recoveries from Steps of the Analytical Method

For 25 experiments, aliquots for radioactivity counting were taken at the end of each stage of the analytical methods and recovery percents were calculated (Equation 7.1). Results of these calculations for tissue, blood and faeces are given in Tables 7.8-7.10 and shown in Figures 7.12-7.14. In each of these, recoveries of dehydroepiandrosterone sulphate (DHEA-S), œstradiol glucuronide or corticosterone are shown. It was drawn from these results that, in general, steroid recoveries obtained by this method were high. This was more significant when the many steps and the complexity of the

Table 7.7: Molecular weights and five peak-mass spectra of steroids studied (TMSi derivatives).

Abbreviated Steroid Name	Structure	MW	Peak 1 m/z	Peak 2 m/z	Peak 3 m/z	Peak 4 m/z	Peak 5 m/z
Androsterone	3α-Hydroxy-5α-androstane-17-one	362	272	271	347	155	129
Androstanediol	3β,17β-Dihydroxy-5α-anderostane	436	129	421	130	241	346
	3β,17β-Dihyroxy-5β-androstane	436	256	129	241	346	215
D H A	3β-Hydroxy-androst-5-ene-17-one	360	129	304	321	360	270
A'-diol	3β,17β-Dihydroxy-androst-5-ene	434	129	215	344	239	254
	3β,17 α-Dihydroxy-androst-5-ene		129	215	344	239	254
P-lone	3α-Hydroxy-5β-Pregnan-20-one	390	215	300	375	285	257
P-diol	3α,20α-Dihydroxy-5β-pregnane	464	117	118	119	284	269
P'-lone	3β-Hydroxy-Pregn-5-ene-20-one	388	129	298	85	259	388
			129	298	259	388	121
P'-diol	3β,20α-Dihydroxy-pregn-5-ene	462	117	129	118	372	462

Table 7.8: Percentage recovery of tissue steroids in different steps of the analytical method.

Number of Experiments	Radio-labelled steroid used	Reversed Phase Column	Hydrolysis		Sep-Pak C_{18} cartridges	Solvolysis		Amberlyst A-26 Column	Final
			Rapid	Slow		Rapid	Slow		
6	^{3}H-DHEA-S†								
	Mean	90.5	84.4*	82.2**	89.6	92.0*	90.6**	87.4	64.1
	Std.D	5.9	15.5	14.3	7.2	5.3	9.2	6.2	6.9
3	^{3}H-Oestradiol - G††								
	Mean	90.7	96.8	94.5	92.6	94.2	-	95.3	80.3
2	^{3}H-Corticosterone†††								
	Mean	91.3	89.0	-	90.4	95.9	-	94.6	82.5

* z and P values for the comparison of the rapid solvolysis with the rapid hydrolysis = 1.2 and P >10%.

** z and P values for the comparison of the slow solvolysis with the slow hydrolysis = 1.4 and P >10%.

† radio-labelled dehydroepiandrosterone sulphate

†† radio-labelled œstradiol-glucuronide

††† radio-labelled corticosterone

Table 7.9: Percentage recovery of blood steroids in different steps of the analytical method.

Number of Experiments	Radio-labelled steroid used	Reversed Phase Column	Hydrolysis		Sep-Pak C_{18} cartridges	Solvolysis		Amberlyst A-26 Column	Final
			Rapid	Slow		Rapid	Slow		
4	^{3}H-DHEA-S								
	Mean	81.1	83.7*	82.0	88.8	96*	-	82.2	55.2
2	^{3}H-Oestradiol - G								
	Mean	88.7	94.1	-	90.3	91.8	-	90.0	70.0
2	^{3}H-Corticosterone								
	Mean	93.1	92.4	75	93.5	92.6	-	98.0	75.0

* z and P values for comparing rapid solvolysis with rapid hydrolysis = 2.2 and P °5%, respectively.

Table 7.10: Percentage recovery of faecal steroids in different steps of the analytical method.

Number of Experiments	Radio-labelled steroid used	Reversed Phase Column	Hydrolysis		Sep-Pak C_{18} cartridges	Solvolysis		Amberlyst A-26 Column	Final
			Rapid	Slow		Rapid	Slow		
3	^{3}H-DHEA-S								
	Mean	91.3	85.0*	70	90.0	93.2*	-	90.5	65.1
2	^{3}H-Oestradiol - G								
	Mean	92.1	95.5	87.3	91.6	94.2	-	93.3	79.9
1	^{3}H-Corticosterone								
	Mean	93.4	92.1	-	94.5	93.8	-	-	76.3

* Comparing rapid solvolysis with rapid hydrolysis, z and P = 1.04 and >10%, respectively.

FIGURE 7.12 : RECOVERY OF LABELLED DEHYDROEPIANDROSTERONE IN EACH STAGE OF THE EXTRACTION PROCEDURE (N = 6, 4 & 3 FOR TISSUE, BLOOD & FAECES RESPECTIVELY).

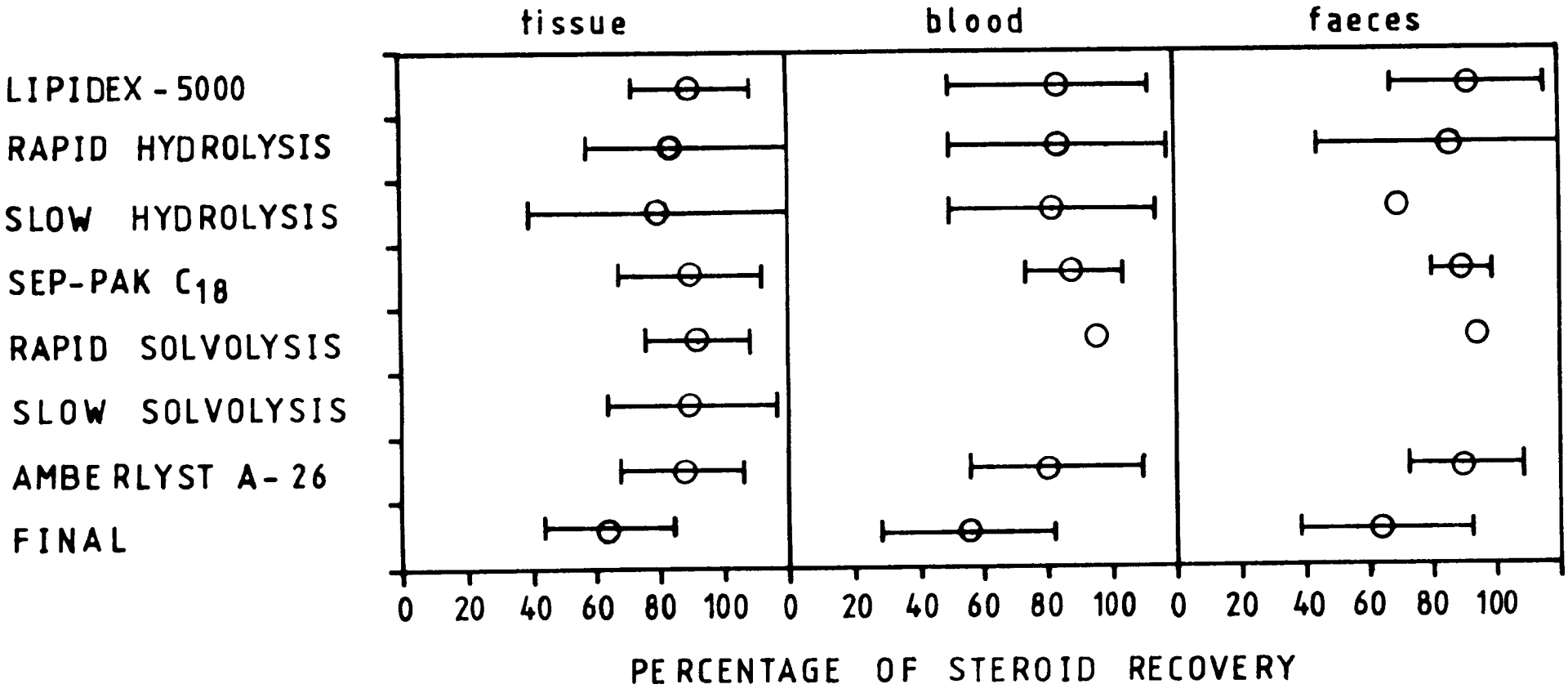


FIGURE 7.13 : RECOVERY OF LABELLED OESTRADIOL IN EACH STAGE OF THE EXTRACTION PROCEDURE (N = 3, 2 & 2 FOR TISSUE, BLOOD AND FAECES RESPECTIVELY).

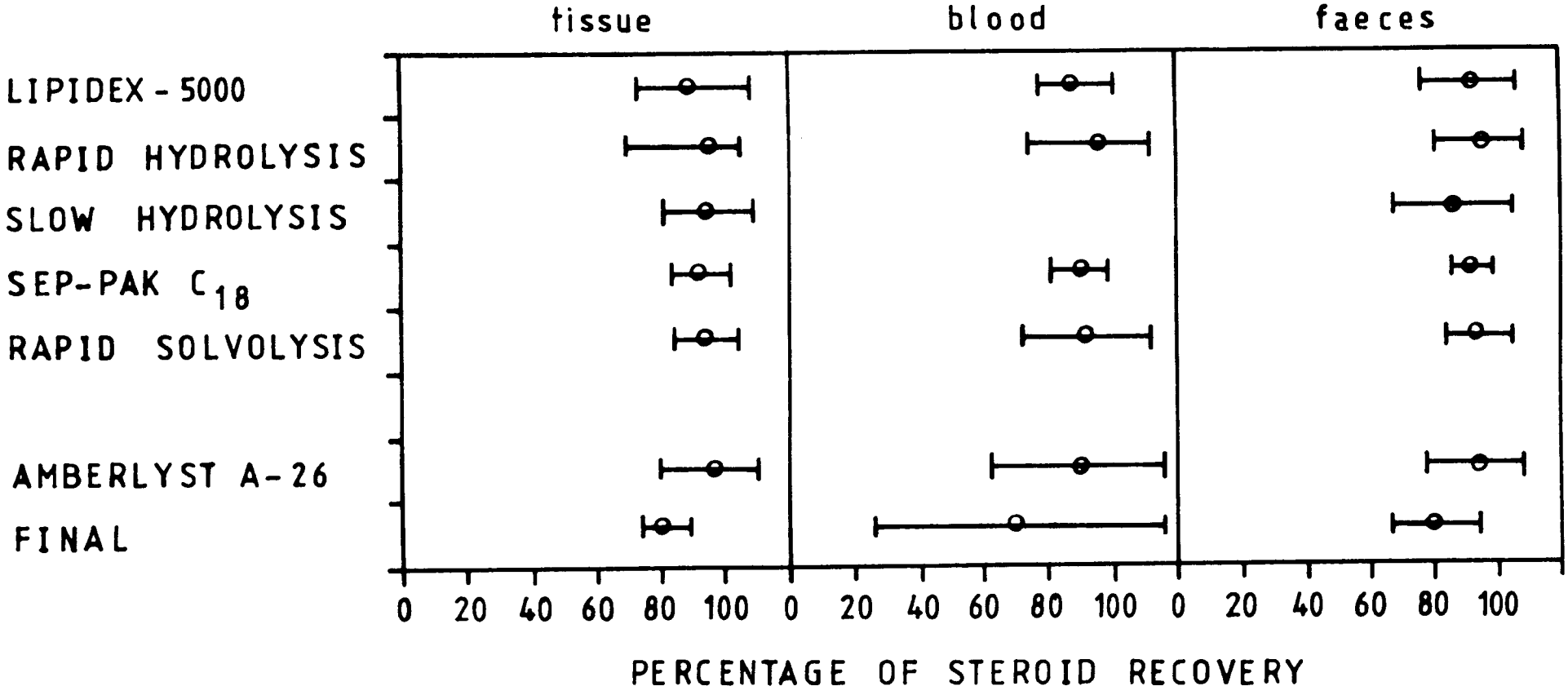
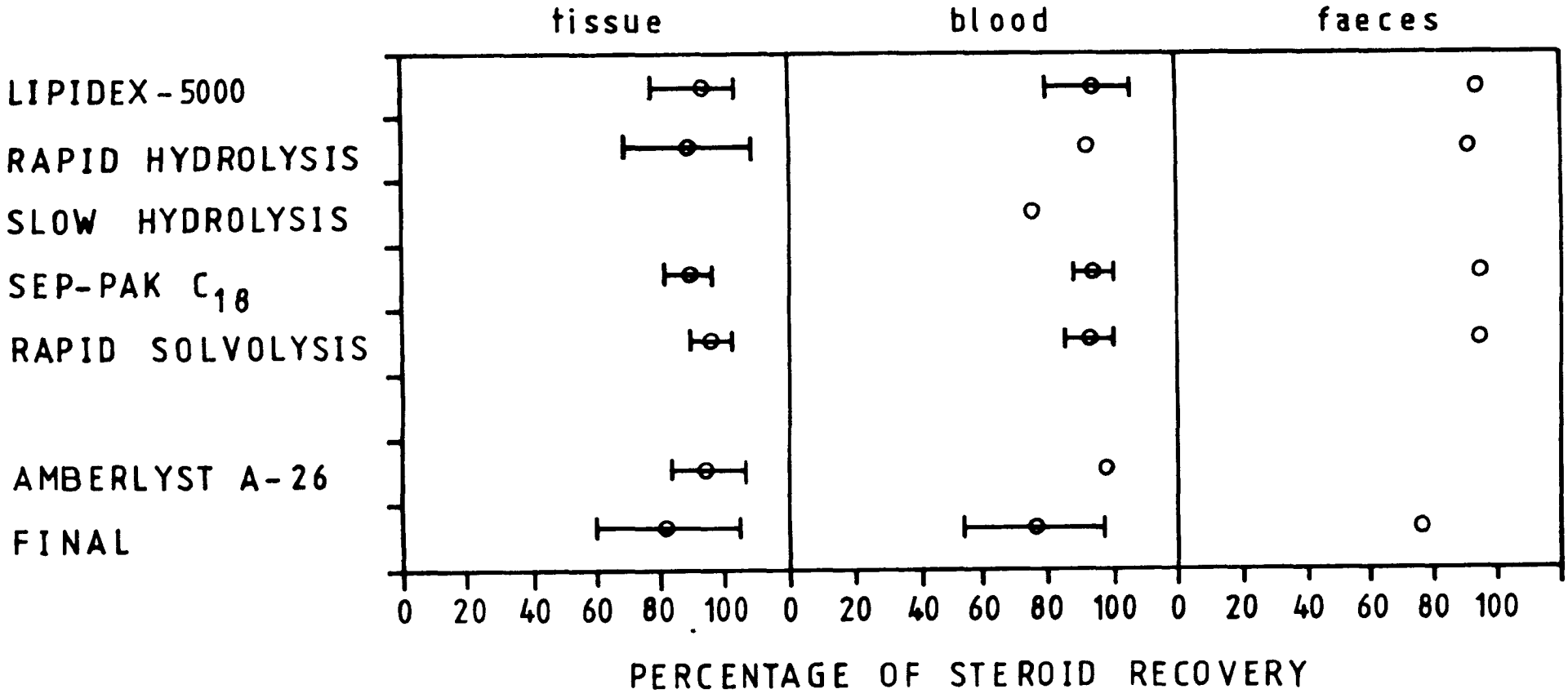


FIGURE 7-14 : RECOVERY OF LABELLED CORTICOSTERONE IN EACH STAGE OF THE EXTRACTION PROCEDURE (N = 2, 2 & 1 FOR TISSUE, BLOOD AND FAECES RESPECTIVELY).



method were considered. The lowest recoveries were noted for blood steroids. Recoveries of tissue and faecal steroids were similar, however, those for tissue steroids were slightly greater. In all samples, the most poorly recovered steroid was dehydroepiandrosterone. Main steroid losses occurred in the enzymatic hydrolysis step and, to a lesser extent, the reversed phase and the Amberlyst A-26 columns. Also, some losses took place during the passage of the aqueous steroid mixture through the Sep-Pak C_{18} cartridges. Recoveries for the straight phase column were not quantitated as the separation of the steroid found in this column was selective and none of the radio-labelled steroids used was similar to those steroids which were collected from the straight phase column for analysis.

It was worth noting that this method was relatively efficient in removing a great amount of contaminants such as cholesterol which is usually present in considerable quantities in tissues, faeces and blood samples. Other sterols were also removed from the extracts during these purification steps, thereby allowing the use of campesterol and ß-sitosterol as internal standards in subsequent quantitative GC-MS measurements. To demonstrate this result the weight of the residue at the end of each step was measured. Weight data are given in Table 7.11 which shows how samples or extracts of many hundreds of milligrammes were reduced to a few milligrammes as a result of the purificiation process.

Table 7.11: Weight of residues collected at the end of each step* of the analytical method.

SAMPLE	Number of Experiments	Weight or volume of sample mg or ml		Weight of Residue (mg) after:							
				Extraction		Lipidex-5000 column		Sep-Pak C_{18} cartridge		Amberlyst A-26 column	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Blood	8	9.75	6.6	219.5	181.7	133.3	83.4	9.95	4	2.9	0.87
Faeces	6	1053	415	143	40	69.3	2.8	22	9.4	9.9	0.14
Tissue (Liver)	11	1625	765	243	190	96.3	57.6	29.9	8.9	7.7	2.9

* The weight of the residue collected at the end of the straight phase column was very small and values could not be obtained accurately.

7.3 Study of Rat Samples

Thirty rats were killed as described earlier (Paragraph 6.4.1). These were divided into two groups: in the first group, samples were collected immediately after death so that they were used to study the steroids at the moment of death. In the second group, the rats were dissected and samples were collected 24 hours after death. The samples collected consisted of liver, blood, adrenal glands and faeces. It was found that the rat was a satisfactory model in respect of the quantity of the liver and faeces sample required for the steroid analyses in this study. However, this was not the case when blood, adrenal tissue and urine were concerned. For instance, urine could not be obtained because the volume was very small and most was lost when the animals were killed. Also, little blood was available for collection, particularly, when cervical dislocation was employed as the method of killing. Samples were processed to prepare extracts which were then purified by the procedures described in Paragraphs 6.4.2-6.4.8.

7.4 Gas Chromatography-Mass Spectrometry (GC-MS)

7.4.1 Steroid Retention Indices

These were established to help in the identification of steroid peaks during the GC-MS analysis. The procedure used in the establishment of these steroid indices was described earlier (Paragraph 6.6). In summary, the indices were obtained by comparing

the retention times of steroid standards with hydrocarbons (C_{16}-C_{34}). The number of carbon atoms contained in these hydrocarbons was known. Thus steroid indices were calculated easily according to this formula:

$$I_s = \frac{R_s - R_{c1}}{R_{c2} - R_{c1}} \times 200 \quad \text{.....Equation 7.2}$$

where I_s = Steroid retention index.

R_s = Retention time for a steorid standard

R_{c1} = Retention time for the first hyrocarbon

and R_{c2} = Retention time for the second hydrocarbon. In fact, a computer programme was developed for these calculations (Appendix I, Programme 11). Indices measured in this way were, therefore, similar to Kovats retention indices except that they were measured using temperature programing instead of isothermal condition. Retention times were obtained by the GC-MS analyses of the steroids and hydrocarbons which were, essentially, conducted under exactly the same conditions. Thus retention indices of 33 major steroids which were the most likely to be encountered in animal samples and of two plant steroids (i.e. campesterol and ß-sitosterol) [537] were calculated. The latter two steroids were used as internal standards during the subsequent GC-MS analyses. These retention indices are given in Table 7.12. For practical purposes indices were approximated to the nearest whole number (shown between the brackets in the Table).

7.4.2 Selective Ion Monitoring (SIM)

This technique was found to be useful to detect

Table 7.12: Steroid retention indices recorded on a BP-1 Capillary Column (MO-TMSi derivatives). (N = 4)
(continued on next page)

STEROID	Index	
	Mean (to nearest whole number)	Std. Dev.
3α-Hydroxy-5α-androstan-17-one (Androsterone)	2581.8 (2580)	12.4
3α-Hydroxy-5β-androstan-17-one (Aetiocholanolone)	2589.3 (2590)	14.2
3β-Hydroxyandrost-5-en-17-one (Dehydroepiandrosterone)	2640 (2640)	23.4
3β-Hydroxy-5α-androstan-17-one (Epiandrosterone)	2656 (2655)	6.6
3β,17β-Dihydroxy-androst-5-ene (Androstenediol)	2665.3 (2665)	13.3
3α-Hydroxy-5α-androstane-11,17-dione (11-Oxoandrosterone)	2679 (2680)	1.0
3α-Hydroxy-5β-androstane-11,17-dione (11-Oxo-aetiocholanolone)	2682.3 (2680)	2.1
3-Hydroxy-oestra-1,3,5(10)-trien-17-one (Oestrone)	2690.3 (2690)	24.5
Androst-4-ene-3,17-dione (Androstenedione)	2737.6 (2740)	32.5
17β-Hydroxy-androst-4-en-3-one (Testosterone or Transtestosterone)	2742.5 (2745)	5.2
3α,17α-Dihydroxy-5β-pregnan-20-one (17α-Hydroxypregnanolone)	2754 (2755)	8.9
3α,11β-Dihydroxy-5α-androstan-17-one (11β-Hydroxyandrosterone)	2762.0 (2760)	9.8
3α,11β-Dihydroxy-5β-androstan-17-one (11β-Hydroxyaetiocholanolone)	2764.8 (2765)	13.4
3α-Hydroxy-5β-pregnan-20-one (Pregnanolone)	2786.5 (2785)	6.9
3β,16-Dihydroxy-androst-5-en-17-one (16α-hydroxy-dehydroepiandrosterone)	2805.3 (2805)	52.3
3α,20α-Dihydroxy-5β-pregnane (Pregnanediol)	2847.0 (2845)	46.5
3β-Hydroxy-pregn-5-en-20-one (Pregnenolone)	2848.0 (2850)	11.3
3α,17α,20α-Trihydroxy-5β-pregnane (Pregnanetriol)	2870.0 (2870)	36.1
3β,20α-Dihydroxy-pregn-5-ene (Pregnenediol)	2883.7 2885)	11.9

Table 7.12: (continued)

STEROID	Index	
	Mean	Std. Dev.
Oestra-1,3,5(10)-triene-3,16α,17β-triol (Oestriol)	2908.5 (2910)	16.8
Pregn-4-ene-3,20-dione (Progesterone)	2926.2 (2925)	38.8
3β,17α,20α-Trihydroxy-pregn-5-ene (Pregnenetriol)	2995.7 (2995)	5.8
3α,17α,21-Trihydroxy-5β-pregnane-11, 20-dione (Tetrahydrocortisone or THE)	3013.2 (3015)	20.7
3α,11β,21-Trihydroxy-5α-pregnan-20-one (Allo-tetrahydrocorticosterone)	3023.8 (3025)	10.7
3α,21,-Dihydroxy-5β-pregnane-11,20-dione (Tetrahydro-11-dehydrocorticosterone or THA)	3050.2 (3050)	47.0
3α,11β,17α,21-Tetrahydroxy-5β-pregnan-20-one (Tetrahydrocortisol or THF)	3056.7 (3055)	1.5
3α,17α,20α,21-Tetrahydroxy-5β-pregnan-11-one (α-cortolone)	3079.3 (3080)	15.0
3α,11β,17α,21-Tetrahydroxy-5α-pregnan-20-one (Allo-Tetrahydrocortisol)	3099.3 (3100)	76.4
3α,17α,20β,21-Tetrahydroxy-5β-pregnan-11-one (β-cortolone)	3116.8 (3115)	4.0
3α,11β,17α,20β-21-Pentalhydroxy-5β-pregnane (β-cortol)	3137.4 (3140)	46.3
18,11-Hemiacetal of 11β-21,dihydroxy-3,20-dioxo-pregn-4-en-18-al(Aldosterone)	3150.0 (3150)	19.0
3α,11β,17α,20α-21-Pentahydroxy-5β-pregnane (α-cortol)	3158.3 (3160)	6.7
11β,17α,21-Trihydroxypregn-4-ene-3,11,20-trione (Cortisol)	3290.0 (3290)	15.6
(24R)-Ergost-5-en-3β-ol (campesterol)	3058 (3060)	
Stigmast-6-en-3β-ol (β-Sitosterol)	3142.6 (3145)	

small quantities of steroids. However, repetitive scanning (another GC-MS technique, usually requiring larger quantities of sample) was necessary when standard steroids and hydrocarbons were analysed to obtain steroid retention indices and to aquire the full mass spectra of steroid peaks. Further discussion of these techniques will be given later (Paragraph 8.4). The SIM software used in this study did not permit more than one set of ions to be analysed at one time. Due to this restriction and, to some extent, the technical difficulties encountered in the sample purification, analysis was planned to be confined to only eight steroids (Table 7.7). As shown in this table, these steroids had 117 and/or 129 ion fragments in common (Figure 8.1), either as a major or a minor peak except for pregnanolone which also showed both ions but as a very minor peaks. Accordingly, an acquisition method was used in which ions at m/z 117 and 129 were detected when a sample was loaded into the capillary column. Thus a steroid peak was identified by the presence of one or both ion fragments and also by its retention index.

Before the sample was analysed, the internal standard (2 μg) was added. This contained 60% β-sitosterol and 40% campesterol. Both steroids were of plant origin and did not interfere with the quantitative analysis of animal steroids. Also they showed 129 and, to a lesser extent, 117 ion fragments in common with other analysed steroids. The sample was, then, derivatised to

form trimethylsilyl ether (TMSi) compounds (Paragraph 6.5.1). This method was found to be easier and faster than the O-methyloxime-trimethylsilyl ether (MO-TMSi) derivatization.

For the purpose of quantitation, the areas of the ion spectra (i.e. 117 and 129 ion fragments), which corresponded to the steroids and the internal standards, were measured and compared. Thus the quantity of a steroid peak was calculated according to this equation:

$$Q_s = \frac{Q_i \cdot A_s}{A_i} \cdot R_s \qquad \text{.....Equation 7.3}$$

where Q_s = quantity of the steroid.

Q_i = quantity of the internal standard.

A_s = measured peak area for the steroid.

A_i = measured peak area for the internal standard.

R_s = response ratio

Results obtained from qualitative and quantitative analysis of liver, adrenal gland, blood and faecal material are given in Tables 7.13-7.16. Peaks were designated by numbers according to the retention indices and relative retention times. By the latter is meant the absolute retention times of steroids relative to the absolute retention time of the internal standard. Also the number of samples in which the steroids were detected is shown by numbers placed between brackets. Examples of the chromatograms based on m/z 117 and 129, for liver tissue, blood and faeces are shown in Figures 7.15 and 7.16. The following inferences were concluded from these experiments:

Table 13a: Results of GC-MS analysis of rat liver samples (continued on next page).
Number in brackets indicates incidence of peak in the chromatograms.

Peak No.	Relative retention time	Retention Index	AT THE MOMENT OF DEATH concentration ng/gm				AT TWENTY-FOUR HOURS AFTER DEATH concentration ng/gm				TENTATIVE IDENTIFICATION
			based on m/z 117		based on m/z 129		based on m/z 117		based on m/z 129		
			Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.	
1	0.35	2580	6.4(2)	-	2.4(1)	-	6.1(1)	-	12.25(2)	-	Androsterone
2	0.36	2585	2.5(3)	-	3.5(6)	1.19	None	None	3.0(1)	-	Androsterone - isomer
4	0.38	2600	7.9(1)	-	5.1(1)	-	37.2(8)	40.3	20.0(10)	23.3	Aetiocholonolone - isomer
5	0.39	2605	1.8(2)	-	3.8(5)	2.6	None	None	None	None	Aetiocholonolone - isomer
6	0.40	2610	8.0(7)	7.3	6.4(5)	7.4	5.0(2)	-	2(2)	-	DHEA- isomer
7	0.41	2615	7.3(2)	-	4.3(4)	4.2	19.5(8)	22.11	25.5(7)	23.9	DHEA- isomer
8	0.43	2625	6.9(2)	-	4.3(4)	0.7	None	None	None	None	DHEA- isomer
9	0.44	2630	8.3(4)	7.8	3.4(1)	-	7.4(3)	-	6.6(1)	-	DHEA- isomer
10	0.45	2635	2.9(2)	-	6.5(3)	-	None	None	2.6(2)	-	DHEA- isomer
11	0.46	2640	3.3(2)	-	4.2(1)	-	None	None	3.7(4)	1.2	DHEA- isomer
12	0.47	2670	None	None	None	None	None	None	6.4(6)	4.2	Androstenedione Or 11-oxoandrosterone
13	0.48	2695	5.2(2)	-	6.6(7)	5.3	None	None	6.9(1)	-	Oestrone
14	0.49	2735	19.4(1)	-	12.4(7)	15.9	None	None	2.3(1)	-	Androstenedione

Table 13a (continued)

Peak No.	Relative retention time	Retention Index	AT THE MOMENT OF DEATH concentration ng/gm based on m/z 117 Average	S.D.	based on m/z 129 Average	S.D.	AT TWENTY-FOUR HOURS AFTER DEATH concentration ng/gm based on m/z 117 Average	S.D.	based on m/z 129 Average	S.D.	TENTATIVE IDENTIFICATION
15	0.50	2770	33.5(1)	-	28.7(3)	-	38.6(3)	-	21 (6)	20.1	11ß-hydroxy-aetiocholonolone
16	0.51	2800	None	None	6.4(5)	6	None	None	8.7(6)	4.4	16-hydroxy-DHEA
17	0.52	2820	None	None	None	None	16.4(2)	-	50.9(3)	-	Pregnenolone-isomer
18	0.53	2840-2845	21.3(3)	-	5.3(7)	4.2	66.1(4)	31.6	149.6(7)	175.2	Pregnenolone
19	0.54	2850	16.5(9)	14.0	26.0(10)	28.5	81.2(10)	54.8	71.3(9)	50.0	Pregnanediol
20	0.55	2855	9.3(2)	-	5.0(1)	-	7.1(1)	-	5.6(1)	-	Pregnanediol-isomer
22	0.58	2870	15.6(5)	13.7	17.4(4)	17.8	42.1(4)	60.4	2.3(2)	-	Pregnantriol
23	0.60	2880-2885	11.1(3)	-	11.9(4)	6.6	69.1(2)	-	21.7(9)	19.5	Pregnenediol
24	0.62	2890	11.1(8)	8.0	3.8(3)	-	12.5(8)	3.5	6.2(1)	-	Pregnenediol-isomer
25	0.63	2900	28.2(4)	15.6	18.7(4)	16.8	31.5(9)	23.0	23.5(7)	10.1	oestriol-isomer
26	0.64	2910	2.7(1)	-	3.2(4)	2.1	5.1(3)	-	7.0(4)	3.1	oestriol
29	0.67	2925	7.9(1)	-	7.8(1)	-	17.5(1)	-	64.3(1)	-	prostesterone
30	0.68	2930	None	None	2.1(2)	-	6.8(2)	-	None	None	

Table 13a (continued)

Peak No.	Relative retention time	Retention Index	AT THE MOMENT OF DEATH concentration ng/gm based on m/z 117 Average	S.D.	based on m/z 129 Average	S.D.	AT TWENTY-FOUR HOURS AFTER DEATH concentration ng/gm based on m/z 117 Average	S.D.	based on m/z 129 Average	S.D.	TENTATIVE IDENTIFICATION
31	0.69	2935	None	None	None	None	7.4(2)	-	None	None	
33	0.71	2945	6.1(5)	2.6	None	None	None	None	9.5(2)	-	
34	0.72	2950	None	None	None	None	18.9(4)	12.8	3.7(1)		
35	0.73	2955	7.1(1)	-	None	None	None	None	None	None	
36	0.74	2960	None	None	2.1(3)	-	18.0(1)	-	7.1(6)	3.4	
37	0.76	2970	None	None	1.6(1)	-	None	None	8.0(1)	-	
38	0.77	2975	181.2(3)	-	5.0(3)	-	11.8(2)	-	10.1(8)	7.1	
39	0.79	2990	None	None	None	None	None	None	16.3(1)	-	
40	0.80	2995	5.6(1)	-	None	None	None	None	None	None	Pregnenetriol
41	0.81	3000	None	None	None	None	10.6(1)	-	10.1(1)	-	Pregnenetriol-isomer
42	0.82	3005	10.7(2)	-	4.5(1)	-	None	None	None	None	THE-isomer
43	0.83	3010	13.5(6)	6.6	16.7(4)	21.5	34.9(10)	23.4	14.1(10)	17.2	Cholesterol
44	0.84	3020	None	None	None	None	None	None	20.8(1)	-	Allo-tetrahydro-corticosterone
46	0.94	3085	None	None	None	None	None	None	2.0(1)	-	α-cortolone
47	0.98	3120	15.1(1)	-	13.3(2)	-	None	None	None	None	ß-cortolone

Table 7.14: Results of GC-MS analysis of rat adrenal samples (continued on next page).
Number in brackets indicates incidence of peak. Peaks Nos. represent the same GC peaks as in Table 7.13a.

Peak No.	Relative retention time	Retention Index	AT THE MOMENT OF DEATH concentration ng/gm based on m/z 117	AT THE MOMENT OF DEATH concentration ng/gm based on m/z 129	AT TWENTY-FOUR HOURS AFTER DEATH concentration ng/gm based on m/z 117	AT TWENTY-FOUR HOURS AFTER DEATH concentration ng/gm based on m/z 129
1	0.35	2580	57.4(2)	37.0(1)	56.2(1)	None
2	0.36	2585	None	11.8(1)	None	None
3	0.37	2590	None	50.0(1)	None	None
4	0.38	2600	None	None	46.0(1)	None
9	0.44	2630	None	None	38.0(1)	33.1(1)
10	0.45	2635	None	9.8(1)	None	None
11	0.46	2640	None	None	61.9(2)	12.8(1)
13	0.48	2695	None	58.1(1)	47.1(1)	59.7(1)
14	0.49	2735	None	22.0(1)	None	39.1(2)
15	0.50	2770	None	66.8(1)	None	None
18	0.53	2840	None	None	192.6(2)	47.9(1)
19	0.54	2850	174.1(1)	None	278.8(2)	None
21	0.57	2860	None	None	None	35.5(1)
23	0.60	2880	121.2(1)	66.5(1)	562.2(1)	None
24	0.62	2890	193.1(1)	None	None	None
26	0.64	2910	43.9(1)	None	119.8(1)	None

Table 7.14 (continued)

Peak No.	Relative retention time	Retention Index	AT THE MOMENT OF DEATH concentration ng/gm based on m/z 117	AT THE MOMENT OF DEATH concentration ng/gm based on m/z 129	AT TWENTY-FOUR HOURS AFTER DEATH concentration ng/gm based on m/z 117	AT TWENTY-FOUR HOURS AFTER DEATH concentration ng/gm based on m/z 129
33	0.71	2945	15.5(1)	None	None	None
34	0.72	2950	None	None	None	11.4
39	0.79	2990	31.4	12.8(1)	None	None
43	0.83	3010	18.5(1)	183.9(1)	None	157.4(1)

Table 7.15: Results of GC-MS analysis of rat blood samples (continued on next page).
Number in brackets indicates incidence of peak. Peak Nos. represent the same GC peaks as in Table 7.13a

Peak No.	Relative retention time	Retention Index	AT THE MOMENT OF DEATH concentration ng/gm based on m/z 117 Average	S.D.	based on m/z 129 Average	S.D.	AT TWENTY-FOUR HOURS AFTER DEATH concentration ng/gm based on m/z 117 Average	S.D.	based on m/z 129 Average	S.D.
1	0.35	2580	19.4(2)	-	15.2(3)	-	126.2(1)	-	31.4(2)	-
2	0.36	2585	60.9(1)	-	1.4(1)	-	None	None	13.5(2)	-
3	0.37	2590	165.7(1)	-	11.2(3)	-	14.4(1)	-	20.0(7)	10.6
4	0.38	2600	26.5(1)	-	33.2(1)	-	None	None	None	None
5	0.39	2605	2.7(2)	-	25.2(1)	-	7.0(2)	-	72.7(5)	64.4
6	0.40	2610	1.9(2)	-	1.4(2)	-	4.4(1)	-	28.3(1)	-
9	0.44	2630	2.3(2)	-	0.4(1)	-	None	None	15.7(1)	-
11	0.46	2640	None	None	None	None	35.9(3)	-	25.8(10)	16.7
12	0.47	2670	None	None	8.6(1)	-	None	None	None	None
13	0.48	2695	2.4(1)	-	2.4(2)	-	None	None	28.2(6)	16.8
14	0.49	2735	None	None	None	None	16.0(1)	-	34.7(7)	19.1
15	0.50	2770	None	None	2.9(3)	-	None	None	None	None
16	0.51	2800	None	None	1.7(1)	-	None	None	19.6(1)	-
17	0.52	2820	None	None	None	None	69.8(2)	-	33.4(1)	-
18	0.53	2845	34.1(5)	17.1	5.5(7)	8.8	88.3(3)	-	117.7(4)	150.4
19	0.54	2850	67.1(3)	-	3.8(4)	5.0	345.4(8)	313.3	31.4(8)	31.9

Table 7.15 (continued)

Peak No.	Relative retention time	Retention Index	AT THE MOMENT OF DEATH concentration ng/gm based on m/z 117 Average	S.D.	based on m/z 129 Average	S.D.	AT TWENTY-FOUR HOURS AFTER DEATH concentration ng/gm based on m/z 117 Average	S.D.	based on m/z 129 Average	S.D.
22	0.58	2870	22.8(2)	-	2.9(4)	1.8	103.2(1)	-	30.0(3)	-
23	0.60	2885	21.0(2)	-	3.3(2)	-	254.0(1)	-	11.9(2)	-
24	0.62	2890	10.6(1)	-	2.0(1)	-	120.7(2)	-	18.6(4)	23.5
25	0.63	2900	36.0(1)	-	13.6(2)	-	77.2(1)	-	10.8(4)	5.8
26	0.64	2910	69.1(4)	108.4	1.8(2)	-	None	None	None	None
27	0.65	2915	16.2(2)	-	1.1(2)	-	None	None	12.9(2)	-
28	0.66	2920	None	None	2.1(1)	-	None	None	12.6(3)	-
29	0.67	2925	None	None	None	None	79.9(2)	-	13.6(3)	-
31	0.69	2935	None	None	2.4(1)	-	None	None	6.9(3)	-
33	0.71	2945	0.9(1)	-	None	None	211.2(1)	-	15.0(3)	-
35	0.73	2955	27.4(2)	-	1.8(1)	-	None	None	None	None
36	0.74	2960	None	None	None	None	None	None	11.5(1)	-
41	0.81	3000	2.9(1)	-	3.5(1)	None	None	None	None	None
43	0.83	3010	10.4(3)	-	19.1(5)	23.7	289.4(5)	164.2	66.1(4)	55.7

Table 7.16: Results of GC-MS analysis of rat faecal samples (continued on next page).
Number in brackets indicates incidence of peak. Peak Nos. represent the same GC peaks as in Table 7.13a

Peak No.	RELATIVE RETENTION TIME	Retention Index	AT THE MOMENT OF DEATH concentration ng/gm based on m/z 117 Average	S.D.	based on m/z 129 Average	S.D.	AT TWENTY-FOUR HOURS AFTER DEATH concentration ng/gm based on m/z 117 Average	S.D.	based on m/z 129 Average	S.D.
2	0.36	2585	None	None	12.4(2)	-	None	None	4.5(2)	-
3	0.37	2590	None	None	None	None	None	None	6.2(1)	-
4	0.38	2600	7.4(4)	8.8	13.1(2)	-	4.1(1)	-	37.3(2)	-
6	0.40	2610	13.0(6)	21.9	40.5(4)	42.3	7.3(3)	-	16.2(4)	13.4
7	0.41	2615	71.0(1)	-	56.9(3)	-	None	None	22.1(2)	-
8	0.43	2625	None	None	16.7(4)	12.7	None	None	20.3(2)	-
12	0.47	2680	5.7(2)	-	59.0(4)	38.3	11.7(1)	-	27.1(2)	-
13	0.48	2695	42.1(2)	-	23.4(6)	20.2	31.7(1)	-	21.2(5)	8.7
14	0.49	2735	123.4(3)	-	50.0(4)	65.3	None	None	None	None
15	0.50	2770	None	None	51.6(1)	-	61.1(3)	-	54.9(4)	73.6
16	0.51	2800	53.4(1)	-	56.9(2)	-	None	None	38.5(1)	-
17	0.52	2820	None	None	None	None	None	None	245.7(1)	-
18	0.53	2845	55.7(3)	-	29.8(10)	22.8	33.5(3)	-	20.5(8)	9.3
19	0.54	2850	70.9(5)	71.4	27.3(3)	-	72.9(4)	61.1	31.9(3)	-
22	0.58	2870	54.7(3)	-	25.3(4)	21.2	None	None	13.0(2)	-

Table 7.16 (continued)

Peak No.	Relative retention time	Retention Index	AT THE MOMENT OF DEATH concentration ng/gm based on m/z 117 Average	S.D.	based on m/z 129 Average	S.D.	AT TWENTY-FOUR HOURS AFTER DEATH concentration ng/gm based on m/z 117 Average	S.D.	based on m/z 129 Average	S.D.
23	0.6	2880	60.0(3)	-	None	None	36.3(1)	-	None	None
24	0.62	2890	182.1(7)	200	66.7(6)	72.3	130.0(7)	152.8	21.0(3)	-
25	0.63	2900	115.2(4)	92.3	21.1(3)	-	75.6(6)	44.5	28.3(3)	-
26	0.64	2910	193.7(4)	105.1	27.2(5)	10.5	None	None	None	None
29	0.67	2925	85.6(5)	87.7	80.6(6)	106.8	90.1(3)	-	26.6(3)	-
32	0.70	2940	None	None	54.4(4)	80.0	None	None	None	None
34	0.72	2950	1586.5(5)	1462	59.3(4)	44.6	883.9(3)	-	80.3(3)	-
37	0.76	2970	131.3(6)	-	106.1(4)	165.6	8.9(1)	-	43.7(3)	-
38	0.77	2975	None	None	19.6(2)	-	None	None	None	None
41	0.81	3000	494.3(4)	370.3	None	None	574.5(3)	609.3	91.3(1)	-
43	0.83	3010	207.2(4)	145.1	25.2(1)	-	100.7(3)	-	77.9(1)	-
45	0.85	3025	None	None	None	None	None	None	54.0(2)	-
47	0.98	3120	14.9(1)	-	None	None	35.9(1)	-	None	None

Table 7.13b: Values of z and P for comparison of chromatographic peak numbers 18, 19 and 13 for liver, which were shown in Table 7.13a.

PEAK NUMBER	m/z 117		m/z 129	
	z	P%	z	P%
18	4.9	<0.2	90.1	<0.2
19	14.6	<0.2	4.8	<0.2
23	8.2	<0.2	4.6	<0.2

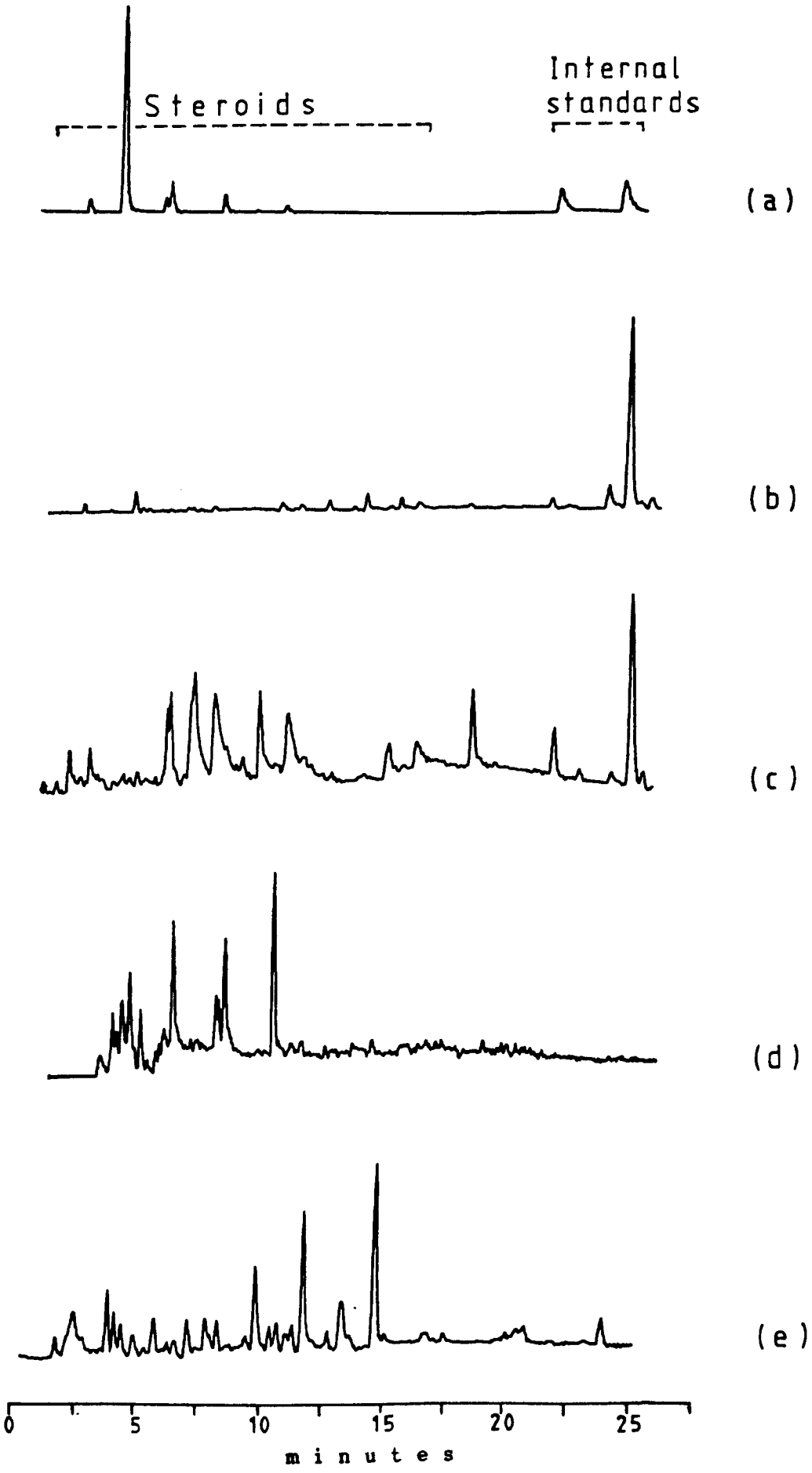


Figure 7.15: Selected ion chromatograms for m/z 129 obtained during GC-MS analysis of steroid extracts from: standard (a), liver (b) and (c), blood (d) and faeces (e).

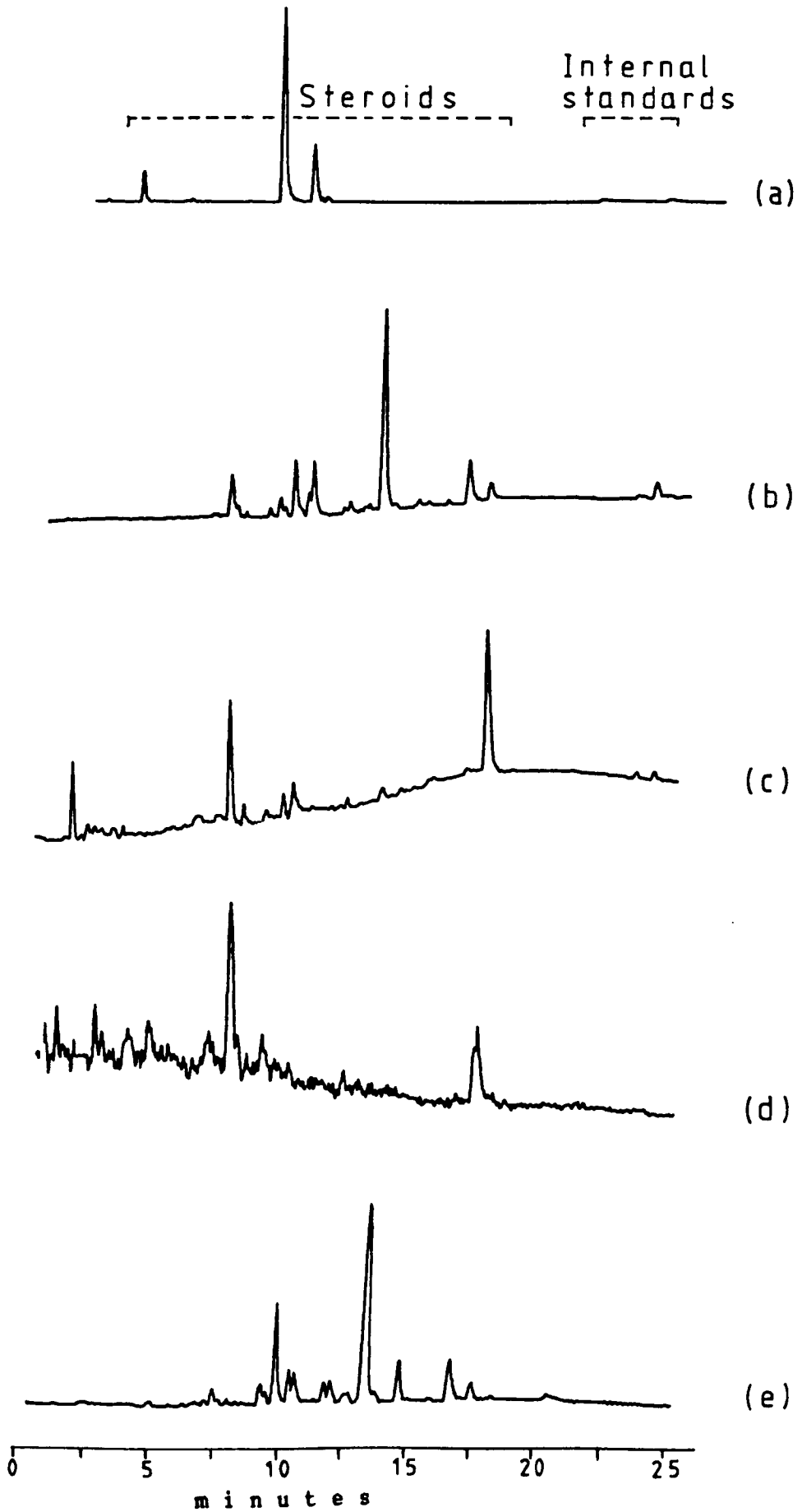


Figure 7.16: Selected ion chromatograms for m/z 117 obtained during GC-MS analysis of steroid extracts from: standard (a), liver (b) and (c), blood (d) and faeces (e).

1. Although an enormous effort was put into the animal experiments and the subsequent GC-MS analysis, the practical usefulness of the results obtained for estimating the post-mortem interval was very limited. For instance, identification of the peaks was tentative and by no means definite. This was due to the restriction imposed by the available facilities of analysis and in particular, software of the SIM technique. Also the technical problems of sample purification resulted in the appearance of many peaks, some of which were probably impurities rather than steroids. For example, in Table 7.13 Peak Nos. 10, 11, 13 and 24 were seen, at the moment of death, in ion chromatograms for both m/z 117 and 129 but at 24 hours post-mortem only peaks for m/z 129 were present. Also peak No. 36 was present for 1-24 hours after death, in ion chromatograms for both fragment m/z 117 and 129 but at the moment of death, it was only seen for m/z 129. Many similar examples were seen in the other tables.
2. If the tentative identification was correct one could say that steroid profiles of biological samples obtained at the moment of death and at 24 hours post-mortem were not the same. It was noted that most changes were quantitative rather than qualitative. This probably reflected technical problems of peak identification rather than an actual situation. However, the statistical significance of these changes

was not certain due to the small number of data encountered in most instances.

3. In spite of the above, there were a few examples where changes at 24 hours after death were more obvious and possibly significant (Table 7.13b). For example, in liver, peak Nos. 18, 19 and 23 (Table 7.13a, tentatively identified as pregnenolone, pregnanediol and pregnenediol respectively) showed moderate to sharp increase at 24 hours post-mortem but peak No. 38 (probably related to progesterone or pregnenetriol) showed a sharp decrease. Similar changes were seen in the blood (Table 7.15, peak Nos. 18 and 19 but not 23). Also in both liver and blood, cholesterol showed a clear increase at 24 hours after death.

CHAPTER 8 : DISCUSSION

8.1 Limitations of Biochemical Methods for Estimating the time of Death

Several investigators have thought that post-mortem chemical changes may provide a solution for the problem of estimating the time since death [94,400], but it was soon realised that the usefulness of biochemical methods to fulfil this aim is limited [316]. Theoretically, if a post-mortem chemical change is to be used reliably as an indicator of the time of death, it should satisfy the criteria:

1. The normal value or range of values of the indicator in a biological sample should be known.
2. The change that takes place in the indicator after death should be dependent on the post-mortem interval and not on other factors such as the cause and the manner of death, or at least the effects of these factors on the indicator should be able to be excluded reliably.
3. The chemical change concerned should occur regularly in all situations and individuals or else the variations of the occurrence and the speed of the change should be low and their magnitudes known.
4. The change should be independent of the intrinsic and extrinsic temperatures and other body and environmental circumstances.

These points and others were discussed by

Lundquist [316] who also pointed out that these criteria were hardly satisfied in the field. In fact these criteria represent some of the limitations of biochemical methods for estimating the post-mortem interval. For instance, knowledge of the normal values of chemical constituents of body fluids and tissues is not always possible: strictly speaking, normal values of the chemicals of vitreous humor are really impossible to obtain during life. Also, there are well-documented variations in normal values of chemical constituents of blood and vitreous humor depending on the site of the withdrawal [41,101,198,239]. In the case of cerebrospinal fluid, the difficulty is to obtain a sample which is free from blood or other contaminants. In addition to that, methods which depend upon the latter fluid are not applied to all cases of head and spinal injuries. Obviously, in many of these cases, which are commonly encountered in forensic patholgy, the question of timing of death is likely to be raised.

Another limitation is derived from the fact that the altered functions of the body during the process of dying or agony may produce abnormal changes in the chemical constituents of body fluids of tissues which are not normally known [316,332,400,438]. This is one reason why animal samples were obtained at the moment of death in the present study and steroids were quantitatively determined for this time. The other reason is that, for many steroids normal values are not known. This is

particularly true for tissues. Many other limitations exist, such as: the continuation of cellular activity during the lapse between somatic and cellular death; the post-mortem action of enzymes and microbials and alterations in the permeability of the dying cell.

Most important of all is the limitation caused by the dependency of many post-mortem chemical changes on the temperature of both the body and the environment [108,316, 400,438]. It has been suggested by some authors that the most useful substances to be studied and used as indicators of time of death are those which are either independent of less sensitive to environmental temperature [316]. It was anticipated that steroidal hormones and metabolites might provide such indicators as discussed below.

8.2 Study of Post-mortem Changes of Steroids

In this research steroid hormones and their metabolites were chosen to be investigated for the following reasons:

1. Steroids have never been studied for the purpose of timing of death. In fact, very little is known about steroidal changes after death.
2. It was supposed, on a scientific basis, that micro-organisms, whether they were present in the body or invaded it after death, might have considerable effect on the steroid profiles of body fluids and tissues. As a matter of fact, there exists

accumulating evidence indicating the important role of micro-organisms in the biosynthesis of post-hepatic steroids in life. For instance, it was found by many workers that the excreted steroids in faeces differed markedly from those presented in bile and that the intestinal micro-flora participated in the entero-hepatic metabolism of steroid hormones [67,247,373]. Other studies showed that metabolism of steroid hormones and bile acids in germ-free and conventional rats, in vivo, was widely different [146,151-154,211, 212,215,266,180]. These studies had demonstrated also that the biological half-lives of steroids were shorter in conventional than in germ-free rats, that plasma levels of some steroids were higher in germ-free than in conventional rats and that the patterns of steroids in faeces and urine samples obtained from germ-free and conventional rats were quite different [146,373]. Two other post-mortem changes might have qualitative and/or quantitative effects on steroids after death. These were the lack of molecular oxygen and also the possible alterations of the enzymes involved in steroid biosynthesis and metabolism.

3. It was hoped that steroid studies might be of value for the estimation of the late post-mortem interval. This was due to two reasons: firstly, steroids were known to be relatively less sensitive to variations in temperature and conditions of storage [538] than many

substances. Analytically, steroids were more resistant to high temperature than many substances. Secondly, the results found by Finlayson indicated that cortisol concentrations in blood were stable for at least 18 hours after death.

This research was conducted in animals so that conditions of study could easily be controlled. An important restriction on conducting the investigations in human beings was the ignorance of the effect of dying processes on steroids as discussed above. Using an animal model, steroids could be determined at the moment of death both qualitatively and quantitatively. This could form the basis for comparison, so that the changes might be detected at subsequent intervals after death. The rat was chosen as a suitable animal model because steroid biochemistry in the rat has been studied in some detail in the past and found to be similar in most respects to that in humans. In addition, it was convenient from the practical viewpoint.

As this study was preliminary and little was known regarding steroids after death, it was planned that investigations would be performed initially at two intervals: the moment of death and twenty-four hours post-mortem. If results were found to be encouraging further investigations, at intermediate intervals, would be required.

8.3 Analytical Method

Steroids in complex biological samples such as tissues have been infrequently studied. The main obstacle is the lack of an efficient analytical method. Difficulties of steroid analysis in these samples are caused by the existance of many impurities which have polarities similar to those of steroids. These contaminants are present ordinarily in much higher concentrations than steroids themselves. As these impurities interfere with the subseqent gas chromatography-mass spectromemtry (GC-MS) of steroids very extensive purification procedures are required. Purification should not cause large steroid losses. Also, very low concentrations of steroid require a highly sensitive and selective detection technique.

8.3.1 Establishment of the Method

For the reasons discussed above, a large part of this research lay in the preparatory experiments to find a multicomponent method to analyse steroid profiles of tissue, blood and faecal material. Before this was achieved, several techniques published by other authors were attempted and their applicability to this study was assessed. This was described in Paragraph 6.2. Some procedures were disregarded due to their impracticability. For instance, the classical method of steroid extraction and purification in separating funnels was found cumbersome and time-consuming; also, it resulted in unpredictable steroid losses due to emulsion formation.

One standard criteria was to find a relatively rapid method. Therefore extraction and purification by the Amberlite XAD-2 column were not considered for subsequent experiments. This was because the flow rate in eluting this column was very slow (0.2 ml/minute). Other procedures, which were found useful, were modified according to the requirements of this study. For example, four to six Sep-Pak C_{18} cartridges, or the equivalent amount of the packing material, were used instead of a single cartridge because impurities in our samples were much greater in quantity than in urine and one cartridge was rapidly overloaded. A single cartridge has been used for urine and some authors have suggested that up to 100 ml urine can be used with one cartridge [455]. However, it was found in this research that the steroid recoveries were significantly greater using 4-6 cartridges than a single cartridge (Tables 7.4-7.6 and Figures 7.7-7.10. z values were 5.5, 25.5, 3.9 for tissue, blood and faeces, respectively, therefore P was <0.2% in all samples).

8.3.2 <u>The Use of Reversed Phase Chromatography</u>

A great part of the impurities present in the samples was found to be of non-polar lipid. This reflected the wide distribution of large quantities of cholesterol in blood and tissues. The function of the reversed phase column chromatography was to remove these non-polar lipids. An efficient reversed phase system should be able to separate steroids from non-polar lipids completely. Also it should recover steroids in high

percentages. A column of hydroxyalkoxypropyl Sephadex (Lipidex-5000) packed in methanol/water/chloroform (90:10:20 v/v/v) was used to satisfy these criteria. Other reversed phase solvent systems were assessed as described earlier (Paragraphs 6.2.2 and 7.2.1). These included methanol/hepatane (95:5 v/v) and methanol/ water/heptane (95:10:5 v/v/v). Results showed that these systems were similar in respect of their abilities to remove cholesterol and recover steroids. However, methanol/water/heptane system was less efficient in this respect than the other two systems. This was because the extract was less soluble in this system than in the other systems, also water was not readily admixed with heptane. Only the methanol/water/chloroform system was used in subsequent experiments. This was for two reasons. Firstly, in this method steroids were extracted from tissue, blood and faecal material into an organic solvent mixture consisting of methanol and chloroform in equal parts by volume; because of this, the residue obtained at the end of the extraction step was more readily dissolved in the methanol/water/chloroform system than in the other two systems which obviously simplified the application of the sample onto the Lipidex-5000 column. Secondly, this type of reversed phase system is widely used by many authors and the results obtained by its use are well confirmed and documented [31]. However, the disadvantage of this system was the difficulty in evaporating its water constituent; this problem was overcome by the addition of

methanol to the round-bottomed flask before of evaporation with a rotary evaporator.

8.3.3 Difficulties of Radioactivity Counting

The efficiency of any spectrometer in counting radioactivity usually depends on many factors, such as concentration of the quenching material, the nature of the sample and also the many different conditions under which analysis is conducted. This means that these factors are too wide to be controlled or compensated for. The quenching correction curves (Figure 7.2), which were developed in this study, were not found to be of practical value. For instance, when these curves were used in some subsequent experiments, several collected AES ratios (i.e. the Y-axis data by which the efficiency was calculated from the quenching correction curve) were found to fall outside the curve. Sometimes the predicted efficiency was unusually high so that the predicted radioactivity (calculated according to the curves) was greater than the radioactivity added to, or present in, the sample. This wide variability in the counting efficiency was thought to be due to either slight differences in the conditions of the experiments or, more likely, to faulty operation of the machine. To avoid this problem, another computerized scintillation spectrometer was used in all subsequent experiments. In fact the radioassays for the assessment of the analytical method were totally dependent on the latter spectrometer.

8.3.4 Enzymatic Hydrolysis and Acidic Solvolysis

Steroids are usually present in conjugated forms in biological materials, either as sulphates or as glucuronides [173,195-197,203]. Gas chromatography-mass spectrometry (GC-MS) requires that these conjugates should be cleaved. This is the function of enzymatic hydrolysis (enzymolysis). For steroid hydrolysis, two techniques existed: the traditional technique is slow and requires that the sample/enzyme mixture is incubated at 37°C for 24-48 hours. The other technique is rapid and requires incubation at a higher temperature (usually 50-60°C) for 3-4 hours [16,28,30]. As steroid recovery from both techniques was similar (Table 7.8-7.10), the rapid technique was used in all subsequent experiments. It is worth noting that similar conclusions have been reported by some other investigators [16,30].

The disadvantage of the traditional hydrolysis is not only its requirement for long periods of incubation but also it may result in uncontrolled losses of steroids because it depends on a rate-limiting step. This has been pointed out by some authors [30].

As steroid sulphates are usually more stable than glucuronide conjugates, the former are incompletely freed by the action of the sulphatase during the enzymatic hydrolysis. Therefore, acidic solvolysis is required to free the remainder of the steroid sulphates. Similar to enzymolysis, acid solvolysis is performed either slowly (incubation at 37-39°C for 16 hours) or rapidly by

incubation at about 50-53°C for 1-2 hours. When both techniques were assessed in this study results were found to be similar (Tables 7.8-7.10). This agreed with the results of some other authors [30]. However, solvolysis did not recover steroid sulphates perfectly (i.e. 100%) and the improvement achieved by including solvolysis compared to enzymolysis alone was not significant. Accordingly, acidic solvolysis was considered as an optional step, but it was an essential one when it was followed by Amberlyst A-26 column chromatography as a final purification procedure. This was because the later column had a poor reproducibility, selectively, for some steroid sulphates [454]. Because of these difficulties, final recoveries of DHEA (added as the sulphate) were less than those of œstradiol (added as the glucuronide conjugate) or of cortocisterone (added as unconjugated form).

8.3.5 Straight Phase Column Chromatography

The procedure and the column used in this chromatography were described in Paragraphs 6.4.8 and 7.2.3. This technique was used to get rid of impurities other than non-polar lipids. Therefore, using this procedure many fatty acids, triglycerides and organic pigments were removed. This column was considered preferable to the Amberlyst A-26 column because it was able to separate steroids into groups selectively [448,451,452]. Also using this technique, the problem of poor recoveries of steroid sulphates by the anion-

exchanger (i.e.Amberlyst A-26) column was avoided. A disadvantage of this straight phase column was that more polar steroids such as corticosterone required the column to be eluted with a large volume of the solvent mixture (usually about 160 ml or more). This consumed time and also may have decreased the purification of the sample.

8.4 Gas Chromatography - Mass Spectrometry (GC-MS)

This is correctly regarded as the most powerful and versatile method of steroid analysis [35,194,468]. Owing to the high selectivity of mass spectrometry, combined GC-MS is also the most specific method for the determination of steroids in biological samples. Thus it is used in the elucidation of the structures of steroids. Also this method is extremely sensitive so that relatively low concentration peaks can be detected. According to the nature of the sample used and the aim of this study, sensitivity and specificity of the method were essential. Firstly, the investigation concerned biological samples, particularly liver tissue and blood, containing very low concentrations of steroids, of the order of ng per gram of sample. Secondly, little was known regarding the effect of the post-mortem interval on steroids, therefore, it was anticipated that quantitative and qualitative analyses of steroids after death might be of equal importance. For these reasons combined GC-MS was used for steroid analysis in this work. However, the drawback of this method was the necessity of performing extensive purification of the biological samples as described earlier.

Mass spectrometry and combined GC-MS are discussed in many published works and textbooks [6,77,81-83,194,203, 322,468,596,533]. Discussion of technical aspects of these methods is beyond the scope of this thesis. The principles of operation of GC-MS were given in Paragraph 5.3. In summary, it is based on a form of gas-liquid chromatography (GLC) in which the steroid solute is present in the gas phase. The effluent gas is subjected to mass spectrometry using electron impact ionization or chemical ionization, so that mass spectra of the individual gas chromatographic peaks can be recorded as the components are eluted from the GC column [194,322]. Two techniques were used for GC-MS analysis: in the first the gas chromatograph eluent was scanned over the full mass range (repetitive scanning mode), while in the second, selective ion monitoring (SIM) was used. Due to the wider scanning, the first technique was less sensitive than the second. Accordingly, this was only used in the experiments performed to find the steroid indices (Paragraph 6.5.2). However, this technique was very useful for qualitative investigation and was also used to record total mass spectra of some major steroids which were relevant to this work (Table 7.7), for example to permit the selection of appropriate ions for SIM.

Selective ion monitoring was required because concentrations in the sample, particularly tissue and blood, were extremely small. Two ions m/z 117 and 129 were selected to be monitored (Figure 8.1). This had twin

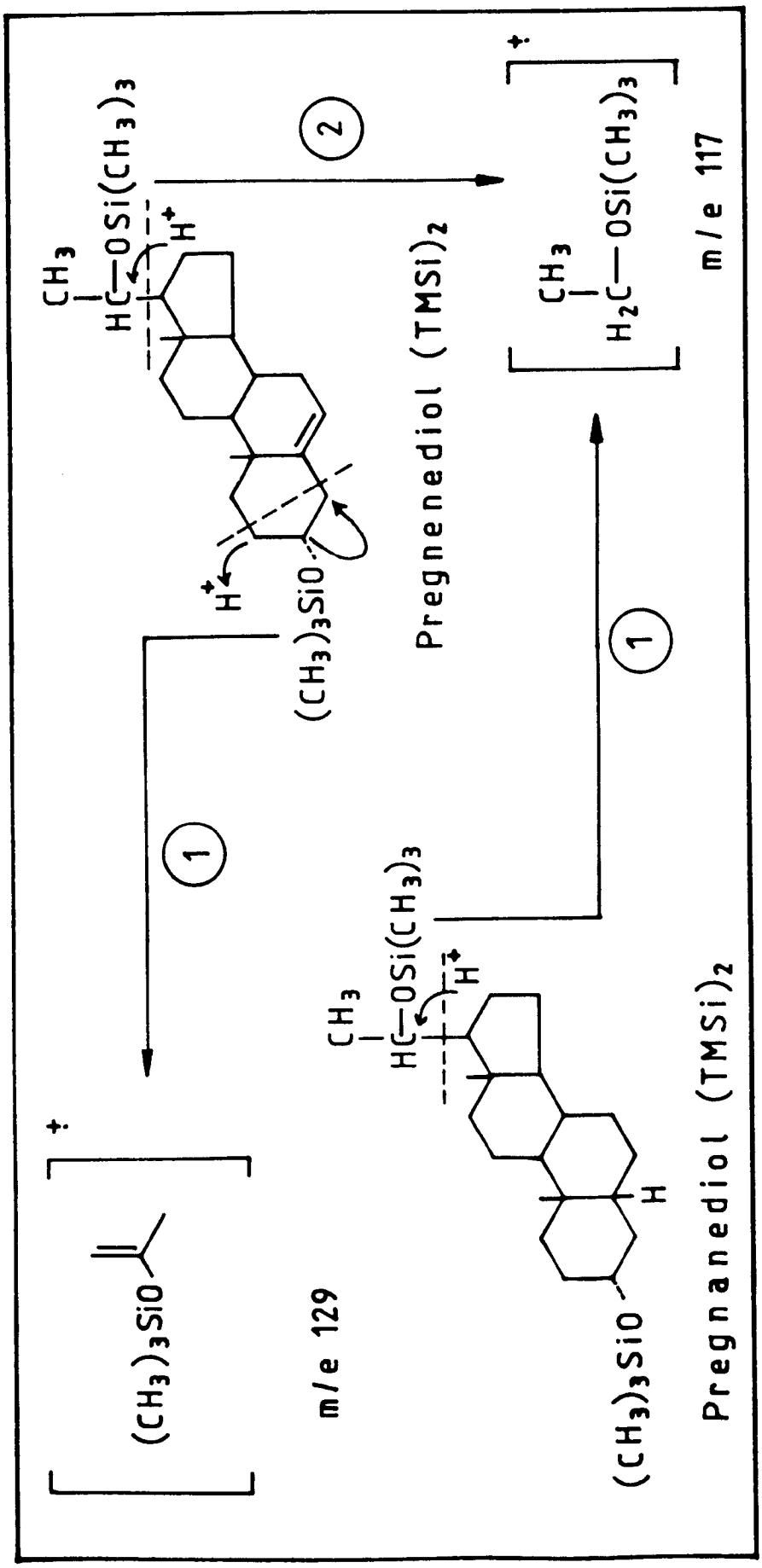


Figure 8.1: Fragmentation scheme for the formation of ions at m/z values 117 and 129.

advantages in that the detection sensitivity was very high because the mass range was narrow and also because the mass spectra of many steroids, particularly those which it was planned to study, involved one or both ions. However, the disadvantage was that these ion fragments were encountered in the mass spectra of some of the impurities. This added difficulties to the process of the peak identification, so that only tentative identification of some detected peaks was possible (Tables 7.13-7.16). To obtain more certain identification, more ions should be monitored but this was difficult and time consuming as the available software did not allow the monitoring of more than one group of ion fragments at one time. Also, if a wider mass range was used the sensitivity would not have been sufficient to detect the small quantities of steroids present.

Prior to GC-MS, the steroids were derivatized. The procedure was given earlier (Paragraph 6.5). This process was used to increase the volatility and thermal stability of steroids and improve their separation on the column. Two methods of derivatization were used, namely - to obtain either trimethylsilyl ether (TMSi), or O-methyloxime-trimethylsilylether (MO-TMSi) compounds. As corticosteroids were not included in this initial study, the former method was more commonly used because it was easier to perform than the latter. The empirical structures of TMSi compounds of some relevant steroids are shown in Figure 8.2.

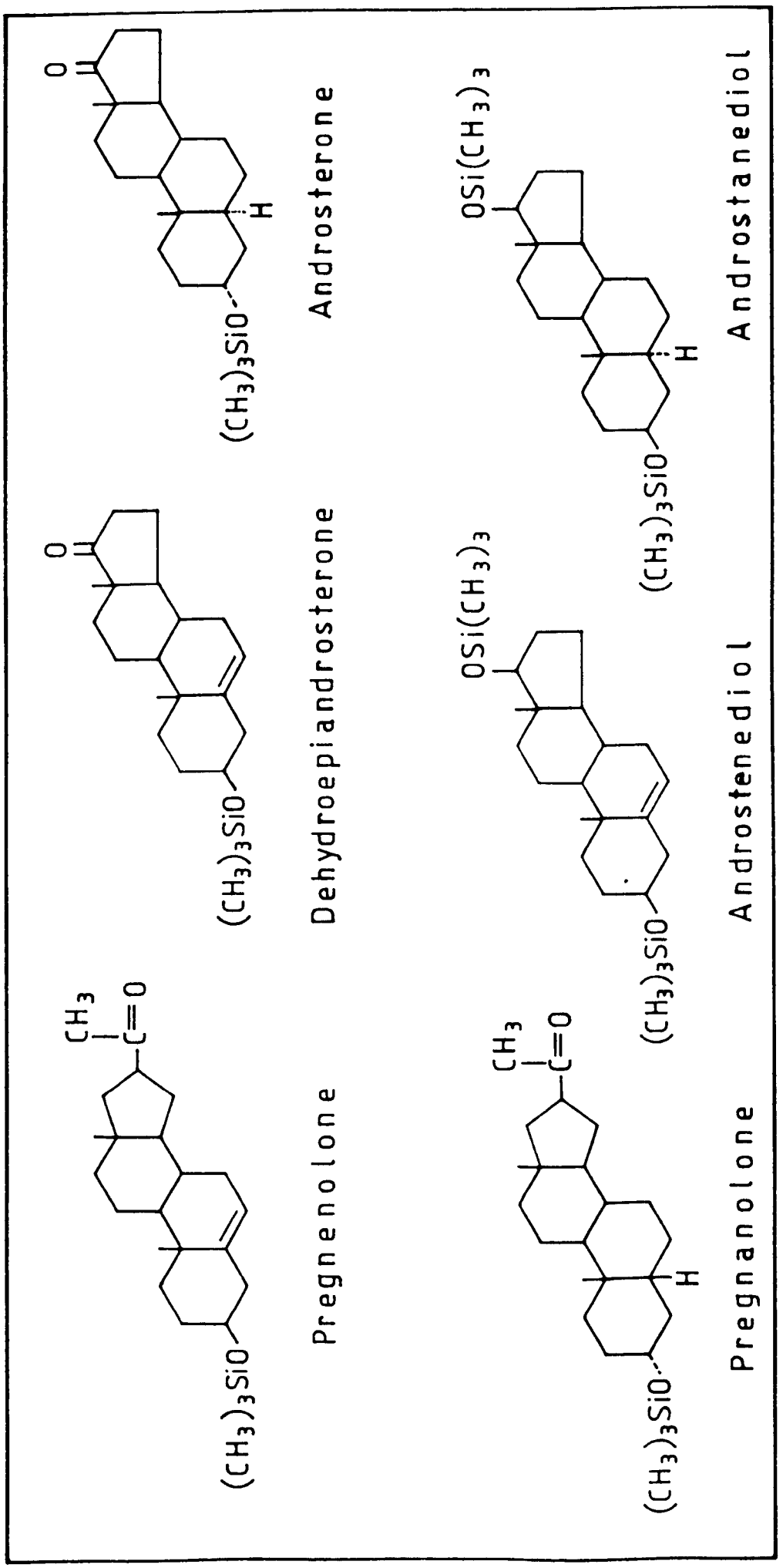


Figure 8.2: Structural formulae of some steroid trimethylsilyl ethers.

The steroid solutes need to be vaporized at suitable temperatures. Usually high temperatures are required depending on the steroid profile to be analysed. A temperature programme rate of 3°C per minute was useful to achieve relatively good separation of steroid peaks. It is worth noting that a final time of 5-10 minutes at 300°C was required to ensure that the internal standards (ß-sitosterol and campesterol) and higher boiling point impurities were eluted from the GC column, otherwise they might contaminate the next sample. However, the disadvantage was that a long time was required to run the sample (about 25-30 minutes).

8.5 Do Steroids Change after Death?

Before answering this question, it is important to discuss a few points:-

1. It is known that many of the reactions involved in the biosynthesis and metabolism of steroids require cytochrome P-450, NADPH and, most relevantly, molecular oxygen for their activity. Therefore, it was anticipated that the more likely changes to be seen after death would be related to the ratio between the oxo- and the hydroxy-groups (oxo/hydroxy group ratio). For example, changes of pregnenolone/ pregnenediol ratio. Steroid pairs of a similar type are numerous.
2. Other anticipated changes were the appearance of new metabolites or the disappearance of those already

present.

3. From the practical point of view, it was not possible to identify the peaks with certainty due to technical difficulties in SIM and sample purification. However, false peaks (non-steroidal peaks) could be identified and excluded. For example in Table 7.13a, peaks No. 19, 11, 13 and 14 seemed false because at the moment of death they were present with both m/z 117 and 129, while at 24 hours after death they were absent for m/z 117. Another example was given in Paragraph 7.4.2 and others can easily be seen in Tables 7.13a-7.16.
4. Another problem which should be noted is that the number of the measurements for many peaks was small and not enough to draw significant conclusions.
5. Bearing in mind the above restrictions, one can find a few examples where changes of steroids between the moment of death and 24 hours post-mortem, were obvious and of significance. For example, in Table 7.13a, steroids represented by peak Nos. 18, 19 and 23 were significantly increased 24 hours after death (Table 7.13b). These findings were also seen in the blood samples but not in the faeces. However, no further significance tests were made because they were not very meaningful. It is interesting to note that, according to the retention indices obtained during SIM, these peaks were tentatively identified as pregnenolone, prenanediol and pregnenediol respectively. However, in these examples, both the

oxo- and the hydroxy groups increased, which cannot be explained on the basis supposed above.

6. It was also noted that the peak, which might represent cholesterol, showed a tendency to increase after 24 hours post-mortem.

It should be emphasised that these conclusions should not be generalized unless more investigations are performed to confirm or discredit them. It is also relevant to consider recent results of analysis of the post-mortem changes in drug levels after death. Several workers have found that the observed levels of drugs, for example in blood, increase after death, possibly due to release of drug from protein binding sites [539].

Although further investigation of this problem is highly interesting and may be of importance from the academic point of view, from the practical point of view this method is of limited use to the practising forensic pathologist because it is cumbersome, time-consuming and also very expensive. Also it is likely to be unreliable as the factors that affect steroids are numerous and the variations are large, as indicated by the standard deviations of data given in the tables above.

REFERENCES

[1] Adelson, L., Sunshine, I., Rushforth, N.B. and Monkoff, M. Vitreous potassium concentration as an indicator of the post-mortem interval. J.Forens.Sci. 1963; 8:503.

[2] Ad Hoc Committee of the Harvard Medical School. A definition of irreversible coma. J.A.M.A. 1968; 205:85-88.

[3] Adjutantis, G. and Coutselinis, A. Estimation of the time of death by potassium levels in the vitreous humor. Forens.Sci. 1972; 1:55-60.

[4] Adjutantis, G. and Coutselinis, A. Changes in magnesium concentration of the vitreous humor of exenterated human eyeballs immersed in sea water. Forens.Sci. 1975; 4:63.

[5] Adlercreutz, H. Quantitative mass spectrometry of endogenous and exogenous steroids in metabolic studies in man. Quant. Mass Spectrom. Life Sci.Proc.Int.Symp.1st. 1976 (pub.1977): 15-28.

[6] Adlercreutz, H. Biomedical application of the mass spectrometry of steroid hormones. In; Quayle, A.ed. Advances in Mass Spectrometry, vol.8B, Hyden and Son Ltd., on behalf of the Institute of Petroleum, London, 1979; 1165-1179.

[7] Ahrens, E.H. Jr. Degradation of the sterol ring structure in the intestine, and the significance of this for sterol balance studies. Gastroenterology. 1966; 51(4):596-597.

[8] Akaishi, S. and Kudo, T. Medico-legal aspects of the post-mortem rectal temperature (Report 1.). The 18th Meeting of Tohoku Association of Forensic Medicine. 1961, Obaraohsen.

[9]. Akaishi, S., Murakami, S., Kudo, T. and Shimizu, M. Medico-legal studies on the post-mortem temperature (III). Jap.J. Legal Med. 1962; 16:124. (cited in Ref.No.250)

[10] Akaishi, S., Murakami, S. and Tsushida, S. Animal experiments on the post-mortem rectal temperature. The 5th Meeting of Tohoku Association of Forensic Medicine.1954, Otakionsen. (cited in Ref.No.250)

[11] Al-Alousi, L.M. and Anderson, R.A. Microwave thermography in forensic medicine. Police Surgeon No. 30, 1986; 30:30-42.

[12] Al-Alousi, L.M. and Anderson, R.A. Post-mortem interval by microwave thermography. Forens.Sci.Int.(in press), also Proceeding on the Uses of Forensic Sciences. Strathclyde University: Glasgow, 1986 (in press).

[13] Ali, W.M. Forensic Medicine: Science and Practice. (in Arabic) 5th ed.Vol.1. Baghdad: Education Press, 1976:166-169.

[14] Anderson, R.A. Studies of lipophillic dextran gels for the chromatographic analysis of steroids. Thesis. University of Glasgow, 1973.

[15] Anderson, R.A., Defaye, G., Madani, C. and Chambaz, E.M. Lipophilic gel and gas-phase analysis of steroid hormones application to the human newborn. J.Chromatography 1974; 99:485-494.

[16] Anderson, S.H.G., Axelson, M., Sahlberg, B.-L. and Sjövall, J. Simplified method for the isolation and analysis of ethynyl steroids in urine. Anal. letters 1981; 14(B10): 783-790.

[17] Anderson, S., Herbring, B.G. and Widman, B. Accidental profound hypothermia. Case report. Br.J.Anaesth.1970; 42:653.

[18] Andersson, B., Grant, R. and Larsson, S. Central control of heat loss mechanisms in the goat. Acta Physiol.Scand.1956; 37:261.

[19] Aoki, T., Yoshiura, M., Iwamoto, T. and Iriyama, K. Post-mortem changes of uric acid in various rat tissues: determination of uric acid by reversed-phase high performance liquid chromatography with electrochemical detection. Anal.Biochem. 1984; 143:119-124.

[20] Arca, M., Montali, A., Ciocea, S., Angelico, F. and Cantaford, A. An improved gas-liquid chromatographic method for the determination of faecal neutral sterols. J.Lipid Res. 1983; 24:332-335.

[21] Arnason, A. and Bjarnason, O. Post-mortem changes of human serum esterases. Acta Pathol.Microbiol.Scand.[A] 1972;80:841.

[22] Arutjonov, A.M. The use of entomological observations in forensic science. Sud.Med.Ekspert. 1963; 6:51. (cited in Ref.No.383)

[23] Astbury, W.T. and Bell, F.O. Nature of the intramolecular fold in alpha-keratin and alpha-myosin. Nature 1941; 147: 696-699.

[24] Astrup, T. The biological significance of fibrinolysis. Lancet, Lond.Sept. 1956; (ii)271(6942):565-568.

[25] Austin, G.M. and Berry, J.W. Observations on one hundred cases of heatstroke. J.A.M.A. 1956; 161:1525.

[26] Axelson, M., Clark, J.H. and Eriksson, H.A. Estrogen binding in target tissues: A GC/MS method for assessing uptake, retention and processing of estrogens in target cell nuclei under in vivo conditions. J.Steroid Biochem.1981; 14: 1253-1260.

[27] Axelson, M., Cromholm, T., Sahlberg, B.-L. and Sjovall, J. Changes in the metabolic profile of steroids in urine during ethanol metabolism in man.J.Steroid Biochem.1981; 14:155-159.

[28] Axelson, M. and Sahlberg, B.-L. Group separation and gas chromatography-mass spectrometry of conjugated steroids in plasma. J.Steroid Biochem. 1983; 18(3):313-321.

[29] Axelson, M. and Sahlberg, B.-L. Solid extraction of steroid conjugates from plasma and milk. Anal.letters 1981; 14(B_{10}):771-782.

[30] Axelson, M., Sahlberg, B.-L. and Sjovall, J. Analysis of profiles of conjugated steroids in urine by ion-exchange separation and gas chromatography-mass spectrometry. J.Chromatography 1981; 224:355-370.

[31] Axelson, M., Schumacher, G. and Sjövall, J. Analysis of tissue steroids by liquid-gel chromatography and computerized gas chromatography-mass spectrometry. J.Chromatographic Sci. 1974; 12:535-540.

[32] Axelson, M., Schumacher, G., Sjövall, J., Gustafsson, B. and Lindell, J.O. Identification and quantitative determination of steroids in bovine corpus luteum during oestrous cycle and pregnancy. Acta Endocrinologica 1975; 80:149-164.

[33] Axelson, M. and Sjövall, J. Separation and computerized gas chromatography-mass spectrometry of unconjugated neutral steroids in plasma. J.Steroid Biochem. 1974; 5:733-738.

[34] Axelson, M. and Sjövall, J. Analysis of unconjugated steroids in plasma by liquid-gel chromatography and glass capillary gas chromatography-mass spectrometry. J.Steroid Biochem.1977; 8:683-692.

[35] Axelson, M. and Sjövall, J. Gas chromatographic-mass spectrometric analysis of metabolic profiles of unconjugated steroids in biological materials. Res.Steroids 1979; 8:119-130.

[36] Axelson, M. and Sjoval, J. Strong non-polar cation exchangers for the separation of steroids in mixed chromatographic systems. J.Chromatography. 1979; 186:725-732.

[37] Baillie, T.A., Anderson, R.A., Sjövall, K. and Sjövall, J. Identification and quantitation of 16 -hydroxy C_{21} steroid sulphates in plasma from pregnant women. J.Steroid Biochem.1976; 7:203-209.

[38] Baillie, T.A., Eriksson, H., Herz, J.E. and Sjövall, J. Specific deuterium labelling and computerized gas chromatography-mass spectrometry in studies on the metabolism in vivo of a steroid sulphate in the rat. Eur.J.Biochem. 1975; 55:157-165.

[39] Back, P., Sjövall, J. and Sjövall, K. Monohydroxy bile acids in plasma in intrahepatic cholestasis of pregnancy. Identification by computerized gas chromatography-mass spectrometry. Med. Biology 1974; 52:31-38.

[40] Balasooriya, B.A.W., Hill, C.A.ST. and Williams, A.R. The biochemical changes in pericardial fluid after death: An investigation of the relationship between the time since death and the rise or fall in electrolyte and enzyme concentrations and their possible usefulness in determining the time of death. Forens.Sci.Int. 1984; 26:93-102.

[41] Balasooriya, B.A.W., Hill, C.A.ST. and Williams, A.R. The biochemistry of vitreous humor. a comparative study of the potassium, sodium and urate concentrations in the eyes at identical time intervals after death. Forens.Sci. Inter. 1984; 26:85-91.

[42] Baldwin, E. Dynamic aspects of biochemistry. London: Cambridge University Press, 1948:329-352.

[43] Barbour, H.G. Heat regulating mechanisms of the body. Physiol.Rev. 1921; 1:295.

[44] Bard, P. Body temperature regulation. In: Mountcastle, V.B. ed. Medical Physiology.Vol.1, 12th ed. Saint Louis: The C.V. Mosby Company, 1968:553-590.

[45] Bard, P. Control of systemic blood vessels. In: Mountcastle, V.B. ed. Medical Physiology. 12th ed. Vol.1. Saint Louis: The C.V. Mosby Co.,Ch.10, 1968:150-208.

[46] Bardzik, S. The efficiency of methods of estimating the time of death by pharmacological means. J.Forens.Med.1966; 13:141.

[47] Barrett, A.H., Myers, P.C. and Sadowsky, N.L. Detection of breast cancer by microwave radiometry. Radio Science. 1977; 12(Suppl.):167-171 (Microwave Radiometry).

[48] Barrett, A.H., Myers, P.C. and Sadowsky, N.L. Microwave thermography in the detection of breast cancer, AJR 1980; 134(2):365-368.

[49] Bate-Smith, E.C. and Bendall, J.R. Rigor mortis and adenosine triphosphate. J.Physiol. 1947; 106:177-185.

[50] Bazett, H.C., Alpers, B.C. and Erb, W.H. Hypothalamus and temperature control. Arch.Neurol.Psychiat. 1933; 30:728.

[51] Bazett, H.C. Blood temperature and its control. Am.J.Med.Sci. 1949; 218:483.

[52] Bazett, H.C. The regulation of body temperatures. In: Newburgh, L.H.ed. Physiology of Heat Regulation and the Science of Clothing. Philadelphia: W.B. Saunders Company, 1949; ch.4.

[53] Bazett, H.C. Theory of reflex controls to explain regulation of body temperature at rest and during exercise. J.Appl.Physiol. 1951; 4:245.

[54] Bazett, H.S., Love, L., Newton, M., Eisenberg, L., Day, R. and Forster, R. Temperature changes in blood flowing in arteries and veins in man. J.Appl.Physiol. 1948; 1:3.

[55] Bedolla-Tovar, N., Rahman, S.A., Cekar, S.Z. and Diczfalusy, E. Assessment of the speficity of norethisterone radio immunoassays. J.Steroid Biochem. 1978; 9:561.

[56] Behnke, A.R. and Yaglou, C.P. Physiological responses of men to chilling in ice water and to slow and fast rewarming. J.Appl. Physiol. 1951; 3:591.

[57] Benedict, F.G. and Carpenter, T.M. The metabolism and energy transformation of healthy man during rest. Carnegie Institute of Washington Publication No.126, 1910.

[58] Benzinger, T.H. On physical heat regulation and the sense of temperature in man. Proc.Nat.Acad.Sci. 1959; 45:645.

[59] Benzinger, T.H. The sensory receptor organ and quantitative mechanism of human temperature control in warm environment. Fed.Proc. 1960; 19, Suppl.5:32.

[60] Benzinger, T.H. and Kitzinger, C. The human thermostat. In: Herzfeld, C.ed. Temperature: Its measurement and control in science and industry. Vol.3. Part 3 New York: Reinhold Publishing Corporation, 1963:637-665.

[61] Benzinger, T.H. and Taylor, G.W. Cranial measurements of human body temperature. In: Herzfeld, C.M.ed. Temperature: its measurement and control in Science and Industry. Vol.3. Part 3 New York: Reinhold Publishing Corporation, 1963: 111-120.

[62] Bergeret (of Arbois). Ann.Hyg.Publ. 2nd ed. (Paris). Ch.4:442-452. (cited in Ref.No. 400)

[63] Björkhem, I. Microsomal 12 -hydroxylation of 7 -[12 , 12β-$^2H_2$]hydroxy-4-cholesten-3-one. Eur.J.Biochem.1975; 51:137-143.

[64] Björkhem, I., Gustafsson, J., Johansson, G. and Persson, B. Biosynthesis of bile acids in man: Hydroxylation of the C_{27} - steroid side chain. J.Clin.Inves. 1975; 55(3): 478-486.

[65] Björkhem, I. and Karlmar, K.E. 18-Hydroxylation of deoxy-corticosterone by reconstituted system from rat and bovine adrenals. Eur.J.Biochem. 1975; 51:145-154.

[66] Blumenfeld, T.A., Blome, W.A. and Devlin, J. Post-mortem vitreous humor concentrations of Na, K, Cl, Ca and Mg in children. Pediatr.Res. 1974; 8:356.

[67] Bokhenheuser, V.D., Winter, J. and Kelly, W.G. Metabolism of biliary steroid by human faecal flora. Am.J.Clin.Nutr. 1978; 31(10,suppl.):221-226.

[68] Bolliger, A. and Carrodus, A.L. Creatine retention in blood and cerebrospinal fluid. Med.J.Aust. 1938; 1:69.

[69] Bonte, W. and Bleifuss, J. Post-mortem dating of putrefied material through ptomaine estimation. J.Forens.Sci. 1977; 22(3):558-572.

[70] Bradlow, H.L. Extraction of steroid conjugates with a neutral resin. Steroids 1968; 11:265-272.

[71] Bradlow, H.L. Modified technique for the elution of polar steroid conjugates from Amerlite XAD-2(R). Steroids 1977; 30(4):581-583.

[72] Bray, M., Luke, J.L. and Blackbourne, B.D. Vitreous humor chemistry in deaths associated with rapid chilling and prolonged freshwater immersion. J.Forens.Sci., JFSCA, 1983; 28(3):588-593.

[73] Brazinsky, J.H. and Kellenberger, R.E. Comparison of immunoglobulin analyses of ante-mortem and post-mortem sera. Am.J. Clin.Pathol. 1970; 54:622.

[74] Brebner, D.F., Kerslake, D.M. and Waddle, J.L. The diffusuon of water vapour through human skin. J.Physiol. 1956; 132:225-231.

[75] Breuer, H. Über die Warneleitung dis muskels und fettes. Arch.ges.Physiol. 1924; 204:442. (cited in Ref.No. 223)

[76] British Medical Association Special Committee. Accidental hypothermia in the elderly. Br.Med.J.1964; ii(2):1255-1258.

[77] Brooks, C.J.W. and Middledich, B.S. Mass spectrometry in steroid analysis. In: Heftmann, E.ed. Modern methods of Steroid Analysis. Academic Press: New York, London 1973:139-198.

[78] Brown, A., Hicks, B., Knight, B. and Nokes, L.D.M. Determination of time since death using the double exponential cooling model. Med.Sci.Law 1985; 25(3):223-227.

[79] Brown, A. and Marshall, T.K. Body temperature as a means of estimating the time of death. Forens.Sci.1974;4:125-133.

[80] Brunner, Ch.A. Collaborative study of the analysis of mestranol in combination with norethindrone or norethynodrel. J.Ass.Off.Anal.Chem. 1971; 54(3): 590-592.

[81] Budzikiewicz, H. Biochemical application of mass spectrometry In: Waller, R.G.ed. Biochemical Applications of Mass Spectrometry, Wiley - Interscience: New York, 1972: 251-290.

[82] Budzikiewicz, H., Djerassi, C. and Williams, D.H. Structure Elucidation of Natural Products by Mass Spectrometry, Vol.II, Holden-Day: San Francisco, 1964.

[83] Budzikiewicz, H., Djerassi, C. and Williams, D.H. Mass Spectrometry of Organic Compounds, Holden-Day: San Francisco, 1967.

[84] Buettner, K. Diffusion of water and water vapour through human skin. J.Appl. Physiol. 1953; 6:229-242.

[85] Burgh, G.E. and Winsor, T. Rate of insensible perspiration (diffusion of water) locally through living and through dead human skin. Arch.Intern.Med. 1944; 74:437-444.

[86] Burman, J.W. The cooling of the body after death. Br.Med.J. 1874; March I:408.

[87] Burman, J.W. On the rate of cooling of the human body after death. Edinb.Med.J. 1880; 25:993.

[88] Burton, A.C. Human calorimetry: Average temperature of tissues of body. J.Nutr.1935; 9:261.

[89] Burton, A.C. Temperature regulation. Ann.Rev.Physiol. 1939; 1:109.

[90] Burton, A.C. Operating characteristics of the human thermoregulatory mechanism. In: American Institute of Physics ed. Temperature: Its Measurement and Control in Science and Industry. New York: Reinhold Publishing Corporation, 1941: 522-528.

[91] Burton, A.C., Snyder, R.A. and Leach, W.G. Damp cold vs. dry cold specific effects of humidity on heat exchange of unclothed man. J.Appl.Physiol. 1955; 8:269.

[92] Burton, J.F. Fallacies in the signs of death. J.Forens.Sci. 1974; 19:529-534.

[93] Cameron, I.L., Finnie, M.F. and Fullerton, G.D. Post-mortem changes in skeletal muscle proton spine-lattice relaxation times. Physiol.Chem.and Physics and Medical NMR.1984; 16:351-354.

[94] Camps, F.E. Establishment of the time of death: a critical assessment. J.Forens.Sci. 1959; 4:73-82.

[95] Carlsten, A. and Grimby, G. Thermal gradients between rectum, oesophagus and right heart blood in man. Preliminary report. Acta.Physiol.Scand. 1957; 42(suppl.145):29.

[96] Carlsten, A. and Grimby, G. Rapid changes in human right heart blood temperature at variation in venous return. Scan.J.Clin. Lab.Invest. 1958; 10:397.

[97] Carslaw, H.S. and Jaeger, J.C. Conduction of Heat in Solids. 2nd ed. Oxford: The Clarendon Press, 1959:1-50.

[98] Carslaw, H.S. and Jaeger, J.C. Conduction of heat in solids. 2nd ed. Oxford: The Clarendon Press, 1959:188-213.

[99] Chacon, M.A. and Tildon, J.T. Mode of death and post-mortem time effects on 3,3',5-triiodothyroxine levels: relevance to elevated post-mortem T_3 levels in SIDS. Life Sciences 1984;35(22): 2219-2225.

[100] Choo-Kang, E., McKoy, C. and Escoffery, C. Vitreous humor analytes in assessing the post-mortem interval and the antemortem clinical status. West Indian Med.J. 1983; 32(1):23-26.

[101] Coe, J.I. Post-mortem chemistries on human vitreous humor. Am.J.Clin.Pathol. 1969; 51:741-750.

[102] Coe, J.I. Use of chemical determinations on vitreous humor in forensic pathology. J.Forens.Sci. 1972; 17:541.

[103] Coe, J.I. Comparison of antemortem and post-mortem serum proteins. Bul.Bell Museum of Pathobiology 1973; 2(1):40.

[104] Coe, J.I. Further thoughts and observations on post-mortem chemistry. Forens.Sci.Gaz. 1973; 5(5):2.

[105] Coe, J.I. Post-mortem values of thyroxine and thyroid stimulating hormone. J.Forens.Sci. 1973; 18:20.

[106] Coe, J.I. Post-mortem chemistry: practical considerations and a review of the literature. J.Forens.Sci. 1974; 19:13.

[107] Coe, J.I. Post-mortem chemistries of blood: particular reference to urea nitrogen, electrolytes and billirubin. J.Forens.Sci. 1974; 19:33.

[108] Coe, J.I. Post-mortem chemistry of blood, cerebrospinal fluid and vitreous humor: In: Tedeschi, C.G., Eckert, W.G. and Tedeschi, L.G. eds. Forensic Medicine: a study in trauma and environmental hazards. Vol.II, Ch.45. Philadelphia: W.B.Saunders Company, 1977:1033-1060.

[109] Conference of Medical Royal Colleges and their Faculties in the UK. Diagnosis of death. Br.Med.J.1976; ii:1187-1188.

[110] Conference of Medical Royal Colleges and their Faculties in the UK. Diagnosis of death. Br.Med.J. 1979; i:3320.

[111] Cooper, K.E. and Kenyon, J.R. A comparison of temperatures measured in the rectum, oesophagus, and on the surface of the aorta during hypothermia in man. Brit.J.Surg. 1957; 44:616.

[112] Cooper, W.C., Halbert, S.P. and Manski, W.J. Immunochemical analysis of vitreous and subretinal fluid. Invest.Opthalmol. 1963; 2:369.

[113] Coutselinas, A. and Boukis, D. The estimation of Mg ion concentration in C.S.F. as a method for diagnosis of drowning in sea water. Forens.Sci. 1976; 7:109.

[114] Cranston, W.I., Gerbrandy, J. and Snell, E.S. Oral, rectal and oesophageal temperatures and some factors affecting them in man. J.Physiol. 1954; 126:347-358.

[115] Daldrup, T. Postmortaler Eiweibzerfall in menschlichen organen: Reaktionen und Zeitliche Zusammenhange. Dissertation, Triltsch, Düsseldorf. (cited in Ref.No.269)

[116] Davis, T.R.A. and Mayer, J. Demonstration and quantitative determination of the contributions of physical and chemical thermogenisis on acute exposure to cold. Am.J.Physiol. 1955; 181:675.

[117] Davy, J. Observations on the temperature of the human body after death. Researches, Physiological and Anatomical. London. 1839; 1:228.

[118] DeBernardi, A. Peroxidase test of cadaveric blood applied to blood crusts & stains. Minerva Med.Leg., Tor.1958; 78(1):24-32. (cited in Ref.No.438)

[119] DeJorge, F.B. and Jose, N.K. Iodine content of normal human eye tissues. Nature(Lond.) 1967; 214:491.

[120] Denborough, M.A., Ebeling, P., King, J.O. and Zapf, P. Myopathy and malignant hyperpyrexia. Lancet 1970; 1:1138.

[121] Denborough, M.A., Forster, J.F.A., Hudson, M.C. and Carter, N.G. Biochemical changes in malignant hyperpyrexia. Lancet. 1970; 1:1137.

[122] Deshpande, N. Enzyme Studies in endocrine tissues. Research on Steroids, Transections of the meeting of the International Study Group for Steroid hormones. 1973; 5:15-27.

[123] Dietrick, R.B. and Fritts, J.M. Comparative measurements of temperature in liver, lung and rectum of the rabbit. Proc.Soc. Exper.Biol. & Med. 1952; 80:293.

[124] Dito, W.R. Transaminases activity in post-mortem cerebrospinal fluid. Am.J.Clin.Pathol. 1964; 42:360.

[125] Dixon, W.J. BMDP Statistical Software, 1983 Revised Printing; University of California Press, 1983.

[126] Doering, G., Korinth, E. and Schmidt, O. Post-mortem glycolysis in muscle. J.Forens.Med. 1962; 9:106-116.

[127] Done, A.K., Ely, R.S. and Kelly, V.C. Studies of 17-hydroxy corticosteroids: XIV plasma 17-hydroxycorticosteroid concentrations at death in human subjects. Am.J.Dis.Child. 1958; 96:655.

[128] Dotzauer, G. Idiomuskulärer Wulst und post-mortal blunting bei plötzlichen to desfällen. [idiomuscular swelling and post-mortal haemorrhage in sudden death]. Deut.Zschr. gerichtl.Med. 1958; 46(5):761-771. (cited in Ref.No.438)

[129] Dowler, B. Experimental research upon febrile caloricity both before and after death: Post-mortem fever (Western Journal of Medicine and Surgery, June and October 1844), Phil.Med. Examiner 1845; Vol.VIII New Series No.VI - June:359.

[130] Dowler, B. History of febrile caloricity, XLVII (Yellow fever dissection CXLIV). Phil.Med.Examiner 1845; Vol.VIII New Series No.VIII - August:455.

[131] Dowler, B. Two cases of febrile caloricity. Phil.Med. Examiner 1845; Vol.VIII, New Series No.IX - September:525.

[132] Dowler, B. Post-mortem caloricity of yellow fever. Phil. Med.Examiner 1845; Vol.VIII New Series October:624.

[133] Dowler, B. Experimental researches into animal heat in the living and the dead body. New Orleans Med.Surg.J.1856; 12: 54-63,205-211,289-308,433-451,470-491,603-626,759-767. (cited in Ref.No.353)

[134] Dubois, E.F. Lane Medical Lectures: The mechanism of heat loss and temperaure regulation. Stanford: Standford University Press, 1937.

[135] Dubois, E.F. Heat loss from the human body. Harvey Lecture. Bull.New York Acad.Med. 1939; 15:143.

[136] Dubois, E.F. The temperature of the human body in health and disease. In: American Institute of Physics ed. Temperature: its measurement and control in science and industry. New York: Reinhold Publishing Corporation, 1941: 24-40.

[137] Dubois, E.F. Fever and the regulation of body temperature. Publ.No.13. American Lecture Series. Springfield: Charles C.Thomas Publisher, 111,1948.

[138] Dubois, E.F. The many different temperatures of the human body and its parts. West.J.Surg.1951; 59:476.

[139] Dubois, D. and Dubois, E.F. A formula to estimate the approximate surface area if height and weight be known. Arch. Int.Med. 1916; 17:863.

[140] Dubois, E.F., Ebaugh, F.G. and Hardy, J.D. Basal heat production and elimination, of thirteen normal women at temperatures from 22°C. J.Nutr.1952; 48:257.

[141] Duguid, H., Simpson, R.G. and Stowers, J.M. Accidental hypothermia. Lancet 1961; ii Dec.:1213-1219.

[142] Dyfverman, A. and Sjövall, J. A novel liquid-gel chromatographic method for extraction of unconjugated steroids from aqueous solutions. Anal.letters 1978; B11(6): 485-499.

[143] Eichna, L.W., Berger, A.R., Rader, B. and Becker, W.H. Comparison of intracardiac and intravascular temperatures with rectal temperatures in man. J.Clin.Invest.1951; 30:353.

[144] Eichna, L.W., Park, C.R., Nelson, N., Horvath, S.M. and Palmer, E.D. Thermal regulation during acclimatization in a hot, dry (desert type) environment. Am.J.Physiol.1950; 163:585.

[145] Ellingboe, J., Nyström, E. and Sjövall, J. Liquid-gel chromatography on lipophilic hydrophobic sephadex derivatives. J.Lipid Res.1970; 11:226-273.

[146] Einarsson, K., Gustaffson, J.A. and Gustafsson, B.E. Differences between germ-free and conventional rats in liver microsomal metabolism of steroids. J.Biol.Chem. 1973; 248(10):3623-3630.

[147] Enticknap, J.B. Biochemical changes in cadaver sera. J. Forens.Med.1960; 7:135.

[148] Enticknap, J.B. Lipids in cadaver sera after fatal heart attack. J.Clin.Pathol.1961; 14:496.

[149] Enticknap, J.B. Fatty acid content of cadaver sera in fatal ischemic heart disease. Clin.Sci.1962; 23:425.

[150] Erdei, Z. and Vass, Z. Chromatographic investigation of free amino acids of the vitreous body. Acta Ophthalmol. 1967; 45:22.

[151] Eriksson, H., Gustafsson, J-A. and Sjövall, J. Steroids in germfree and conventional rats: 4.identification and bacterial formation of 17- -pregnane derivatives. Eur.J. Biochem.1968; 6:219-226.

[152] Eriksson, H., Gustaffson, J.-A. and Sjövall, J. Steroids in germfree and conventional rats: free steroids in faeces from conventional rats. Eur.J.Biochem.1969; 9:286-290.

[153] Eriksson, H., Gustafsson, J.-A. and Sjövall, J. Steroids in germfree and conventional rats: 21-dehydroxylation by intestinal micro-organisms. Eur.J.Biochem.1969; 9:550-554.

[154] Eriksson, H., Gustafsson, J.-A. and Sjövall, J. Studies on the structure, biosynthesis and bacterial metabolism of 15-hydroxylated steroids in the female rat. Eur.J. Biochem.1971; 19:433-441.

[155] Erman, A. Travels in Siberia (Russian observed No.1), Ayer Co. Publisher Inc.: Salem, NH 03079.1970 Repr.of 1848 ed.

[156] Evans, W.E. Some histological findings in spontaneously preserved bodies. Med.Sci.Law 1962; 2:155.

[157] Evans, W.E. Adipocere formation in a relatively dry environment. Med.Sci.Law 1963; 3:145.

[158] Evans, W.E.D. The Chemistry of Death. Springfield III., Charles C. Thomas,1963.

[159] Fairclough, R.J., Rabjohns, M.A. and Peterson, A.J. Chromatographic separation of androgens, estrogens and progestogens on hydroxyalkoxy-propyl-sephadex (Lipidex). J.Chromat.1977; 133:412.

[160] Fatteh, A. Histochemical distinction between antemortem and post-mortem skin wounds. J.Forens.Sci.1966;11(1):17-27.

[161] Fatteh, A. Distinction between antemortem and post-mortem wounds: A study of elastic fibres in human skin. J.Forens. Sci.1971; 16(3):393-396.

[162] Faucette, W.E. and Cawley, Leo.P. Effective adsorption of dehydroepiandrosterone (DHA) sulfate and other 17-keto steroid conjugates from urine by Amberlite. Clin.Biochem. 1971; 4:287-296.

[163] Fekete, J.F. and Brunsdon, D.F.V. The use of routine laboratory tests in post-mortem examinations. Can.Soc. Forensic Sci.J.1974; 7:238.

[164] Fekete, J.F. and Kerenyi, N.A. Post-mortem blood sugar and blood urea nitrogen determinations. Can.Med.Assoc.J. 1965; 92:970.

[165] Fell, H.B. and Danielli, J.F. The enzymes of healing wounds: 1.The distribution of alkaline phosphomono-esterase in experimental wounds and burns in the rat. Br.J.Experimental Pathology.1943; 24:196-203.

[166] Fiddes, F.S. and Patten, T.D. A percentage method for representing the fall in body temperature after death: its use in estimating the time of death. J.Forens.Med. 1958; 5(1):2-15.

[167] Finlayson, N.B. Blood cortisol in infants and adults: a post-mortem study. J.Pediatr.1965; 67:284.

[168] Fisher, H. Report of an inquiry by the Hon.Sir Henry Fisher, 1977, London: Her Majesty's Stationary Office:60.

[169] Fisher, R.S. and Petty, C.S. Forensic Pathology. Washington: US Department of Justice, 1977:57.

[170] Foley, F.D., Moncrief, J.A. and Mason, A.D., Jr. Pathology of the lung in fatally burned patients. Ann.Surg.1968; 167:251.

[171] Folkow, B., Ström, G. and Uvnäs, B. Cutaneous vasodilatation elicited by local heating of the anterior hypothalamus in cats and dogs. Acta Physiol.Scand.1949;17:317.

[172] Fourier, J. The analytical theory of heat. 1822. Trans. by Freeman, A. 1888. New York: Dover, 1955. (cited in Ref.Nos. 97 & 350)

[173] Fotherby, K. Biosynthesis of the oestrogens. In: Makin, H.L.J. ed. Biochemistry of Steroid Hormones. 2nd ed. Blackwell Scientific Publications: Oxford, 1984:207-229.

[174] Fotsis, T., Adlercreutz, H., Järvenpää, P., Setchell, K.D.R., Axelson, M. and Sjövall, J. Group separation of steroid conjugates by DEAE-Sephadex anion exchange chromatography. J.Steroid Biochem. 1981; 14:457-463.

[175] Frache, G. Tanatologia forense: nuove acquisizioni e moderni orienta menti. Minerva medicoleg. (Atti Assoc.ital.medleg. e assicurazioni), 1953; 73:25-51. (cited in Ref.No.438)

[176] Franschini, F., Muller, E. and Zanaboni, A. Post-mortem increase of potassium in human cerebrospinal fluid. Nature(Lond.) 1963; 98:1208.

[177] Fusco, M.M., Hammel, H.T. and Harely, J.D. Range of physiologic variations in hypothalamic temperature of the resting dog. Fed.Proc. 1959; 18:49.

[178] Fusco, M.M., Harely, J.D. and Hammel, H.T. Interaction of central and peripheral factors in physiological temperature regulation. Am.J.Physiol. 1961; 200:572.

[179] Gagge, A.P. and Herrington, L.P. Physiological effets of heat and cold. Ann.Rev.Physiol.1947; 9:403.

[180] Ganong, W.F. Review of Medical Physiology. 11th ed. Lange Medical Publications: California 94022, 1983:195-199.

[181] Gantner, G.E., Gaffrey, P.R. and Sturner, W.Q. Ascorbic acid levels in the post-mortem vitreous humor: their use in the estimation of time of death. J.Forens.Med.1962; 9:156.

[182] Gaskell, S.J., Brownsey, B.G., Collins, C.J., Leith, H.M. and Thorne, G.C. Steroid determinations using immuno adsorption and high selectivity gas chromatography-mass spectrometry. Inter.J.Mass.Spectrometry and Ion Physics. 1983; 48:245-248.

[183] Gee, D. Deaths from physical and chemical injury, starvation and neglect. In: Mant, A.K. Taylor's Principles and Practice of Medical Jurisprudence. 13th ed. Edinburgh: Churchill Livingstone, 1984: 249-281.

[184] Gerbrandy, J., Cranston, W.I. and Snell, E.S. The initial process in the action of bacterial pyrogens in man. Clin. Sci.1954; 13:453.

[185] Gerbrandy, J., Snell, E.S. and Cranston, W.J. Oral, rectal and oesophageal temperatures in relation to central temperature control in man. Clin.Sci.1954; 13:615.

[186] Gibson, P.H. and Tomlinson, B.E. Vacuolation in the human cerebral cortex and its relationship to the interval between death and autopsy and to synapse numbers: an electron microscopic study. Neuropathol.Appl.Neurobiol. 1979; 5(1):1-7.

[187] Gilder, S.S.B. Twenty-second World Medical Assembly. Br. Med.J.1968; iii:493-494.

[188] Glaister, J. Medical Jurisprudence and Toxicology. 9th ed. 1953, Churchill Livingston: Edinburgh :28.

[189] Glanville, J.N. Post-mortem serum cholesterol levels. Br. Med.J. 1960; 2:1852.

[190] Goffin, Y. Post-mortem variations and effect of autolysis on some hydrolytic enzymes of the skin and skin appendages. Acta path.microbiol.Scandinav.1968; 73:351-358.

[191] Gonzales, T.A. Vance, M., Helpern, M. and Umberger, C. Legal Medicin: Pathology and Toxicology. 2nd ed. Appleton-Century-Crofts inc.: New York, 1954.

[192] Goodhart, J.F. Thermometric observations in clinical medicine. Guy's Hosp.Rep. 1870 third series; vol.15:365-419.

[193] Gordon, I. and Shapiro, H.A. Forensic Medicine: A guide to principles. 2nd ed. Edinburgh: Churchill Livingstone, 1982:1-63.

[194] Görög, S. Studies in Analytical Chemistry 5: Quantitative Analysis of Steorids. Elsevier Scientific Publishing Company: Amsterdam, 1983.

[195] Gower, D.B. Steroid Hormones, Croom Helm: London, 1979.

[196] Gower, D.B. Biosynthesis of the androgens and other C_{19} steroids. In: Makin, H.L.J. ed. Biochemistry of Steroid Hormones. 2nd ed. Blackwell Scientific Publications: Oxford, 1984:170-206.

[197] Gower, D.B. Biosynthesis of the corticosteroids in: Makin, H.L.J.ed. Biochemistry of Steroid Hormones. 2nd ed. Blackwell Scientific Publications: Oxford, 1984:117-169.

[198] Graf, W. Patterns of human liver temperature. Acta Physiol.Scand.1959; 46(suppl.160):1-135.

[199] Graf, W. and Graf, K. The rectum-liver temperature gradient in man. Acta Physiol.Scand.1957; 41:139.

[200] Graf, W., Porjé, I.G. and Allgoth, A.M. Observations on the temperature of human liver parenchyma. Gastroenterologia. 1955; 83:233.

[201] Graham, J.H. Collaborative Study of a spectrophotometric method for the determination of dienestrol in pharmaceuticals. J.Ass. Off.Anal.Chem.1973; 56(3):674-676.

[202] Grant, J.K. The Gas-Liquid Chromatography of Steroids, Cambridge University Press, 1967.

[203] Grant, J.K. and Bestall, G.H. Clinical Biochemistry of Steroid Hormones: Methods and Applications. Croom Helm: London & Canberra, 1983; 1-29.

[204] Grant, R. Physiological effects of heat and cold. Ann.Rev. Physiol.1951; 13:75.

[205] Green, M.A. and Wright, J.C. Post-mortem interval estimation from body temperature data only. Forens.Sci. Int.1985; 28:35-46.

[206] Green, M.A. and Wright, J.C. The theoretical aspects of the time dependent Z equation as a means of post-mortem interval estimation using body temperature data only. Forens.Sci.Int. 1985; 28:53-62.

[207] Greenwood, B.M. The host response to infection In: Weatherall, D.J., Leadingham, J.G.G. and Warrell, A.D. eds. Oxford Textbook of Medicine Vol.1, Oxford University Press: Oxford, 1983: pp 5.21, 5.470, 5.471.

[208] Grinsted, P. Combined accidental hypothermia and barbiturate poisoning. Ugeskr.Laeger.1970; 132:933.

[209] Gupta, H., Dixit, P.C. and Chandra, J. Relationship of phosphoglucomutase in post-mortem blood with time since death and cause of death. Indian J.Med.Res.1983; 77:154-62.

[210] Gustafsson, J.A. Enerath, P., Hökfelt, T. and Skett, P. Central control of hepatic steroid metabolism: Effect of discrete hypothalamic lesions. Endocrinology 1978; 103(1): 141-151.

[211] Gustafsson, B.E., Gustafsson, J.A. and Sjövall, J. Intestinal and faecal sterols in germfree and conventional rats. Acta Chem.Scand.1966; 20:1827-1835.

[212] Gustafsson, B.E., Gustafsson, J.-A. and Sjövall, J. Steroids in germfree and conventional rats: 2. identification of 3 -,16 -dihydroxy-5 -pregnan-20-one and related compounds in faeces from germfree rats. Eur.J. Biochem.1968; 4:568-573.

[213] Gustafsson, J.A., Lisboa, B.P. and Sjövall, J. Studies on the metabolism of C_{19} - steroids in rat liver: 3. Isolation and biosynthesis of 5 -androstanetriols in rat liver microsomes. Eur.J.Biochem.1968; 6:317-324.

[214] Gustafsson, J.A., Lisboa, B.P. and Sjövall, J. Studies on the metabolism of C_{19} steroids in rat liver: 2. Biosynthesis of hydroxylated derivatives of 17ß-hydroxy-5 -androstan-3-one in rat liver microsomes. Eur.J.Biochem. 1968; 5:437-443.

[215] Gustafsson, J.A. and Sjövall, J. Steroids in germfree and conventional rats: 5. Identification of C_{19} steroids in faeces from germfree rats. Eur.J.Biochem. 1968; 6:227-235.

[216] Habrioux, G., Desfosses, B., Condom, R., Faure, B. and Jayle, M.F. Simultaneous radioimmunoassay of 5 -androstane-3 ,17ß-diol and 5 -androstane-3ß,17ß-diol unconjugated and conjugated in human serum. Steroids 1978; 32:61.

[217] Hall, W.E.B. The medico-legal application of the serum transaminase test. J.Forens.Sci. 1958; 3:117.

[218] Hammel, H.T., Wyndham, C.H. and Hardy, J.D. Heat production and heat loss in the dog at 8-36°C environmental temperatures. Am.J.Physiol.1958; 194:99.

[219] Hannius, P. von, Mallach, H.J. and Mittmeyer, H.J. Uber quantitative Druckmessungen an Totenflecken Zur Bestimmung des to Deszeit. Z.Rechtsmed.1973; 73:235-244. (cited in Ref.No.387)

[220] Hanson, L., Votila, V., Lindfors, R. and Laiho, K. Potassium content of the vitreous body as an aid in determining the time of death. J.Forens.Sci.1966; 11:390.

[221] Hardy, J.D. The physical laws of heat loss from the human body. Proc.Natl.Acad.Sci.1937; 12:631.

[222] Hardy, J.D. Control of heat loss and heat production in physiologic temperature regulation. Harvey Lecture Series No.44, Academic Press, New York, 1953.

[223] Hardy, J.D. Physiology of temperature regulation. Physiol.Rev. 1961; 41:521-606.

[224] Hardy, J.D. The 'set point' concept in physiological temperature regulation In: Yamamoto, W.S. and Brobeck, J.R. eds. Physiological Controls and Regulations. Philadeplphia: W.B. Saunders CO., Ch 6, 1965.

[225] Hardy, J.D. and DuBois, E.F. The technique of measuring radiation and convection. J.Nutr.1938; 15:461.

[226] Hardy, J.D. and DuBois, E.F. Basal metabolism, radiation, convection, and vaporization at temperatures of 22 to 35°C. J.Nutr. 1938; 15(5):477-497.

[227] Hardy, J.D., Milhorat, A.T. and DuBois, E.F. Basal metabolism and heat loss of young women at temperatures from 22°C to 35°C. J.Nutr.1941; 21:383.

[228] Hardy, J.D. and Soderstrom, G.F. Heat loss from the nude body and peripheral blood flow at temperatures of 22°C to 35°C. J.Nutr.1938; 16(5):493-510.

[229] Harkonen,M., Adlercreutz, H. and Groman, E.V. Enzymic techniques in steroid assay. J.Steroid Biochem.1974; 5:717.

[230] Hart, J.S. Metabolic alterations during chronic exposure to cold. Fed.Proc.1958; 17:1045.

[231] Hart, J.S. Physiological response to cold in nonhibernating homeotherms. In: Herzfeld, C.M.ed. Temperature: Its Measurement and Control in Science and Industry. Vol.3, Part 3. New York: Reinhold Publishing Corporation, 1963: 373-406.

[232] Hartiala, J. Steroid metabolism in adult lung. Agents and Actions.1976; 6/4:522-526.

[233] Harvey, O.L. and Crockett, H.E. Individual differences in temperature changes of women during the course of menstrual cycle. Human.Biol.1938; 4:453.

[234] Hass, R. Experimentelle Untersuchungen für postmortalen Todeszeitpunktbestimmung aus dem überleben von nebenhodenschwannzspermien. Munich, Dissertation. (cited in Ref.No.387)

[235] Hatch, T. Assessment of heat stress In: Herzfeld ed. Temperature: its measurement and control in science and industry. Vol.3, part 3. New York: Reinhold Publishing Corporation, 1963:307-318.

[236] Hatfield, H.S. and Pugh, L.G.C. Thermal conductivity of human fat and muscle. Nature 1951; 24:918-919.

[237] Hegnaner, A.H. Lethal hypothermic temperatures for dog and man. Ann. N.Y. Acad.Sci. 1959; 80:315.

[238] Henriques, F.C. Jr. and Moritz, A.R. Studies of thermal injury: Conduction of heat to and through skin and temperatures attained therein: theoretical and experimental investigation. Am.J.Pathol.1947;23:531.

[239] Henry, B.J. and Smith, F.A. Estimation of the post-mortem interval by chemical means. The American J. of Forensic Medicine & Pathology 1980; 1(4):341-47.

[240] Hensel, H. Thermal conductivity of tissues. Ergeb.Physiol. 1952; 47:180.

[241] Hensley, B. Experiments on the temperature of bodies after death. Phil.Med.Examiner 1846; Vol.II New Series (XV. March):1951.

[242] Henssge, C. Die Präzision von Todeszeitchätzungen durch die mathematische Bschreibung der rektalen Leichenabkuhlüng. Z.Rechtsmed.1979; 83:49-67. (also cited in Ref.No.205)

[243] Henssge, C. Todeszeitschätzungen durch die mathematische beschreibung der rektalen Leichenabkuhlüng unter verschiedenen Abkühlungsbedingungen. Z. Rechtsmed.1981; 87:147-178. (also cited in Ref.No.205)

[244] Herrington, L.P. and Gagge, A.P. Temperature regulation. Ann.Rev.Physiol.1943; 5:295.

[245] Hertzman, A.B. Heat and cold. Ann.Rev.Physiol.1955; 17:79.

[246] Hertzman, A.B. Regulation of cutaneous circulation during body heating In: Herzfeld, C.M. ed. Temperature: Its measurement and Control in Science and Industry. Vol.3 Part 3., New York: Reinhold Publishing Coroporation, 1963:559-570.

[247] Hill, M.J. The role of colon anaerobes in the metabolism of bile acids and steroids and its relation to colon cancer. Cancer, 1975; 36(6, suppl.):2387-2400.

[248] Hirabayashi, A. Arch.Hist. 1953; 5:63. (cited in Ref.No.438)

[249] Hiraiwa, K., Kudo, T., Kuroda, F., Ohno, Y., Sebetan, I.M. and Oshida, S. Estimation of post-mortem interval from rectal temperature by use of computer: Relationship between the rectal and skin cooling curves. Med.Sci.Law 1981; 21(1):4-9.

[250] Hiraiwa, K., Ohno, Y., Kuroda, F., Sibetan, I.M. and Oshida, S. Estimation of post-mortem interval from rectal temperature by use of computer. Med.Sci.Law 1980; 20(2): 115-125.

[251] Hirvonen, J. Systemic and local effects of hypothermia. In: Tedischi, C.G., Eckert, W.G. and Tedischi, L.G. eds. Forensic Medicine: a study in trauma and environmental hazards. Vol.1, Philadephia: W.B. Saunders Co., 1977: 758-774.

[252] Hodgkinson, A. and Hambleton, J. Elevation of serum calcium concentration and changes in other blood parameters after death. J.Surg.Res.1969; 9:567.

[253] Hoffman, S.B. Rate of cellular autolysis in post-mortem bone marrow. Am.J.Clin.Path.1964; 41:281-286.

[254] Holbrook, M.M., Dale, S.L. and Melby, J. Peripheral plasma steroid concentrations in rats sacrificed by anoxia. J.Steroid Biochem.1980; 13:1355-1358.

[255] Honour, J.W., Tourniaire, J., Biglier, E.G. and Shackleton, C.H.L. Urinary Steroid excretion in 17 -hydroxylase deficiency. J.Steroid Biochem.1978; 9:495-505.

[256] Horvath, S.M., Rubin, A. and Foltz, E.L. Thermal gradients in the vascular system. Am.J.Physiol.1959; 161:316-322.

[257] Hughes, W. Levels of potassium in the vitreous humor after death. Med.Sci.Law 1965; 5:150.

[258] Hutchins, G.M. Body temperature is elevated in the early post-mortem period. Human Path.1985; 16:560-561.

[259] Isaacs, H. and Barlow, M.B. Malignant hyperpyrexia during anaesthesia: possible association with subclinical myopathy. Br.Med.J.1970; 1:275.

[260] Ishikawa, M. On some researches concerning the estimation of time after death. Acta Med.Leg.Soc.1957; 10:743.

[261] IUPAC-IUB Revised Tentative Rules for Steroid Nomenclature (1967), copyright by the Am.Chem.Soc.(1969), published Biochemistry 1969; 8:2227 and Biochem.J.1969; 113:5.

[262] IUPAC-IUB Definitive Rules for Nomenclature of Steroids (1971), published Pure Appl.Chem.1972; 31:85.

[263] Ivy, A.C. What is normal or normality. Quart.Bull. Northw. Univ.Med.School 1944; 18:22.

[264] Jaffe, F. Chemical post-mortem changes in the intraocular fluid. J.Forens.Sci. 1962; 7:231.

[265] James, W.R.L. and Knight, B.H. Errors in estimating time since death. Med.Sci.Law 1965; 5:111-116.

[266] Janne, O.A., Laatikainen, T.J. and Vihko, R.K. Effect of reduction of the intestinal microflora on the excretion of neutral steroids in human faeces and urine. Eur.J.Biochem. 1971; 20:120-123.

[267] Janne, O., Vikho, R., Sjövall, J. and Sjövall, K. Determination of steroid mono- and disulfates in human plasma. Clin.Chim.Acta 1969; 23:405-412.

[268] Janne, O. and Vihko, R. Identification of 7-hydroxylated C steroid sulphates in human urine. J.Steroid Biochem. 1979; 1:177-184.

[269] Janssen, W. Forensic Histopathology, Translated by Forster, S. Springer-Verlag Berlin Heidelberg: New York, 1984: 46-47.

[270] Janssen, O.M. Diagnosis of uraemia post-mortem. Dan.Med. Bull., Suppl.1969, VIII:1.

[271] Jetter, W.W. Post-mortem biochemical changes. J.Forens. Sci.1959; 4:330.

[272] John, P.D.L. Railroading in retinal vessels: correspondence. Br.Med.J.1967; 4:357.

[273] Johnson, C.C. and Guy, A.W. Non-ionizing electromagnetic wave effect in biological materials and systems. Proc. IEEE, 1972; 60(6): 692-718.

[274] Johnston, A.M. Observations of the cooling rate of deer carcases. Veterinary Record 1984; 114:89.

[275] Joling, R.J. Property of the dead body In: Tedeschi, C.G., Eckert, W.G. and Tedeschi, L.G. Forensic Medicine: a study in trauma and environmental hazards. Vol.2. Philadephia: W.B. Saunders Company, 1977:967-977.

[276] Joseph, A.E.A. and Schickele, E. A general method for assessing factors controlling post-mortem cooling. J. Forens.Sci.1970; 15(3):364-391.

[277] Katsumata, Y., Sato, K., Yada, S., Uematus, T., Oya, M. and Yoshino, M. Anaerobic metabolism in dogs after organismal death. JPN J. Legal Med.1983; 37(1):75-78.

[278] Keatringe, W.R. Heat and cold and drowning In: Weatherall, D.J., Ledingham, J.G.G. and Warrell, A.D. eds. Oxford Textbook of Medicine Vol.1, Oxford University Press: Oxford, 1983: pp 6.52.

[279] Kellie, A.E. Structure and nomenclacture In: Makin, H.L.J. ed. Biochemistry of Steroid Hormones. 2nd ed. Blackwell Scientific Publications: Oxford, 1984:1-19.

[280] Kellogg, T.F. Faecal bile acids and neutral sterols of gnotobiotic, antibiotic fed normal, and normal human children. Germfree Res: Biol.Eff.Gnotobiotic Environ. Proc.Int.Symp. 4th 1972 (Pub.1973) 79-81.

[281] Kevorkian, J. The eye in death. Ciba Clin.Symp.1961; 13:51.

[282] Kevorkian, J. The fundus oculi as a 'post-mortem clock'. J.Forens.Sci.1961;6:261-272.

[283] Knapp, D.R. Steroid, Ch.11 In: Knapp, D.R. ed. Handbook of Analytical Derivatization Reaction, Wiley-Interscience, John Wiley and Sons: New York, 1979:449-514.

[284] Knight, B. The determination of the time since death. Police Surgeon, 1979; No.1:29-32.

[285] Knight, B. Methods of dating skeletal remains. Med.Sci. Law 1969; 9:247-252.

[286] Knight, B. Legal Aspects of Medical Practice. 3rd ed. Edinburgh: Churchill Livingstone, 1982:116-126.

[287] Knight, B. The Moment of Death. In: Polson, C.J., Gee, D.J. and Knight, B.eds. the Essentials of Forensic Med. 4th ed. Oxford: Pergamon Press, 1985, ch.23, Part 2:667-672.

[288] Komura, S. and Oshiro, S. Potassium levels in aqueous and vitreous humor after death. Tohoku J.Exp.Med. 1977; 122: 65-68.

[289] Kopp, R.H. Disposable resin columns in analytical chemistry. Ind.Eng.Chem.Prod.Res.Dev. 1975; 14(2):96-98.

[290] Korpela, J.T. Capillary gas-liquid chromatography of faecal free and esterified neutral sterols. Scand.J. Clin.Lab. Invest. 1982; 42:529-534.

[291] Kuchn, L.A., Tikuisis, P., Livingstone, S. and Limmr, R. Body cooling after death. Aviation, Space, Env.Med.1980: 965-969.

[292] Kuroda, F., Hiraiwa, K., Oshida, S. and Akaishi, S. Estimation of post-mortem interval from rectal temperature by use of computer (III): Thermal conductivity of the skin. Med.Sci.Law 1982; 22(4):285-289.

[293] Kuroda, F., Hiraiwa, K., Oshida, S. and Akaishi, S. Estimation of post-mortem interval from rectal temperature by use of computer (IV): Thermal diffusivity of the rabbit. Med.Sci.Law 1983; 23(2):125-130.

[294] Laiho, K. and Penttilä, A. Autolytic changes in blood cells and other tissue cells of human cadavers. I. viability and ion studies. Forens.Sci.Int.1981; 17(2): 109-120.

[295] Land, D.V. A_3G_z clinical microwave thermography system. IEE Proc., 134 Pt.A, 1987; 193-200.

[296] Land, D.V. Factors affecting temperature measurements in microwave thermography, in "Recent Developments in Medical and Physiological Imaging", J.Med.Eng. and Technology (suppl.) 1986: 102-108.

[297] Land, D.V. amd Brown, V.J. Tissue thermal and electromagnetic modelling for microwave thermography IEE Digest 1986/73, "Industrial and medical application of microwaves", 1986: 16/1-16/4.

[298] Land, D.V., Fraser, S.M. and Shaw, R.D. A review of clinical experience of microwave thermography, in "Recent Developments in Medical and Physiological Imaging", J.Med.Eng. and Technology (suppl.) 1986.

[299] Laufman, H. Profound accidental hypothermia. J.A.M.A. 1951; 147:1201.

[300] Laurence, D.R. Clinical Pharmacology. 4th ed. Edinburgh: Churchill Livingstone, 1973: pp31.8.

[301] Laves, W. Agonal changes in blood serum. J.Forens.Med. 1960; 7:70.

[302] Leading Article: Accidental hypothermia. Br.Med.J.1966; 2:1471.

[303] Leading Article: Malignant hyperpyrexia. Br.Med.J.1968; 3:69.

[304] Leading Article: Brain death. Br.Med.J.1975; 1:356.

[305] Leahy, M.S. and Farber, E.R. Post-mortem chemistry of human vitreous humor. J.Forens.Sci.1967; 12:214.

[306] Levonen, E., Raekallio, J. and Saikkonen, J. Post-mortem determination of blood creatinine and urea. J.Forens.Med. 1963; 10:22.

[307] Lie, J.T. Changes of potassium concentration in the vitreous humor after death. Am.J.Med.Sci.1967; 254:136.

[308] Lind, A.R. Tolerable limits for prolonged and intermittent exposures to heat In: Herzfeld ed. Temperature: its measurement and control in science and industry. Vol.3, Part 3 New York: Reinhold Publishing Corporation, 1963: 337-345.

[309] Lindquist, O. Determination of insulin and glucose post-mortem. Forens.Sci.1973; 2:55.

[310] Lindquist, O. and Rammel, L. Insulin in post-mortem blood. Z.Rechtsmed.1975; 75:275.

[311] Lisboa, B.P. and Sauer, H.D. Metabolism of oestradiol-17β in the human myometrium. J.Steroid Biochem.1975; 6:1131-1136.

[312] Lisboa, B.P. and Sauer, H.D. Progesterone metabolism in the human myometrium. J.Steroid Biochem.1975; 6:1137-1144.

[313] Lorenzen, S.A. and Lawson, R.L. A possible new approach for determining the post-mortem interval. J.Crim.Law and Criminol. 1971; 62:560.

[314] Ludwig, J. Current Methods of Autopsy Practice. Philaldephia: W.B. Saunders Co.1972:220.

[315] Lund, A. Adrenaline and noradrenaline in blood from cases of sudden, natural or violent death In: Proceedings of the Third International Meeting in Forensic Immunology, Medicine, Pathology and Toxicology. London, April, 1963.

[316] Lundquist, F. Physical and chemical methods for the estimation of the time of death. Acta Med.Leg.Soc.1959;9: 205-213.

[317] Lyle, H.P. and Cleveland, F.P. Determination of the time of death by body heat loss. J.Forens.Sci.1956; 4:11-23.

[318] Lyle, H.P., Stemmer, K.L. and Cleveland, F.P. Determination of the time of death: a consideration of post-mortem physical changes. J.Forens.Sci.1959; 4:167-175.

[319] Lythgoe, A.S. Post-mortem activity of alkaline phosphatase in serum from different sites of the cadaver cardiovascular system. J.Forens.Sci.Soc.1981; 21(4): 337-340.

[320] Macleod, J. Davidson's Principles and Practice of Medicine: a textbook for students and doctors. 11th ed. Churchill Livingstone: Edinburgh, 1975:51.

[321] Magoun, H.W., Harrison, F., Brobeck, J.R. and Ranson, S.W. Activation of heat loss mechanisms by local heating of the brain. J.Neurophysiol.1938; 1:101.

[322] Makin, H.L.J. Methods of steroid analysis: group estimations and separation techniques In: Makin, H.L.J. ed. Biochemistry of Steroid Hormones. 2nd ed. Blackwell Scientific Publications: Oxford:478-514.

[323] Malik, M.O.A. Histochemical changes as evidence of the antemortem origin of skin burns. J.Forens.Sci.1970; 15(4): 489-499.

[324] Mallach, H.J. and Mittmeyer, H.J. Totenstarre und Totenflecken. Z.Rechtsmed.1971; 69:70-78. (cited in Ref.No.387)

[325] Mant, A.K. Recent work on changes after death: Lapse of time from chemical changes in cerebrospinal fluid. Factors influencing changes after burial, fluidity of blood after death In: Simpson, K. ed. Modern Trends in Forensic Medicine. Butterworths: London, 1953:80-98.

[326] Mant, A.K. Post-mortem changes In: Mant, A.K., ed. Taylor's Principles and Practice of Medical Jurisprudence. 13th ed. Edinburgh: Churchill Livingstone, 1984; 128-155.

[327] Mant, A.K. and Furbank, R. Adipocere: a review. J.Forens.Med. 1957; 4:18.

[328] Marshall, T.K. The cooling of the body after death. M.D. thesis, Leeds, 1959.

[329] Marshall, T.K. Estimating the time of death: the use of body temperature in estimating the time of death. J.Forens.Sci.1962;7(2): 211-221.

[330] Marshall, T.K. Temperature methods of estimating the time of death. Med.Sci.Law 1965; 5:224-232.

[331] Marshall, T.K. The use of body temperature in estimating the time of death and its limitations. Med.Sci.Law 1969; 9:178-183.

[332] Marshall, T.K. Changes after death In: Camps, F.E., Robinson, A.E. and Lucas, B.G.B. eds. Gradwohl's Legal Medicine. 3rd ed. Bristol: John Wright & Sons Ltd., 1976: 78-100.

[333] Marshall, T.K. The problem of timing death from the body temperature. Police Surgeon. 1979; No.1:21-28.

[334] Marshall, T.K. Timing death: an illustrated case. Police Surgeon. 1981; No.1:38-48.

[335] Marshall, T.K. Post-mortem changes: Lectures of the postgraduate course for Diploma in Medical Jurisprudence. London Hospital, University College. London, 1983. (private communication)

[336] Marshall, T.K. and Hoare, F.E. Estimating the time of death: the rectal cooling after death and its mathematical expression. J.Forens.Sci.1962; 7(1):56-81.

[337] Mason, J.K. Post-mortem changes and timing of death In: Mason, K.D. ed. Forensic Medicine for Lawyers. 2nd ed. London: Butterworths, 1983(Revised reprint 1986) :47-53.

[338] Mason, J.K. Transplantation of organs and the Human Tissue Act. In: Mason, J.K. ed. Forensic Medicine for Lawyers. 2nd ed. London: Butterworths, 1983 :117-124.

[339] Mason, J.K., Klyne, W. and Lennox, B. Potassium levels in the cerebrospinal fluid after death. J.Clin.Pathol.1951; 4:231.

[340] Matsumoto, H., Tsuganezawa, T., Ohki, H. and Kitahama, M. An application of heat conduction model to fall of post-mortem temperature. Rep.Nat.Inst.Pol.Sci.1977; 30:10-17. (Cited in Ref.No.250)

[341] McIntyre, I.M. and Stanley, M. Post-mortem and regional changes of serotonin, 5-hydroxyindoleacetic acid, and tryptophan in brain. J.Neurochem.1984; 42(6):1588-1592.

[342] Mead, J. and Bonmarito, C.L. Reliability of rectal temperature as an index of internal body temperature. J.Appl.Physiol.1949; 2:97-109.

[343] Mellette, H.C. Skin, rectal and intravascular temperature adjustments in exerise. Am.J.Physiol.1950; 163:734.

[344] Millington, D.S. New techniques in quantitative mass spectrometry. J.Reprod.Fertil.1977; 51(1):303-308.

[345] Milnor, W.R. Blood supply of special regions In: Mountcastle, V.B. ed. Medical Physiology. Vol.1, 12th ed. Saint Louis: The C.V. Mosby Company ch.10, 1968 :221-243.

[346] Minard, D. and Copman, L. Elevation of body temperature in health. In: Herzfeld, C.M. ed. Temperature: Its measurement and control in science and industry. Vol.3, part 3 New York: Reinhold Publishing Corporation, 1963:527-543.

[347] Moar, J.J. Determining the so-called moment of death in homicide victims: the significance of antemortem wounds and the post-mortem interval. S.Afr.Med.J. 1982; 62(2):64-6 .

[348] Mole, R.H. The relative humidity of the skin. J.Physiol. 1948; 107:399-411.

[349] Molnar, G.W. Newton's thermometer: a model for testing Newton's law of cooling. Physiologist 1969; 12:9-19.

[350] Molnar, G.W. Newton's law of cooling applied to Newton's ingot of iron and to other solids. Physiologist.1969; 12:137-151.

[351] Molnar, G.W. Tutorial: Factors which cause deviation from Newton's law of cooling. J.Ass.Adv.Med.Instru.1970; 4(3): 89-93.

[352] Molnar, G.W. Analysis of the rate of digital cooling. J.de Physiologic.1971; 63:350-352.

[353] Molnar, G.W., Hurley, H.J. and Ford, R. Application of Newton's law to body cooling. Pflugers Arch.1969; 311: 16-24.

[354] Moraru, I., Belis, V., Streja, D., Droc, I. and Ivanovici, V. The study of blood cholinesterase in the cadaver in various kinds of death. In: Proceedings of the Third International Meeting in Forensic Immunology, Medicine, Pathology and Toxicology. London, April, 1963.

[355] Morgan, A.D. Misleading Evidence: Time of death. Br.Med. J. 1945; ii:25.

[356] Moritz, A.R., Henriques, F.C.Jr., Durta, F.R. and Weisiger, J.R. Studies of thermal injury. IV. An exploration of the casualty-producing attributes of conflagrations; local and systemic effects of general cutaneous exposure to excessive circumambient (air) and circumradiant heat of varying duration and density. Arch.Pathol.1947; 43:466.

[357] Moritz, A.R., Henriques, F.C., Jr., and McLean, R. The effect of inhaled heat on the air passages and lungs: An experimental investigation. Am.J.Pathol.1945; 21:311.

[358] Mueller, B. Todeszeitbestimmung auf grund der äusseren besichtigung und untersuchung der leiche. Münch.Med. Wschr.1937; 84:1021. (cited in Ref.No.269)

[359] Mueller, B. Das verhalten der mastdarmtemperatur der leiche unter verschiedenen äusseren bedingungen. Deutsch.Z. ges.gerichtl. Med.1938; 29:158. (cited in Ref.Nos. 269 and 461)

[360] Mueller, B. Gerichtliche medizin Springer, Berlin Heidelberg: New York, 1975. (Cited in Ref.No.269).

[361] Murakami, S., Jin, H., Furusawa, M., Sasaki, S., Wakamatsu, K. and Tsushida, S. On the post-mortem rectal temperature. Practice and Research of Forensic Medicine. 1957; 4:7-19 (cited in Ref.No.250)

[362] Murakami, S. and Kudo, T. Medicolegal study of the post-mortem rectal temperature. The 19th Meeting of Tohoku Association of Forensic Medicine, 1961, Akita. (Cited in Ref.No.).

[363] Murray, D. Cortisol binding to plasma protein in man in health, stress and at death. J.Endocr.1967; 39:571-591.

[364] Murray, E. and Hordynsky, W. Potassium levels in cerebrospinal fluid and their relation to duration of death. J.Forens.Sci. 1958; 3:480.

[365] Naeve, W. and Apel, D. Hirntemperatur und Todeszeit. Z.Rechtsmed. 1973; 73:159-169. (cited in Ref.No.387 & 461)

[366] Nanikawa, R. and Janssen, W. Uber das postmortale verhalten dersuccinodehydrogenase-aktivitä in geweben und leukocyten. Deustch Z. ges. gerichtl.Med. (English summary) 1965; 56: 44-56. (also cited in Ref.No.269)

[367] Naumann, H.N. Urobilinogen in cerebrospinal fluid. Proc.Soc. Exp.Biol.Med. 1947; 65:72.

[368] Naumann, H.N. Studies on post-mortem chemistry. Am.J. Clin.Pathol.1949; 47:70.

[369] Naumann, H.N. Cerebrospinal fluid electrolytes after death. Proc.Soc.Exp.Biol.Med.1958; 98:16.

[370] Naumann, H.N. Post-mortem chemistry of the vitreous body in man. A.M.A. Arch.Ophthalmol.1959; 62:356.

[371] Naumann, H.N. and Young, I.M. Comparative bilirubin levels in vitreous body, synovial fluid, cerebrospinal fluid and serum after death. Proc.Soc.Exp.Biol.Med.1960; 105:70.

[372] Nixon, D.A. Cerebrospinal fluid inositol and its rise in post-mortem specimens. J.Physiol.(Lond.)1955; 129:272.

[373] Nes, W.R. and McKean, M.L. Biochemistry of Steroids and Other Isopentenoids. University Park Press, Baltimore, 1977:444.

[374] Nes, W.R. and McKean, M.L. Biochemistry of steroids and other isopentenoids. University Park Press, Baltimore, 1977:587-88 and 325-410.

[375] Newburgh, L.H. and Johnston, M.W. The insensible loss of water. Physiol.Rev.1942; 22:1-18.

[376] Newton, I. Scala graduum caloris. Calorum desriptiones and signa. Philosophical Trans.Roy.Soc.1701:832-829, London (cited in Ref.No.79).

[377] Nicati, M. A certain sign of death. Med.Rec.1894; 45:480.

[378] Nielsen, M. Die Regulation der Korpertemperatur bei Muskelurbeit. Skand.Arch.Physiol.1938; 79:193.

[379] Nokes, L.D.M., Brown, A. and Knight, B. A self-contained method for determining time since death from temperature measurements. Med.Sci.Law 1983; 23(3):166-169.

[380] Nokes, L.D.M., Hicks, B. and Knight, B.H. The post-mortem temperature plateau - fact or fiction? Med.Sci.Law 1985; 25(4):263-264.

[381] Novotny, M., Maskarinec, M.P., Steverink, A.T.G. and Farlow, R. High-resolution gas chromatography of plasma steroidal hormones and their metabolites. Anal.Chemistry 1976; 48:468-472.

[382] Nuorteva, P. Age determination of a blood stain in a decaying shirt by entomological means. Forens.Sci.1974; 3:89.

[383] Nuorteva, P. Sarcosaprophagous insects as forensic indicators. In: Tedeschi, C.G., Eckert, W.G. and Tedeschi, L.G. eds. Forensic Medicine: a study in trauma and environmental hazards. Vol.2. Philadelphia: W.B. Saunders Company, 1977: 1072-1095.

[384] Nourteva, P., Isokoski, M. and Laiho, K. Studies on the possibilities of using blowflies (Dipt.) as medicolegal indicators in Finland. Ann.Entomol.Fenn.1967; 33:217.

[385] Nuorteva, P., Schumann, H., Isokoski, M. and Laiho, L. Studies on the possibilities of using blowflies (Diptera, Calliphoridese) as medicolegal indicators in Finland:2. Four cases where the species identification was performed from larvae. Ann. Entomol.Fenn.1974; 40:70.

[386] O'Connor, J.M. On the mechanism of chemical temperature regulation. Proc.Royal Soc. (London) 1915; 89:201.

[387] Oever, R.V.D. A review of the literature as to the present possibilities and limitations in estimating the time of death. Med.Sci.Law 1976; 16(4):269-276.

[388] Olaison, B. Post-mortem decrease in brain temperature. Z.Rechtsmed. 1979; 83:253-257.

[389] Pallis, C. ABC of brain-stem death; series of nine articles. Br.Med.J. Nov.1982-Jan.1983: 285, 6354, 1558.

[390] Parker, L.N. Grover, P.K., and Odell, W.D. An improved radio-immunoassay for 4-androstene-3,17-dione. Steroids 1977; 29:715.

[391] Paton, D. Time of death (letter on the unreliability of the "usual calculation" and the cooling curves according to Gradwhol). The Police Surgeon 1970; 15:116.

[392] Peck, C.C., Beal, S.L., Sheiner, L.B. and Nichols, A.I. Scientific commentary: extended least squares non-linear regression: a possible solution to the "choice of weights" problem in analysis of individual pharmacokinetic data. J.Pharmacokinetics and Biopharmaceutics. 1984; 12(5): 545-558.

[393] Penttilä, A. and Laiho, K. Autolytic changes in blood cells of human cadavers. II Morphological Studies. Forens. Sci.Int.1981; 17(2):121-132.

[394] Petersdorf, R.G. Alternations in body temperature: Disturbances of heat regulation, chills and fever In: Thorn, G.W., Adams, R.D., Braunwald, E., Isselbacher, K.J. and Petersdorf, R.G. eds. Harrison's Principles of Internal Medicine. 8th ed., International Student Edition, McGraw-Hill Kogakusha Ltd., A Blakiston Publication: Tokyo, 1977.

[395] Petrove, B.A. Transfusion of cadaver blood. Surgery 1959; 46:651.

[396] Pex, J.O., Meneely, K.D. and Andrews, F.C. Time of death estimation in blacktail deer by temperature and aqueous glucose. J.Forens.Sci.JFSCA 1983; 28(3):594-600.

[397] Pickering, G. Regulation of body temperature in health and disease. Lancet 1958; 1(1):59.

[398] Pinson, E.A. Evaporation from human skin with sweat glands inactivated. Am.J.Physiol. 1942; 137:492-503.

[399] Point of death; no judicial definition. Br.Med.J. 1981; 282: 1083-1084.

[400] Polson, C.J. The signs of death In: Polson, C.J., Gee, D.J. and Knight, B. eds. The Essentials of Forensic Medicine. 4th ed. Oxford: Pergamon Press, 1985; 3-39.

[401] Popwassilew, I. and Palm, W. Uber die Todeszeitbestimmung in den esten 10 Stunden. Z.ärztl.Fortbild 1960; 54:734 (Cited in Reference No 438).

[402] Porteous, I.B. Persistence of motility in bone marrow cells from the cadaver. Nature (Lond.) 1961; 192:569-570.

[403] Praetorius, E., Paulsen, H. and Dupont, H.I. Uric acid, xanthine and hypoxanthine in the cerebrospinal fluid. Scand.J. Clin.Lab. Invest.1957; 9:133.

[404] Prescott, L.F., Peard, M.C. and Wallace, I.R. Accidental hypothermia: a common condition. Br.Med.J. 1962; 2:1367-1370.

[405] Price, D.B. The significance of the diameter of the pupils in the cadaver with reference to the causes and time of death. Proc.Int.Meet.Forens.Immunol.Med.Path.and Toxicol: Report of the 3rd international meeting. International Congress Series No.80, Excerpta Medica Foundation: Amsterdam, London, New York, 1963:181.

[406] Prokop, O. Lehrbuch der Gerichtlichen Medizin, Verlag Volk und Gesundheit, Berlin, 1960 (cited in Ref.No.438).

[407] Prokop, O. Forensische Medizin. Berlin, Volk u. Gesundheit. 1966 (cited in Ref.No.269).

[408] Pullar, P. The Histopathology of Wounds. In: Mant, A.K.ed. Modern Trends in Forensic Medicine. 3, London: Butterworths, 1973.

[409] Pullar, P., and Liadsky, C. Dihydrogenase system of human foetal skin. British J.Dermatology 1965; 77:314-321.

[410] Raekallio, J. Histochemical distinction between antemortem and post-mortem skin wounds. J.Forens.Sci. 1964; 9(1):107-117.

[411] Raekallio, J. Application of histochemistry to forensic medicine. Med.Sci.Law 1966; 6:142-146.

[412] Raekallio, J. Enzyme histochemistry of vital and post-mortem skin wounds. J.Forens.Med.1966; 13(3):85-91.

[413] Raekallio, J. Determination of the age of wounds by histochemical and biochemical methods. Forens.Sci.1972; 1:3-16.

[414] Raekallio, J. Timing of the wound. In: Tedeschi, C.G., Eckert, W.G. and Tedeschi, L.G. eds. Forensic Medicine: a study in trauma and environmental hazards. Vol.1. Philadephia: W.B. Saunders Company, 1977:22-28.

[415] Raekillo, J. Histochemical and biochemical estimation of the age of injuries In: Perper, J.A. and Wecht, C.H. eds. Microscopic Diagnosis in Forensic Pathology. Thomas, Charles, C.: Springfield, IL 62717, 1980:17-35.

[416] Rainy, H. On the cooling of dead bodies as indicating the length of time that has elapsed since death. Glasg.Med.J. New Series 1868/69; 1:323.

[417] Raszeja, S. and Bardzik, S. Die Bewertung der Brauchbarkeit interlataler Reaktionen der Schweissdrusen zur Bestimmung der Todeszeit. Z.Rechtsmed.1970; 67:223. (cited in Ref.No.387)

[418] Reimann, H.A. The problems of long continued low-grade fever. J.Am.Med.Assn.1936; 107:1089.

[419] Reimann, W. Uber den Auskühlmodus der Leiche. Beitr. Gerichtl. Med.1968; 24:57-62 (Cited in Reference No 387).

[420] Rentoul, E. and Smith, H. Glaister's Medical Jurisprudence and Toxicology. 13th ed. Edinburgh: Churchill Livingstone, 1973; 113-139.

[421] Reynolds, J.W. and Mirkin, B. Urinary Steroid levels in Newborn Infants with Intra-Uterine Growth Retardation. J.Clin. Endocrinol.Metab. 1973; 36:576-581.

[422] Rhead, W.J., Cary, E.E., Allaway, W.H., Saltzstein, S.L. and Schrauzer, G.N. The vitamin E and selenium status of infants and the sudden infant death syndrome. Bioinorganic Chemistry 1972; 1:289.

[423] Rhead, W.J., Schrauzer, G.N., Saltzstein, S.L., Cary, E.E. and Allaway, W.H. Vitamine E, Selenium, and the Sudden Infant Death Syndrome. J.Pediatr.1972; 81:415.

[424] Ricci, P. Ulteriori osservazioni Sulle Modificazioni Putrifactive Degli Elementi Figurati Del Sangue Periferico. Med.legale e assicuraz 1957; 5:63. Medicina Legale e delle assicurazion: 5533.610 (Cited in Ref.No. 294 and 438).

[425] Riddle, J.M. and Barnhart, M.I. Ultrastructural Study of fibrin dissolution via emigrated polymorphonuclear neutrophils. Am.J. Path.1964; 45:805-815.

[426] Robinsen, D.M.Jr. and Kellenberger, R.E. Comparison of electrophoretic analyses of antemortem and postmortem serums. Am.J. Clin.Pathol.1962; 38:371.

[427] Robins, S. Physiological adjustment to heat. In: Newburg, L.H.ed. Physiology of Heat Regulation and the Science of Clothing. Ch.5, Philadelphia: W.B. Saunders Company, 1949.

[428] Robinson, S. Physiology of muscular exercise In: Mountcastle, V.B. ed. Medical Physiology. Vol.1. 12th ed. Saint Louis: the C.V. Mosby Company, 1968:520-552.

[429] Romeo, J.A Heatstroke. Milit.Med.1966; 131:669.

[430] Roos, R.W. Determination of conjugated and estrified estrogens in pharmaceutical tablet dosage forms by high-pressure normal-phase partition chromatography. J.Chromat. Sci.1976; 14(11):505-512.

[431] Rubenstein, E., Meub, D. and Eldridge, F. Common carotid blood temperature. J.Appl.Physiol.1960; 15:603-605.

[432] Ruse, J.L. and Solomon, S. The in vivo metabolism of 16 - hydroxy progesterone Biochemistry. 1966; 5(3):1065-1071.

[433] Ruse, J.L. and Solomon, S. The isolation and origin of urinary 16 -hydroxyprogesterone. Biochemistry 1966; 5(3): 1072-1078.

[434] Salisbury, C.R. and Melvin, G.S. Ophthalmoloscopic signs of death. Br.Med.J.1936; 1:1249-1251.

[435] Saram, G.S.W.de, Webster, G. and Kathirgamataby, N. Post-mortem temperature and the time of death. J.Crim.Law and Criminol. 1955; 46:562-577.

[436] Saram, G.S.W. de Estimation of time of death by medical criteria. J.Forens.Med. 1957; 4(2):47.

[437] Sasaki, S., Tsunenari, S. and Kanda, M. The estimation of the time of death by non-protein nitrogen (NPN) in cadaveric materials. Report 3: Multiple regression analysis of NPN values in human cadaveric materials. Forens.Sci.Int. 1983; 22:11-22.

[438] Schleyer, F. Determination of time of death in the early post-mortem interval In: Lundquist, F. ed. Methods of Forensic Science.Vol.2 New York: Interscience Publishers, 1963:253-293.

[439] Schourup, K. Dodstidsbestemmelse, Copenhagen: Dansk Videnskabs Forlag, 1950-1 (cited in Ref.No 438).

[440] Schwarke, R. Die postmortale rectumtemperatur und ihre gerichtsmedizinische verwertbarkeit zur todeszeitbertimmung. Dtsch.Z.ges.gerichtl.med.1939; 31:256. (cited in Ref.Nos. 438 amd 461)

[441] Schwarz, F. and Heidenwolf, H. Post-mortem cooling and its relation to the time of death. Int.Crim.Pol.Rev.1953; 73: 339-344.

[442] Scott, J.C. and Bazett, H.C. Temperature regulation. Ann. Rev.Physiol.1941; 3:107.

[443] Sellier, K. Determination of the time of death by extra-polation of the temperature decrease curve. Acta Med.Leg. Soc. 1958; 11: 279-302.

[444] Sendroy, J.Jr and Cecchini, L.P. Determination of human body surface area from height and weight. J.Appl.Physiol. 1954; 7(1):1-12.

[445] Serio, M., Calabresi, E., Mannelli, M. and Pazzagli, M. Testicular Secretion in Mammals and in Man In: Serio, M. and Martini, L. eds. Animal Models in Human Reproduction, Raven Press: New York, 1980: 121-133.

[446] Setchell, K.D.R., Almé, B., Axelson, M. and Sjövall, J. The multicomponent analysis of conjugates of neutral steroids in urine by liphophilic ion exchange chromatography and computerized gas chromatography-mass spectrometry. J.Steroid Biochem.1976; 7:615-629.

[447] Setchell, K.D.R., Axelson, M., Simarina, A.I. and Gontscharow, N.P. Urinary steroid excretion and conjugation by the baboon (Papio hamadryas): a comprehensive study. J. Steroid Biochem. 1976; 7:809-816.

[448] Setchell, K.D.R. and Shackleton, C.H.I. The group separation of plasma and urinary steroids by column chromatography on Sephadex LH-20. Clin.Chim.Acta 1973; 47:381-388.

[449] Seydeler, R. Nektrothermometrie, Vjschr.Prakt.Heilk. (Prague) 1869; 104:137 (Cited in Ref.No. 79).

[450] Shackleton, C.H.L. Urinary Steroid Metabolite Analyses: Potential for renaissance. Clin.Chemistry 1981; 27(3): 509-511.

[451] Shackleton, C.H.L., Gustafsson, J.-A. and Mitchell, F.L. Steroids in newborns and infants: the changing pattern of urinary steroid excretion during infancy. Acta Endocrinologica 1973; 74:157-167.

[452] Shackleton, C.H.L. and Honour, J.W. Simultaneous estimation of urinary steroid by semi-automated gas chromatography. Investigation of neonatal infants and children with abnormal steroid synthesis. Clin.Chim.Acta 1976; 69: 267-283.

[453] Shackleton, C.H.L., Honour, J.W., Dillon, M. and Milla, P. Multicomponent gas chromatographic analysis of urinary steroids excreted by an infant with a defect in aldosterone biosynthesis. Acta Endocrinologica 1976; 81: 762-773.

[454] Shackleton, C.H.L., Sjövall, J. and Wisen, O. A simple method for the extraction of steroids from urine. Clin. Chim.Acta, 1970; 27:354-356.

[455] Shackelton, C.H.L. and Whitney, J. Use of Sep-pak cartridges for urinary steroid extraction: evaluation of the method for use prior to gas chromatograph analysis. Clin. Chim.Acta 1980; 107: 231-243.

[456] Shapiro, H.A. Medicolegal Mythology. J.Forens.Med.1954;1:144.

[457] Shapiro, H.A. The post-mortem temperature plateau.1965; 12(4): 137-141.

[458] Shapiro, H.A. Criteria for determining that death has occurred: the Philadephian Protocol. J.Forens.Med.1969; 16(1):1.

[459] Shibolet, S., Coll, R., Gilat, J. and Sohar, E. Heatstroke: its clinical picture and mechanism in 36 cases. Q.J.Med. 1967; 36:525.

[460] Shikata, I. Japan J.leg.Med.1958; 12:227 (Cited in Ref.No. 438).

[461] Simonson, J., Voigt, J. and Jeppeson, N. Determination of the time of death by continuous post-mortem temperature measurement. Med.Sci.Law 1977; 17(2):112-122.

[462] Simpson, C.K. Rex v Dobkin: the baptist church cellar murder. Med.Leg.J. 1943; XI:132.

[463] Simpson, C.K. Taylor's Principles and Practice of Medical Jurisprudence, 12th ed. London: Churchill Livingstone, 1965:70-103.

[464] Simpson, K. The moment of death: a new medicolegal problem. Acta Anasth.Scand.1968; 29(suppl.):361-379.

[465] Simpson, K. and Knight, B. Forensic Medicine 9th ed. London: Edward Arnold, 1985.

[466] Simpson, L.R. Thyrotoxicosis: Post-mortem diagnosis in an unexpected death. J.Forens.Sci.1976; 21:831.

[467] Sivaloganathan, S. Ante-mortem injury or post-mortem? : Diagnosis using histamine as a marker. Med.Sci.Law 1982; 22(2):119-125.

[468] Sjövall, J. and Axelson, M. General and Selective isolation procedure for GC-MS analysis of steroids in tissues and body fluids. J.Steroid Biochem. 1979; 11(1A):129-134.

[469] Sjövall, J. and Axelson, M. New approaches to the isolation, identification and quantitation of steroids in biological materials. Vit.Horm.1982; 39:31-144.

[470] Sjövall, J. and Vikho, R. Analysis of solvolyzable steroids in human plasma by combined gas chromatography-mass spectrometry. Acta Endocrinologica 1968; 57:247-260.

[471] Sjövall, K. Gas chormatographic determination of steroid sulphates in plasma during pregnancy. Annals Clin.Res. 1970; 2(4):393-408.

[472] Sjövall, K. and Sjövall, J. Estimation of dehydroepiandrosterone sulfate in human serum by gas-liquid chromatography. Anal.Biochem.1966; 14:337-346.

[473] Skegg, P.D.G. Irreversibly comatosed individuals: alive or dead? Cambridge Law J.1974; 33:130.

[474] Smith, S. and Fiddes, F.S. Forensic Medicine: a textbook for students and practitioners 10th ed. J & A. Churchill Ltd.: London, 1955:14-37.

[475] Sommer, A.G. De signis mortens hominis absolutam ante putredims accessum indicatatibus. Copenhagen: Hauniae, 1833. (cited in Ref.No.332)

[476] Spiteller, G. Combination of chromatographic and mass spectrometric methods in the search for metabolic compounds. Advances in the Bioscience 1968 (Pub.1969); 2:41-68.

[477] Spitz, W.U., Petty, C.S. and Fisher, R.S. Physical activity until collapse following fatal injury by firearms and sharp pointed weapons. J.Forens.Sci.1961; 6:290.

[478] Stenberg, A. Developmental, diuranal and oestrous cycle-dependent changes in the activity of liver enzymes. J.Endocr. 1976; 68:265-272.

[479] Straumfjord, J.V. and Butler, J.J. Evaluation of ante-mortem acid-base status by means of determining the pH of post-mortem blood. Am.J.Clin.Pahol.1957; 23:165.

[480] Stubbe, P., Mentzel, H. and Wolf, H. Growth hormone fluctuations in the paramortal period. Horm.Metab. Res. 1973; 5:163.

[481] Stupfel, M. and Severinghaus, J.W. Internal body temperature gradients during anaesthesia and hypothermia and effects of vagotomy. J.Appl.Physiol.1956; 9:380-386.

[482] Sturner, W.Q. and Dempsey, J.L. Sudden infant death: chemical analysis of vitreous humor. J.Forens.Sci.1973; 18:12.

[483] Sturner, W.Q. and Gantner, G.E. The post-mortem interval: a study of potassium in the vitreous humor. Am.J.Clinc.Pathol. 1964; 42:137.

[484] Swatland, H.J. Low temperature activation of post-mortem glycogenolysis in bovine skeletal muscle fibres. Histochem.J. 1979; 11(4):391-398.

[485] Swift, P.G.E., Worthy, E. and Emergy, J.L. Biochemical state of the vitrous humor of infants at necropsy. Arch. Dis.Child.1974; 49:680.

[486] Szent-Gyorgyi, A. Chemistry of muscular contraction. New York: Academic Press, 1947:77.

[487] Takatori, T. and Yamoaka, A. The mechanism of adipocere formation: 1. Identification and chemical properties of hydroxy fatty acids in adipocere. Forens.Sci.1977; 9:63.

[488] Takatori, T. and Yamaoaka, A. The mechanism of adipocere formation: II. Separation and identification of oxo fatty acids in adipocere. Forens.Sci.1977; 10:117-125.

[489] Tanner, J.M. The relationship between the frequency of the heart, oral temperature and rectal temperature in man at rest. J.Physiol.1951; 115:391-409.

[490] Taylor, A.S. and Wilks, S. On the cooling of the human body after death. Guy's Hospital Rep. 3rd series. 1863; 9:180.

[491] Taylor, N.F. and Shackleton, C.H.L. Gas chromatographic steroid analysis for diagnosis of placental sulfatase deficiency: a study of nine patients. J.Clin. Endocrinol.Metabolism 1979; 49(1):78-86.

[492] Tedeschi, G. Systemic and localized hyperthermic injury. In: Tedischi, C.G., Eckert, W.G. and Tedischi, L.G. eds. Forensic Medicine: a study in trauma and environmental hazards. Vol.1, Ch.20, Philadelphia: W.B. Saunders Company, 1977: 701-729.

[493] Tetsuo, M., Axelson, M. and Sjövall, J. Selective isolation procedures for GC-MS analysis of ethynyl steroids in biological material. J.Steroid Biochem.1980; 13:847-860.

[494] Thomson, W.A.R. Definition of death In: Camps, F.E., Robinson, A.E. and Lucas, B.G.B. eds. Gradwohl's Legal Medicine. 3rd ed. Bristol: John Wright & Son Ltd., 1976: 50-57.

[495] Thurston, G. and Burton, J.C. Death. In: Mant, A.K.ed. Taylor's Principles and Practice of Medical Jurisprudence. 13th ed. Edinburgh: Churchill Livingstone, 1984: 29-38.

[496] Tilesius. On the Mammoth, or Fossil Elephant, found in the Ice, at the mouth of the River Lena. In: Siberia, from the fifth volume of the memoirs of of the Imperial Academy of Sciences of St.Petersburg. Q.J. Science and arts 1820; 8:95-108.

[497] Tomlin, P.J. "Railroading" in retinal vessels. Br.Med.J. 1967; 3:722-723.

[498] Tomlin, P.J. Retinal changes after cardiac arrest. Br.Med. J. 1967; 4:110.

[499] Tongue, J.I. and Wannan, J.S. The post-mortem blood sugar. Med.J.Aust.1949; 1:439.

[500] Traupe, A. Die postmortale rektumtemperaiur und ihre beziehungen zur todeszeit. Inaug.Dissert., Göttingen, 1937 (Cited in Ref.No.336).

[501] Trolle, C. A study of the insensible perspiration in man and its nature. Skand.Arch.Physiol.1937; 76:225-246.

[502] Tsunenari, S., Lythgoes, A.S. and Gee, D.J. The activity of the rate-limiting glycolytic enzymes in the erythrocytes with relation to time after death. J.Forens.Sci. Soc.1981; 21(4): 333-336.

[503] Umberger, E.J. Isonicotinoyl hydrazine as a reagent for determination of ^{4}-3-keto steroids - determination of progesterone and testosterone propionate in oil solutions. Anal.Chem.1955; 27:768-773.

[504] United States Pharmacopoeia XX, USP Convention Inc., Rockville, Md., 1980; 296-326: 556-587 and 741-774.

[505] University of Glasgow. Microwave Thermography. Publication of University of Glasgow, June 1985.

[506] Vandenheuvel, W.J.A., Smith, J.L., Albers-Schönberg, G., Plazonnet, B. and Belanger, P. Derivatization and gas chromatography in the mass spectrometry of steroids In: Heftmann, E. ed. Modern Methods of Steroid Analysis, Academic Press: New York, 1973:199-219.

[507] Van der Bogert, F. and Moravel, C.L. Body temperature variations in apparently healthy children. J.Paediatr.1937;10:466.

[508] Vermeulen, A. Plasma levels and secretion rate of steroids with anabolic activity in man. Environ.Qual. (Suppl.) 1976; 5:171-180.

[509] Voisin, C., Wattel, F., Scherpereel, P., Gosselin, B. and Chopin, C. Enzymes in the cerebrospinal fluid in diagnosis of brain death. Resuscitation 1975; 4:61.

[510] Wada, M. and Takagaki, T. A simple and accurate method for detecting the secretion of sweat. Tohoku J.Exp. Med.1948; 49:284 (also cited in Ref.No. 438).

[511] Walla, B.N.S., Sarin, G.W., Chandra, R.I. and Ghai, O.P. Preterminal and post-mortem changes in serum potassium of children. Lancet, 1963; 1:1187.

[512] Walther, J., Bishop, F.W. and Warren, S.L. The Temperature Pattern of Laboratory animals in normal and febrile states. In: American Institute of Physics ed. Temperature: its Measurement and Control in Science and Industry. New York: Reinhold Publishing Corporation, 1941: 474-486.

[513] Watson, D.R. and Spaziani, E. Rapid isolation of ecolysteroids from crustacean tissues and culture media using Sep-Pak C_{18} cartridges. J.Liquid Chromatography 1982; 5(3):525-535.

[514] Whitney, J.O. and Thaler, M.M. A simple liquid chromatographic method for quantitative extraction of hydrophobic compounds from aqueous solutions. J.Liquid Chromatography 1980; 3(4):545-556.

[515] Wilkins, T.D. and Hackman, A.S. Two pattern of neutral steroid conversion in the faeces of normal North Americans. Cancer Res.1974; 34:2250-2254.

[516] Williamson, E.D. and Adam, L.H. Temperature distribution in solids during heating and cooling. Phys.Rev.2nd series. 1919; 14(2):99-114.

[517] Winslow, C.A. and Gagge, A.P. Influence of physical work on physiological reactions to the thermal environment. Am.J.Physiol.1941; 134:664.

[518] Winslow, C.E.A., Herrington, L.P. and Gagge, A.P. Physiological reactions of the human body to varying environmental temperatures. Am.J.Physiol.1937; 120:1.

[519] Winslow, C.E.A., Herrington, L.P. and Gagge, A.P. Physiological reactions of the human body to various atmospheric humidifiers. Am.J.Physiol.1937; 120:288-299.

[520] Wissler, E.H. An analysis of factors affecting temperature levels in the nude human. In: Herzfeld ed. Temperature: Its measurement and control in Science and Industry.Vol.3, Part 3, New York: Reinhold Publishing Corporation, 1963: 603-612.

[521] Wolff, K. and Schellander, F.G. Enzyme-histochemical studies on the healing progress of split skin grafts: 1. Aminopeptidase, Diphosphopyridine-nucleotide-diphorase and succinic dehydrogenase in autografts. J.Investigative Dermatology 1965;45(1): 38-45.

[522] Womack, F. The rate of cooling of the body after death. St.Bart's Hosp.Rep.1887; 23:193.

[523] Wood, M.J. fever. Medicine International 1984; 2(1):10.

[524] Wright, S. Applied Physiology. 7th ed.Oxford: Oxford University Press, 1945:592.

[525] Wroblewski, B. and Ellis, M. Eye changes after death. Brit.J. Surg.1970; 57:69-71.

[526] Wyndham, C.H., Bouwer, W.V.d.M., Devine, M.G. and Paterson, H.E. Physiological responses of African laborers at various saturated air temperatures, wind velocities and rates of energy expenditure. J.Appl. Physiol.1952; 5:290-298.

[527] Wyndham, C.H., Bouwer, W.V.d.M., Devine, M.G., Paterson, H.E. and MacDonald, D.C. Examination of use of heat-exchange equation for determining changes in body temperature. J.Appl. Physiol.1952; 5:299.

[528] Wyndham, C.H., Strydom, N.B., Morison, F.D. du Toit, and Kraan, J.G. Responses of unacclimatized men under stress of heat and work. J.Appl.Physiol.1954; 6:681.

[529] Yapo, E.A., Barthelemy-Clavey, A., Racadot, A. and Mizon, J. J.Chromat.Biomed.Appl.1978; 145:478. (cited in Ref.No.194)

[530] Yasoshima, S. Jap.J.Leg.Med.1955; 9:19 (Cited in Ref.No. 438).

[531] Yashimato, K., Itoh, N., Hashimoto, Y., Irizawa, Y. and Komura, S. Studies on the estimation of post-mortem time: An application of Interactive Image analysis system. Hiroshima J.Med.Sci.1984; 33(4):615-618.

[532] Young, E. and Solomon, S. The In Vivo Metabolism of 16 - hydroxydehydroisoandrosterone in man. Biochemistry 1967; 6(7):2040-2052.

[533] Zaretskii,V.I. Mass Spectrometry of Steroids, Wiley: New York, 1976.

[534] Zenker, N., Chalon, M.A. and Tildon, J.T. Mode of death effect on rat liver iodothyronine 5'deiodinase activity: role of adenosine 3',5' monophosphate, Life Sciences 1984; 35(22):2213-2217.

[535] Morgan, R., McClean, W. and Rosell, J. Interfacing your BBC microcomputer. Chapter 6, Analogue inputs, Englewood Cliffs, NJ London: Prentice-Hall International (U.K.) Ltd.; 1985:61-78.

[536] Webb, P.A., Terry, H.J. and Jee, D.J. A method for time of death determination using ultrasound - A preliminary report. J.Forens.Sci.Soc.1986; 26:393-399.

[537] Windholz, M., Budavari, S., Stroumtsos, L.Y. and Fertig, M.N. eds. The Merck Index: An Encyclopedia of Chemicals and Drugs, 9th ed., Published by Merck & Co., INC., Rahway, N.J., U.S.A.; 1976:218 and 1105.

[538] Fonda, E.S., Rampacek, G.B., Kraeling, R.R. an Hart, M.A. Effect of storage time and temperature on steroid and protein hormone concentrations in porcine plasma and serum. Theriogenology 1982; 18(6):711-721.

[539] Jones, G.R. Post-mortem changes in drug levels: A common phenomenon? The International Association of Forensic Toxicologists, Proc.23rd European Intrnational Meeting Terrorism: Analysis and detection of explosives, 1986: Ghent, Belgium:29-35.

A P P E N D I C E S

I. COMPUTER PROGRAMMES

II. TABLES

Appendix I: Programme 1: The data logging control programme.

```
 10 REM PROGRAM READS 4 ANALOGUE
 20 REM INPUTS AT INTERVALS t;OUTPUTS
 30 REM THE RESULTS AT INTERVALS
 40 REM dumptime AND ENDS AT
 50 REM HOUR=duration OR WHEN
 60 REM "CTR S" IS PRESSED.
 70 REM "CTR R" ALLOWS PARAMETERS
 80 REM TO BE CHANGED AT START.
 90 PROCinitialise
100 REM OPEN FILE
110 *FX 138,0,13
120 D=OPENOUT("data")
130 *FX 15,1
140 CLS
150 DIM TEMP(4,dumptime DIV t)
160 TIME=0
170 REPEAT
180 X=1
190 PROCread
200 FOR X=2 TO (dumptime DIV t)
210 PROCtimecheck:REM WAIT t MINS
220 PROCread
230 NEXT X
240 PROCstore
250 PROCtimecheck
260 UNTIL FNHR>=duration
270 CLOSE#D:PRINT " "
280 PRINT "STOP AT ";FNHR;" HOURS ",
290 PRINT FNMIN MOD 60;" MINUTES"
300 END
310 DEFPROCread
320 FOR A=1 TO 2
330 Y=0
340 FOR J=1 TO 300
350 temp=ADVAL(A)/16
360 Y=Y+temp
370 NEXT J
380 temp=Y/300
390 IF (temp-INT(temp))>=0.5 THEN temp  =temp+1:REM ROUND UP
400 TEMP(A,X)=INT(temp)
410 IF A=3 THEN 490 ELSE 420
420 NEXT A
430 A=3
440 REPEAT
450 U=ADVAL(0) AND 3
460 UNTIL U=1 OR U=2
470 TEMP(4,X)=U+64
480 GOTO 330
490 W=ADVAL(0) AND 3
500 IF W=U THEN 510 ELSE 430
510 PRINT FNHR;" HRS ";
520 PRINT FNMIN;" MINS: READ"
530 PRINT TEMP(1,X),TEMP(2,X),TEMP(3,X),TEMP(4,X)
540 ENDPROC
550 DEFPROCtimecheck
```

```
560 min=min+t:REM NEXT READ-TIME
570 REPEAT
580 PROCstopcheck
590 UNTIL (FNMIN+60*FNHR)>=min
600 ENDPROC
610 DEFPROCstore
620 *MOTOR1
630 FOR X=1 TO (dumptime DIV t)
640 FOR A=1 TO 4
650 PRINT#D,TEMP(A,X)
660 NEXT A
670 NEXT X
680 *MOTOR0
690 PRINT "STORE AT:-"
700 PRINT FNHR;" HOURS   ",
710 PRINT FNMIN MOD 60;" MINUTES"
720 X=0:REM FLAG FOR tidystop
730 ENDPROC
740 DEF FNHR=(TIME DIV 360000)MOD 100
750 ENDPROC
760 DEF FNMIN=((TIME DIV 6000)MOD 60)
770 ENDPROC
780 DEFPROCstopcheck
790 stop$=INKEY$(0)
800 IFstop$=CHR$(19)THEN PROCtidystop
810 ENDPROC
820 DEFPROCtidystop
830 IF X=0 THEN GOTO 270:REM NOTHING    TO STORE
840 PROCstore
850 GOTO 270
860 ENDPROC
870 DEFPROCinitialise
880 min=0:REM PRESENT MINUTE
890 t=5:REM INTERVAL IN MINUTES BETWEE N READINGS
900 dumptime=60
910 duration=60:REM RUNTIME IN HOURS
920 ENDPROC
```

Appendix I: Programme 2: Used for manual digitization of the data

```
 10 REM THIS PROGRAMME IS CALLED HIPLOT
 20 REM ITS FUNCTION IS TO DIGITISE DATA ON FOUR-PEN CHART TO DISK

 30 *FX7,5
 40 *FX2,2
 50 @%=&20206:DIM Y(4,150),COL$(4),CUR$(4),THERE(4)
 60 CLS:PRINT:PRINT"Digitising program"
 70 FOR I=1 TO 4:FOR J=1 TO 70:Y(I,J)=0:NEXT:NEXT
 80 COL$(1)="GREEN":COL$(2)="RED":COL$(3)="BLUE":COL$(4)="BROWN"
 90 CUR$(1)="ENVIRONMENT":CUR$(2)="BRAIN":CUR$(3)="LIVER":CUR$(4)="RECTAL"
100 PRINT:PRINT"Which curves are on chart:"
110 FOR I=1 TO 4:PRINT "  ";CUR$(I);" (";COL$(I);") ";
120 INPUT R$:IF R$="Y" THERE(I)=1 ELSE IF R$="N" THERE(I)=0 ELSE 120
130 NEXT
140 INPUT"Total time for chart: "XFINAL:XSTART=0:XST=XSTART:FIRST=TRUE
150 INPUT"Name of file to store data: "F$:F%=OPENOUT(F$)
160 PRINT:PRINT"Prepare first section:":INITIALISED=FALSE
170 PRINT"Digitise first point on axis":PROCGETDIG:X0=XD:Y0=YD
180 PRINT"Digitise second point on axis":PROCGETDIG:X1=XD:Y1=YD
190 DX=X1-X0:DY=Y1-Y0:DS=SQR(DX^2+DY^2):CST=DX/DS:SNT=DY/DS
200 INPUT"Enter times: "T0,T1
210 XF=(T1-T0)/DS:IF NOT FIRST THEN 240 ELSE FIRST=FALSE
220 PRINT"Digitise two points to give temperature scale (50 deg)"
230 PROCGETDIG:XL=XD:YL=YD:PROCGETDIG:YF=50/((YD-YL)*CST-(XD-XL)*SNT)
240 INITIALISED=TRUE:IF T1<XFINAL XEND=T1 ELSE XEND=XFINAL
250 PRINT"Start time for this section is ";STR$(XST);", stop time ";STR$ XEND
260 FOR C=1 TO 4
270 IF THERE(C)=1 THEN PROCGETPTS
280 NEXT:PRINT"End of section.  Last Time was ";XEND:PRINT"WAIT":PROCSAVE
290 IF XEND<XFINAL XST=XEND+5:PRINT:PRINT"Prepare next chart section":GOTO170
300 PRINT"END OF DIGITISING":CLOSE#F%:END
310 DEF PROCGETPTS:PRINT:PRINT"Digitise start point of ";COL$(C);" curve"
320 K=0:XNEXT=XST:PROCGETVAL:IF X>XNEXT VDU7:PRINT"Too far left":GOTO 320
330 REPEAT XP=X:YP=Y:PROCGETVAL:UNTIL X>XNEXT OR NOT GOTTHEM
340 IF NOT GOTTHEM PRINT"ABANDON";CHR$(7):END
350 K=K+1:IF X=XP Y(C,K)=Y ELSE Y(C,K)=YP+(Y-YP)/(X-XP)*(XNEXT-XP)
360 PRINT"PT ";STR$(K),XNEXT,Y(C,K):IF XNEXT>=XEND VDU7:ENDPROC
370 XNEXT=XNEXT+5:IF X>XNEXT THEN 350 ELSE 330
380 DEF PROCSAVE:FOR I=1 TO K:FOR C=1 TO 4:PROCPUT(Y(C,I)):NEXT:PROCNEWL
390 NEXT:ENDPROC
400 DEF PROCPUT(X):LOCAL I:X$=STR$(INT(X*100+.5)/100)
410 FOR I=1 TO LENX$:BPUT#F%,ASC MID$(X$,I,1):NEXT:BPUT#F%,32:ENDPROC
420 DEF PROCNEWL:BPUT#F%,13:BPUT#F%,10:ENDPROC
430 DEF PROCGETVAL:PROCGETDIG:X=T0+((XD-X0)*CST+(YD-Y0)*SNT)*XF
440 Y=(-(XD-X0)*SNT+(YD-Y0)*CST)*YF:ENDPROC
450 DEF PROCGETDIG:GOTTHEM=FALSE
460 *FX21,1
470 REPEAT UNTIL ADVAL-1>0 OR ADVAL-2>0:IF ADVAL-2>0 THEN 490
480 ESCAPE=GET:IF INITIALISED ENDPROC ELSE END
490 *FX2,1
500 CONTRL=GET:PROCDIG:XD=D:PROCDIG:YD=D
510 *FX2,2
520 GOTTHEM=TRUE:ENDPROC
530 DEF PROCDIG:LOCAL I,D$:D$="":FOR I=1 TO 6:D$=D$+CHR$(GET-128):NEXT
540 D=VAL D$:ENDPROC
```

Appendix I: Programme 3: Used for transferring data from cassettes to disks.

```
  1 REM PROGRAMME CALLED "READTAP"
  2 REM TO TRANSFER DATA FROM CASSETTE TO DISK
  3 REM THIS IS ONLY FOR POST-MORTEM TEMPERATURE WORK
 10 REM  Copy cassette file from mortuary into text file on disk.
 20 REM  Tape:
 30 REM       Col 1:  Rectal
 40 REM       Col 2:  Environment
 50 REM       Col 3:  Liver    or    Brain
 60 REM       Col 4:   65      or     66
 70 REM  Disk:
 80 REM       Col 1:  Environment
 90 REM       Col 2:  Brain
100 REM       Col 3:  Liver
101 REM       Col 4:  Rectal
110 *TAPE
120 F$="data":MODE 6:DIM R(750),E(750),L(750),B(750):X%=OPENUP(F$)
130 J=1:REPEAT INPUT#X%,R(J),E(J),LB,ILB:R(J)=R(J)/1.669:E(J)=E(J)/1.669
140 IF ILB=65 L(J)=LB/83.45:B(J)=0 ELSE L(J)=0:B(J)=LB/83.45
150 PRINT R(J),E(J),L(J),B(J):J=J+1:UNTIL EOF#X%
160 *DISK
170 N=J-1
180 INPUT"Disk file name: "F$:Y%=OPENOUT(F$)
190 FOR J=1 TO N:PROCWRITEN(Y%,E(J)):PROCWRITEN(Y%,B(J)):PROCWRITEN(Y%,L(J))
200 PROCWRITEN(Y%,R(J)):PROCNEWLINE(Y%):NEXT:CLOSE#Y%:END
210
220 DEF PROCWRITEN(C%,X):LOCAL N$,J%:N$=STR$(X):BPUT#C%,32
230 J%=INSTR(N$,"."):IF J%>0 N$=LEFT$(N$,J%+2)
240 FOR I%=1 TO LEN(N$):BPUT#C%,ASC(MID$(N$,I%,1)):NEXT:ENDPROC
250
260 DEF PROCNEWLINE(C%):BPUT#C%,13:BPUT#C%,10:ENDPROC
```

Appendix I: Programme 4: Used for displaying temperature traces on the monitor screen

```
  1 REM PROGRAMME CALLED "DISPLAY"
  2 REM TO DISPLAY PLOT OF FOUR TEMPERATURE DATA VERSUS TIME IN MINUTE
 10 @%=2:DIM C(4),T(4),S(4),K0(4),C$(4):C(1)=2:C(2)=5:C(3)=4:C(4)=1
 11 C$(1)="E":C$(2)="B":C$(3)="L":C$(4)="R"
 20 XO=150:XR=1080:YO=100:YR=800
 30 INPUT"Name of data file: "F$:F%=OPENUP(F$)
 35 IF F%=0 PRINT"FILE NOT FOUND":END
 40 INPUT"Title: "TITLE$
 50 INPUT"Max. data time: "DT:IF DT=0 DT=3600
 55 INPUT"Time shift: ",TSH
 60 FAC=XR/DT:HOURS=DT/60
 70 MODE2:PROCAXES:PROCREAD:FOR I=1 TO 4:S(I)=T(I):NEXT
 80 FOR I=2 TO 3:IF T(I)=0 K0(I)=-1 ELSE K0(I)=1
 90 NEXT I
100 K=5:REPEAT PROCREAD:PROCPLOT:K=K+5:UNTIL K>DT OR EOF#F%
110 CLOSE#F%:VDU7:PROCdump:END
120
130 DEF PROCREAD:FOR I=1 TO 4:T(I)=FNREADN(F%):NEXT:ENDPROC
180
190 DEF PROCAXES:VDU5:CLS:FOR Y=0 TO YR STEP 20:PLOT 69,XO,YO+Y:NEXT
200 FOR K=10 TO 40 STEP 10:MOVE 0,K*20+YO:PRINT K;:NEXT
210 FOR X=0 TO XR STEP 12:FOR K=0 TO 3:PLOT 69,X+XO,K*200+YO:NEXT K,X
220 FOR H=10 TO HOURS STEP 10:MIN=H*60:PROCMV(MIN,0):PROCDR(MIN,.8)
240 PROCMV(MIN,-1.2):VDU8:PRINT H:NEXT:MOVE 1000,30:PRINT"hrs"
250 MOVE 300,900:PRINT LEFT$(TITLE$,12)
252 MOVE 0,200:PRINT"o";:PLOT0,-10,-25:PRINT"C"
255 FOR I=1 TO 4:MOVE 1210,700+50*I:GCOL0,C(I):PRINTC$(I):NEXT
260 VDU4:ENDPROC
270
280 DEF PROCPLOT:FOR I=1 TO 4 STEP 3
282 GCOL0,C(I):PROCMV(K-5+TSH,S(I)):PROCDR(K+TSH,T(I)):S(I)=T(I):NEXT
284 FOR I=2 TO 3:IF T(I)=0 THEN 290
286 IF K0(I)>-1 GCOL0,C(I):PROCMV(K0(I)+TSH,S(I)):PROCDR(K+TSH,T(I))
288 K0(I)=K:S(I)=T(I)
290 NEXT:ENDPROC
299
300 DEF PROCMV(X,Y):MOVE X*FAC+XO,Y*20+YO:ENDPROC
310 DEF PROCDR(X,Y):DRAW X*FAC+XO,Y*20+YO:ENDPROC
320
900 DEF FNREADN(C%):LOCAL N$,C$:N$=""
910 REPEAT C$=CHR$(BGET#C%):UNTIL (C$)="0" AND C$<="9") OR C$="." OR EOF#C%
920 N$=C$
930 REPEAT C$=CHR$(BGET#C%):N$=N$+C$
940 UNTIL (C$)"9" OR C$("0") AND C$<>"."
950 IF EOF#C% GOTO 980
960 REPEAT C$=CHR$(BGET#C%):UNTIL (C$)="0" AND C$<="9") OR C$="." OR EOF#C%
970 IF NOT EOF#C% THEN PTR#C%=PTR#C%-1
980 =VAL(N$)
1020DEF PROCdump
1030A$=GET$:IFA$="Q" THEN ENDPROC ELSE IF A$<>"S" AND A$<>"L" THEN 1030
1040IF A$="L" lineend=80:pixelinc=2 ELSE lineend=40:pixelinc=4
1050VDU29,0;0;:PROCcoldump
1060ENDPROC
```

```
1070REM COLOURJET BBC DUMP-MODES 0,1,2,4,5
1080DEF PROCcoldump
1090PROCassemble:REM ASSEMBLE CODE
1110VDU19,0,7,0,0,0:VDU19,7,0,0,0,0
1130*FX6,0
1150*FX5,1
1160VDU2
1170*FX3,10
1180CALL DUMP
1190*FX6,10
1200*FX3,4
1210ENDPROC
1220DEF PROCassemble
1230OSWORD=&FFF1:OSWRCH=&FFEE:OSBYTE=&FFF4
1260P%=&80:xlow=P%:xhi=P%+1:ylow=P%+2:yhi=P%+3:LGCOL=P%+4
1320DOTCTR=P%+10:buffptr=P%+11:datacount=P%+12
1350DIM COD% 700
1360FORPASS=0TO2STEP2
1370P%=COD%:redram=P%:greenram=P%+80:blueram=P%+160:P%=P%+240
1410[OPT PASS
1420.DUMP LDX #3:STX yhi:LDX #&FE:STX ylow
1440.NWLIN LDA yhi:BPL NWLIN1:LDA #&A:JMP OSWRCH
1460.NWLIN1 LDX #0:STX buffptr:STX xlow:STX xhi:INX:STX datacount
1480.getbyte LDA #8:STA DOTCTR
1490.GTBYT DEC DOTCTR:BMI testwhite:LDY #(xlow DIV256):LDX #(xlow MOD256)
1510 LDA #9:JSR OSWORD:LDA LGCOL:BPL GTPAL:LDA #0:BEQ putbuff
1540.GTPAL LDY#(LGCOL DIV 256):LDX#(LGCOL MOD 256)
1550 LDA #&B:JSR OSWORD:LDA LGCOL+1
1570.putbuff LDX buffptr
1580 LSR A:ROL redram,X:LSR A:ROL greenram,X:LSR A:ROL blueram,X
1610 CLC:LDA xlow:ADC #pixelinc:STA xlow:LDA xhi:ADC #0:STA xhi:JMP GTBYT
1620.testwhite LDX buffptr:LDA redram,X:AND greenram,X:AND blueram,X
1630 INC buffptr:CMP #&FF:BEQ nextbyte:INX:STX datacount
1650.nextbyte LDA buffptr:CMP #lineend:BNE getbyte
1660.sendrow LDA #27:JSR OSWRCH:LDA #&59:JSR OSWRCH
1680 LDA datacount:CMP #1:BNE out:JSR sendnull:JMP nexty
1710.out JSR OSWRCH
1720. sendred LDX #0
1730.red1 LDA redram,X:BNE red2:JSR sendnull
1750.red2 JSR OSWRCH:INX:CPX datacount:BNE red1
1760.sendgreen LDX #0
1770.grn1 LDA greenram,X:BNE grn2:JSR sendnull
1790.grn2 JSR OSWRCH:INX:CPX datacount:BNE grn1
1800.sendblue LDX #0
1810.blu1 LDA blueram,X:BNE blu2:JSR sendnull
1830.blu2 JSR OSWRCH:INX:CPX datacount:BNE blu1
1840.nexty LDA ylow:SEC:SBC #pixelinc:STA ylow
1850 LDA yhi:SBC #0:STA yhi:JMP NWLIN
1870.sendnull TXA:PHA:LDA #6:LDX#7:JSR OSBYTE:LDA #0:JSR OSWRCH
1880 LDA #6:LDX#0:JSR OSBYTE:PLA:TAX:LDA #0:RTS
1890]:NEXT PASS
1900ENDPROC
```

Appendix I: Programme 5: Used to calculate the body site temperature at the moment of death

```
  1 REM PROGRAMME CALLED "SCALER"
  2 REM TO CALCULATE BODY SITE TEMPERATURE AT MOMENT OF DEATH (T0)
 10 REM E,B,L,R
 20 P=1:M=2:DIM E(M,M),S(2*P),T(M)
 30 INPUT"Data file:"F$:F%=OPENUP(F$):IF F%=0 CLOSE#F%:PRINT"Not found":END
 40 INPUT"Output file:"O$:O%=OPENOUT(O$)
 50 INPUT"Time shift: ",TS
 60 REPEAT INPUT"Rectal, Brain, Liver (R/B/L) "R$
 70 RECTAL=LEFT$(R$,1)="R":BRAIN=LEFT$(R$,1)="B":LIVER=LEFT$(R$,1)="L"
 80 UNTIL RECTAL OR BRAIN OR LIVER:PROCSTARTVAL:PROCOUTPUT:END
 90
100 DEFPROCSOLVE:LOCAL I,J,K,F
110 FOR I=1 TO M-1:FOR J=I+1 TO M:F=E(J,I)/E(I,I)
120 FOR K=I TO M:E(J,K)=E(J,K)-F*E(I,K):NEXT K:T(J)=T(J)-F*T(I):NEXT J,I
130 T(M)=T(M)/E(M,M)
140 FOR I=M-1 TO 1 STEP -1:FOR J=I+1 TO M:T(I)=T(I)-E(I,J)*T(J):NEXT J
150 T(I)=T(I)/E(I,I):NEXT:ENDPROC
160
170 DEF PROCREAD:X=5*N+TS
180 ENV=FNREADN(F%):BR=FNREADN(F%):LIV=FNREADN(F%):RECT=FNREADN(F%)
190 IF N=0 TE0=ENV
200 IF RECTAL Y=RECT ELSE IF LIVER Y=LIV ELSE Y=BR
210 NULL=Y=0:ENDPROC
220 IF RATIO Y=(Y-ENV)/DENOM ELSE IF SUBE Y=Y-ENV
230
240 DEF PROCOUTPUT:FIN=FALSE:N=0:F%=OPENIN(F$):@%=&20208
250 REPEAT PROCREAD:IF NULL THEN 260
255 Y=(Y-ENV)/DENOM:FIN=Y<=0:IF Y>0 PROCW(X):PROCW(Y):PROCNL
260 N=N+1:UNTIL EOF#F% OR FIN:CLOSE#F%:CLOSE#O%:ENDPROC
270
280 DEF FNREADN(C%):LOCAL N$,C$:N$=""
290 REPEAT C$=CHR$(BGET#C%):UNTIL (C$)="0" AND C$<="9") OR C$="." OR EOF#C%
300 N$=C$
310 REPEAT C$=CHR$(BGET#C%):N$=N$+C$:UNTIL (C$)"9" OR C$("0") AND C$<>"."
320 IF EOF#C% GOTO 350
330 REPEAT C$=CHR$(BGET#C%):UNTIL (C$)="0" AND C$<="9") OR C$="." OR EOF#C%
340 IF NOT EOF#C% THEN PTR#C%=PTR#C%-1
350 =VAL(N$)
360
370 DEF PROCSTARTVAL:PP=P:M=2:P=1:STARTUP=TRUE
380 FOR I=0 TO 2*P:S(I)=0:NEXT:FOR J=0 TO P:T(J)=0:NEXT
390 N=0:FIRST=TRUE:REPEAT PROCREAD:IF NULL THEN 430
400 FOR K=0 TO 2*P:S(K)=S(K)+(X^K):NEXT
410 IF Y>0 YY=LN(Y) ELSE PRINT"NEGATIVE Y":CLOSE#F%:END
420 XP=1:FOR K=1 TO M:T(K)=T(K)+YY*XP:XP=XP*X:NEXT
430 N=N+1:UNTIL N>36 OR EOF#F%:CLOSE#F%
440 FOR I=1 TO M:FOR K=1 TO M:E(I,K)=S(I+K-2):NEXT K,I:PROCSOLVE
450 PRINT"T0 = "EXP(T(1)),"   Te0 = ",TE0
460 DENOM=EXP(T(1))-TE0:P=PP:M=P+1:STARTUP=FALSE:ENDPROC
999
1000 DEF PROCW(X):LOCAL I,J,LD:X=(X+.005)/1000:IF X>=10 PRINT"ERROR":ENDPROC
1010 LD=TRUE:BPUT#O%,32:FOR I=1 TO 6:J=INTX:IF LD AND J=0 J=32 ELSE J=J+48:LD=FALSE
1015 BPUT#O%,J:X=(X-INTX)*10:IF I=4 BPUT#O%,46:LD=FALSE
1020 NEXT:ENDPROC
1040 DEF PROCNL:BPUT#O%,13:BPUT#O%,10:ENDPROC
```

Appendix I: Programme 6: Used to fit various mathematical functions to the data

```
10REM E,B,L,R
20 VDU2:@%=5:XL=200:XR=1200:YB=200:YT=1000:DIM C(4)
30 *FX6,10
40 INPUT"Data file:"F$:F%=OPENUP(F$):IF F%=0 CLOSE#F%:PRINT"Not found":END
50 INPUT"Time shift: ",TS
55 INPUT"Minimum X-value",XMIN
60 INPUT"Maximum X-value",XMAX:IF XMAX=0 XMAX=3600
70 XFAC=(XR-XL)/(XMAX-XMIN)
80 REPEAT INPUT"Rectal, Brain, Liver (R/B/L) "R$
90 RECTAL=LEFT$(R$,1)="R":BRAIN=LEFT$(R$,1)="B":LIVER=LEFT$(R$,1)="L"
100 UNTIL RECTAL OR BRAIN OR LIVER
110 INPUT"Subtract Env. Temp (Y/N) ",R$:SUBE=R$="Y"
130 INPUT"Subtract Const. Temp (Y/N) ",R$:SUBC=R$="Y":IF SUBC INPUT"Value",TD
150 YMAX=48:YFAC=(YT-YB)/YMAX
160 REPEAT INPUT"Polynomial, Exponential or Hybrid exp. fit (P/E/H)",R$
170 POLY=LEFT$(R$,1)="P":XPON=LEFT$(R$,1)="E":HYBR=LEFT$(R$,1)="H"
180 UNTIL POLY OR XPON OR HYBR
190 IF HYBR P=4 ELSE IF XPON P=1 ELSE INPUT"Order of Polynomial",P
200 INPUT"Title of case:"TITLE$:VDU3
210 M=P+1:DIM E(M,M),S(2*P),T(M)
220 FOR I=0 TO 2*P:S(I)=0:NEXT:FOR J=0 TO P:T(J)=0:NEXT
230 MODE 1:CLS:PROCAXES
240 N=0:FIRST=TRUE:REPEAT:PROCREAD:IF NULL OR X<XMIN THEN 290
250 FOR K=0 TO 2*P:S(K)=S(K)+(X^K):NEXT
260 IF FIRST PROCMV(X,Y):FIRST=FALSE ELSE PROCDR(X,Y)
266 IF NOT XPON YY=Y:GOTO 270
268 IF Y>0 YY=LN(Y) ELSE PRINT"NEGATIVE Y":CLOSE#F%:END
270 XP=1:FOR K=1 TO M:T(K)=T(K)+YY*XP:XP=XP*X:NEXT
290 N=N+1:UNTIL X>XMAX OR EOF#F%:CLOSE#F%:POINTS=N-1:XMAX=X
300 FOR I=1 TO M:FOR K=1 TO M:E(I,K)=S(I+K-2):NEXT K,I:PROCSOLVE
310 *FX3,10
320 VDU2:@%=&50A:IF POLY PROCPOLY ELSE IF XPON PROCEXPO ELSE PROCHYBR
330 PROCERROR:VDU3
340 *FX3,0
350 GCOL0,1:PROCPLOT
360 CHAIN "COLDUMP"
370 END
380
390 DEFPROCSOLVE:LOCAL I,J,K,F
400 FOR I=1 TO M-1:FOR J=I+1 TO M:F=E(J,I)/E(I,I)
410 FOR K=I TO M:E(J,K)=E(J,K)-F*E(I,K):NEXT K:T(J)=T(J)-F*T(I):NEXT J,I
420 T(M)=T(M)/E(M,M)
430 FOR I=M-1 TO 1 STEP -1:FOR J=I+1 TO M:T(I)=T(I)-E(I,J)*T(J):NEXT J
440 T(I)=T(I)/E(I,I):NEXT:ENDPROC
450
460 DEF PROCPOLY:PRINT:PRINT"Polynomial fit, order ";P
470 PRINT"Coefficients:":FOR I=1 TO M:PRINT T(I):NEXT:PRINT:ENDPROC
480
490 DEF PROCEXPO:PRINT:PRINT"Exponential fit":A=EXP(T(1)):B=T(2)
500 PRINT"Function = ";A;" * EXP (";B;" * T )":PRINT:ENDPROC
510
520 DEF PROCHYBR:PRINT:PRINT"Hybrid Exp. fit":A=T(1):B=T(2)/T(1)
530 FOR I=1 TO 5:PRINT T(I):NEXT
```

```
540 PRINT"Function = ";A;" * EXP (";B;" * T )":PRINT:ENDPROC
550
560 DEF PROCPLOT:PROCMV(XMIN,FNVAL(XMIN))
570 FOR X=XMIN TO XMAX STEP (XMAX-XMIN)/100:PROCDR(X,FNVAL(X)):NEXT:ENDPROC
580
590 DEF FNVAL(X):IF POLY THEN 600 ELSE =A*EXP(X*B)
600 LOCAL F:F=0:FOR I=1 TO M:F=F+T(I)*X^(I-1):NEXT:=F
610
620 DEF PROCREAD:X=5*N+TS
630 ENV=FNREADN(F%):BR=FNREADN(F%):LIV=FNREADN(F%):RECT=FNREADN(F%)
640 IF RECTAL Y=RECT ELSE IF LIVER Y=LIV ELSE Y=BR
641 NULL=Y=0:IF NULL ENDPROC
642 IF SUBE Y=Y-ENV
644 IF SUBC Y=Y-TD
645 IF Y<.1 THEN Y=.1
650 ENDPROC
660
720 DEF PROCMV(X,Y):MOVE (X-XMIN)*XFAC+XL,Y*YFAC+YB:ENDPROC
730 DEF PROCDR(X,Y):DRAW (X-XMIN)*XFAC+XL,Y*YFAC+YB:ENDPROC
740
750 DEF PROCAXES:CLS:VDU5
760 PROCMV(XMIN,0):PROCDR(XMAX,0)
770 FOR X=XMIN TO XMAX STEP FNSCALE(XMAX):PROCMV(X,0):PLOT 0,0,-30
780 VDU8,8,8,8:PRINT X;:PROCMV(X,0):PLOT 1,0,10:NEXT
790 PROCMV(XMIN,0):PROCDR(XMIN,YMAX)
800 FOR Y=0 TO YMAX STEP FNSCALE(YMAX):PROCMV(XMIN,Y):PLOT 0,0,20
810 VDU8,8,8,8,8:PRINT Y:PROCMV(XMIN,Y):PLOT 1,10,0:NEXT
815 MOVE 1100,120:PRINT "min.":MOVE 30,1000:PRINT"Temp"
816 MOVE 1000,1000:PRINT"Data":MOVE 1000,950:GCOL0,1:PRINT"Fitted":GCOL0,3
820 MOVE 30,30:PRINT TITLE$:VDU4:ENDPROC
830
840 DEF PROCERROR:F%=OPENUP(F$):U=0:D=0:PR=TS+XMIN
844 PRINT"        TIME       DATA VAL.          FITTED VAL.          DIFF"
850 FOR N=0 TO POINTS:PROCREAD:IF NOT NULL D=Y-FNVAL(X):U=U+D^2:NC=NC+1
856 IF X>=PR AND NOT NULL PRINT X,Y;" ",FNVAL(X);" ",D:PR=PR+15
860 NEXT N:CLOSE#F%
870 SIGMA=SQR(U/NC):PRINT"RMS Error:";:@%=&50A:PRINT SIGMA:ENDPROC
880
890 DEF FNSCALE(X):S=10^(INT LOG X)
900 IF X>6*S =S*2 ELSE IF X>3*S =S ELSE IF X>1.5*S =S/2 ELSE =S/5
910
920 DEF FNREADN(C%):LOCAL N$,C$:N$=""
930 REPEAT C$=CHR$(BGET#C%):UNTIL (C$)="0" AND C$(="9") OR C$="." OR EOF#C%
940 N$=C$
950 REPEAT C$=CHR$(BGET#C%):N$=N$+C$:UNTIL (C$)"9" OR C$("0") AND C$()"."
960 IF EOF#C% GOTO 990
970 REPEAT C$=CHR$(BGET#C%):UNTIL (C$)="0" AND C$(="9") OR C$="." OR EOF#C%
980 IF NOT EOF#C% THEN PTR#C%=PTR#C%-1
990 =VAL(N$)
1000
```

Appendix I: Programme 7: Used to transfer data from BBC microcomputer to the mainframe computer

```
 1 REM PROGRAMME CALLED "EMUSEND"
 2 REM TO TRANSFER DATA FROM BBC TO MAIN FRAME 2988 GUME
10 OSBYTE=&FFF4:PROCASSEMBLE:LON=FALSE:XOFF=FALSE
20 MODE 3:PRINT:PRINT:PRINT"GUCS BBC Terminal Emulation":PROCKEYS:END
30
40 DEF PROCKEYS:PRINT
50 *KEY0"PROCOPENLOG|M"
60 *KEY1"PROCSENDFILE|M"
70 *KEY9"PROCEMU|M"
80 PRINT "f0 - Open Log     f1 - Send file                          f9 - Emulator
90 PRINT:ENDPROC
100
110 DEF PROCEMU:VDU11:PRINT"< Enter Emulator >":Y%=0
120 *FX18
130 *FX229,1
140 REPEAT
150 *FX2,1
160 IF ADVAL-2=0 THEN 240
170 IF ADVAL-2>100 THEN 200
180 L%=GET:VDUL%:IF LON BPUT#F%,L%
190 IF ADVAL-2>0 THEN 170 ELSE 240
200 Y%=19:CALL SEND
210 L%=GET:VDUL%:IF LON BPUT#F%,L%
220 IF ADVAL-2>0 THEN 210
230 Y%=17:CALL SEND
240 IF ADVAL-1=0 THEN 160
250 *FX2,2
260 Y%=GET:IF Y%<>27 CALL SEND
270 UNTIL Y%=27
280 K%=0:PRINT:PRINT"< Leave Emulator >":IF LON CLOSE#F%:LON=FALSE
290 *FX229,0
300 PROCKEYS:ENDPROC
310
320 DEF PROCOPENLOG:VDU11:PRINT"< Open Disc Log File >      ";
330 INPUT"Name: "F$:F%=OPENOUT(F$):LON=TRUE:PRINT:PRINT:PROCEMU:ENDPROC
340
350 DEF PROCSENDFILE:VDU 11:PRINT"< Send File >             ";
360 INPUT"BBC File Name: "F$:F%=OPENUP(F$)
370 IF F%=0 THEN PRINT"File not found":ENDPROC
380 INPUT"                    Mainframe File Name: "F$:F$="INPUT(NAME="+F$+")
390 *FX229,1
400 USERESC=FALSE:PROCSEND(F$):REPEAT PROCSENDLINE:UNTIL EOF#F% OR USERESC
410 IF USERESC PRINT:PRINT"< User Escape >" ELSE PROCSEND("****")
420 *FX229,0
430 PRINT:PRINT"< End of Send >":PRINT:CLOSE #F%:PROCEMU:ENDPROC
440 ENDPROC
450
460 DEF PROCSENDLINE
470 REPEAT K%=BGET#F%:IF K%<>10 Y%=K%:CALL SEND
480 UNTIL K%=13 OR EOF#F%
490 IF K%<>13 Y%=13:CALL SEND
500 REPEAT PROCWAITREPLY:UNTIL E%=13 OR USERESC
510 REPEAT PROCWAITREPLY:UNTIL E%=45 OR USERESC
520 ENDPROC
```

```
530
540 DEF PROCSEND(S$):FOR I%=1 TO LEN(S$):Y%=ASC(MID$(S$,I%,1)):CALL SEND:NEXT
550 Y%=13:CALL SEND
560 REPEAT PROCWAITREPLY:UNTIL E%=13 OR USERESC
570 REPEAT PROCWAITREPLY:UNTIL E%=45 OR USERESC
580 ENDPROC
590
600 DEF PROCWAITREPLY
610 *FX2,1
620 IF ADVAL-2>0 E%=GET:VDU E%
630 *FX2,2
640 IF ADVAL-1>0 K%=GET:USERESC= K%=27
650 ENDPROC
660
670 DEF PROCASSEMBLE:DIM P% 20
680 [:.SEND LDA #138:LDX#2:JSR OSBYTE:RTS:]:ENDPROC
```

Appendix I: Programme 8: Used to calculate the rate of cooling after death

```
 1 REM TO CALCULATE HEAT LOSS IN A GIVEN TIME FOR A GIVEN SITE
 5 REM PROGRAMME CALLED 'DIFFER'
10 @%=&20208
20 DIM C$(4),T(4),P(4):C$(1)="E":C$(2)="B":C$(3)="L":C$(4)="R"
30 INPUT"Name of data file: "F$:F%=OPENUP(F$)
40 IF F%=0 PRINT"FILE NOT FOUND":END
50 INPUT"Time Interval: "DT,"Time Shift: "SHIFT:T=SHIFT-5:T0=SHIFT
60 PRINT
70 PRINT"   Time        Env.            Brain          Liver             Rectal"
80 PRINT"          ";:FOR I=1 TO 4:PRINT"  Data    Diff  ";:NEXT:PRINT:PRINT
90 FIRST=TRUE:REPEAT PROCREAD:IF T=T0 PROCPRINT:T0=T0+DT
100 UNTIL EOF#F%
110 CLOSE#F%:END
120
130 DEF PROCREAD:T=T+5:FOR I=1 TO 4:TT=FNREADN(F%):IF TT>0 T(I)=TT
140 NEXT:ENDPROC
150
160 DEF PROCPRINT:IF NOT FIRST PRINT" ",;:FOR I=1 TO 4:PRINT " ",T(I)-P(I);:NEXT:PRINT
170 PRINT T;:FOR I=1 TO 4:PRINT T(I);" ",;:P(I)=T(I):NEXT:PRINT
180 FIRST=FALSE:ENDPROC
190
200 DEF FNREADN(C%):LOCAL N$,C$:N$=""
210 REPEAT C$=CHR$(BGET#C%):UNTIL (C$)="0" AND C$<="9") OR C$="." OR EOF#C%
220 N$=C$
230 REPEAT C$=CHR$(BGET#C%):N$=N$+C$
240 UNTIL (C$)"9" OR C$<"0") AND C$<>"."
250 IF EOF#C% GOTO 280
260 REPEAT C$=CHR$(BGET#C%):UNTIL (C$)="0" AND C$<="9") OR C$="." OR EOF#C%
270 IF NOT EOF#C% THEN PTR#C%=PTR#C%-1
280 =VAL(N$)
```

Appendix I: Programme 9: Used to display plots of site temperature ratios versus post-mortem interval

```
 1 REM PROGRAMME CALLED "DISPRAT"
 2 REM TO DISPLAY PLOT OF SITE TEMPERATURE RATIOS VERSUS TIME IN MINUTE
10 @%=2:DIM C(4),T(4),S(4),K0(4),C$(4):C(1)=2:C(2)=5:C(3)=4:C(4)=1
11 C$(1)="E":C$(2)="B/R":C$(3)="L/R":C$(4)="R"
20 X0=150:XR=1080:Y0=100:YR=800
30 INPUT"Name of data file: "F$:F%=OPENUP(F$)
35 IF F%=0 PRINT"FILE NOT FOUND":END
40 INPUT"Title: "TITLE$
50 INPUT"Max. data time: "DT:IF DT=0 DT=3600
55 INPUT"Time shift: ",TSH
60 FAC=XR/DT:HOURS=DT/60
70 MODE2:PROCAXES:PROCREAD:FOR I=1 TO 4:S(I)=T(I):NEXT
80 FOR I=2 TO 3:IF T(I)=0 K0(I)=-1 ELSE K0(I)=1
90 NEXT I
100 K=5:REPEAT PROCREAD:PROCPLOT:K=K+5:UNTIL K>DT OR EOF#F%
110 CLOSE#F%:PROCdump:END
120
130 DEF PROCREAD:FOR I=1 TO 4:T(I)=FNREADN(F%):NEXT
140 T(2)=20*T(2)/T(4):T(3)=20*T(3)/T(4):ENDPROC
180
190 DEF PROCAXES:VDU5:CLS:FOR Y=0 TO YR STEP 20:PLOT 69,X0,Y0+Y:NEXT
200 FOR K=1 TO 2:MOVE 0,K*400+Y0:PRINT K;:NEXT
210 FOR X=0 TO XR STEP 12:FOR K=0 TO 3:PLOT 69,X+X0,K*200+Y0:NEXT K,X
220 FOR H=10 TO HOURS STEP 10:MIN=H*60:PROCMV(MIN,0):PROCDR(MIN,.8)
240 PROCMV(MIN,-1.2):VDU8:PRINT H:NEXT
250 MOVE 400,900:PRINT LEFT$(TITLE$,12);:MOVE 1000,30:PRINT"Hrs"
255 FOR I=2 TO 3:MOVE 1000,1000-50*I:GCOL0,C(I):PRINTC$(I):NEXT
260 VDU4:ENDPROC
270
280 DEF PROCPLOT:FOR I=2 TO 3:IF T(I)=0 THEN 290
286 IF K0(I)>-1 GCOL0,C(I):PROCMV(K0(I)+TSH,S(I)):PROCDR(K+TSH,T(I))
288 K0(I)=K:S(I)=T(I)
290 NEXT:ENDPROC
299
300 DEF PROCMV(X,Y):MOVE X*FAC+X0,Y*20+Y0:ENDPROC
310 DEF PROCDR(X,Y):DRAW X*FAC+X0,Y*20+Y0:ENDPROC
320
900 DEF FNREADN(C%):LOCAL N$,C$:N$=""
910 REPEAT C$=CHR$(BGET#C%):UNTIL (C$>="0" AND C$<="9") OR C$="." OR EOF#C%
920 N$=C$
930 REPEAT C$=CHR$(BGET#C%):N$=N$+C$
940 UNTIL (C$>"9" OR C$<"0") AND C$<>"."
950 IF EOF#C% GOTO 980
960 REPEAT C$=CHR$(BGET#C%):UNTIL (C$>="0" AND C$<="9") OR C$="." OR EOF#C%
970 IF NOT EOF#C% THEN PTR#C%=PTR#C%-1
980 =VAL(N$)
1020DEF PROCdump
1030A$=GET$:IFA$="0" THEN ENDPROC ELSE IF A$<>"S" AND A$<>"L" THEN 1030
1040IF A$="L" lineend=80:pixelinc=2 ELSE lineend=40:pixelinc=4
1050VDU29,0;0;:PROCcoldump
1060ENDPROC
1070REM COLOURJET B&C DUMP-MODES 0,1,2,4,5
1080DEF PROCcoldump
1090PROCassemble:REM ASSEMBLE'CODE
1110VDU19,0,7,0,0,0:VDU19,7,0,0,0,0
```

```
1130*FX6,0
1150*FX5,1
1160VDU2
1170*FX3,10
1180CALL DUMP
1190*FX6,10
1200*FX3,4
1210ENDPROC
1220DEF PROCassemble
1230OSWORD=&FFF1:OSWRCH=&FFEE:OSBYTE=&FFF4
1260P%=&80:xlow=P%:xhi=P%+1:ylow=P%+2:yhi=P%+3:LGCOL=P%+4
1320DOTCTR=P%+10:buffptr=P%+11:datacount=P%+12
1350DIM COD% 700
1360FORPASS=0TO2STEP2
1370P%=COD%:redram=P%:greenram=P%+80:blueram=P%+160:P%=P%+240
1410[OPT PASS
1420.DUMP LDX #3:STX yhi:LDX #&FE:STX ylow
1440.NWLIN LDA yhi:BPL NWLIN1:LDA #&A:JMP OSWRCH
1460.NWLIN1 LDX #0:STX buffptr:STX xlow:STX xhi:INX:STX datacount
1480.getbyte LDA #8:STA DOTCTR
1490.GTBYT DEC DOTCTR:BMI testwhite:LDY #(xlow DIV256):LDX #(xlow MOD256)
1510 LDA #9:JSR OSWORD:LDA LGCOL:BPL GTPAL:LDA #0:BEQ putbuff
1540.GTPAL LDY#(LGCOL DIV 256):LDX#(LGCOL MOD 256)
1550 LDA #&B:JSR OSWORD:LDA LGCOL+1
1570.putbuff LDX buffptr
1580 LSR A:ROL redram,X:LSR A:ROL greenram,X:LSR A:ROL blueram,X
1610 CLC:LDA xlow:ADC #pixelinc:STA xlow:LDA xhi:ADC #0:STA xhi:JMP GTBYT
1630.testwhite LDX buffptr:LDA redram,X:AND greenram,X:AND blueram,X
1630 INC buffptr:CMP #&FF:BEQ nextbyte:INX:STX datacount
1650.nextbyte LDA buffptr:CMP #lineend:BNE getbyte
1660.sendrow LDA #27:JSR OSWRCH:LDA #&59:JSR OSWRCH
1680 LDA datacount:CMP #1:BNE out:JSR sendnull:JMP nexty
1710.out JSR OSWRCH
1720. sendred LDX #0
1730.red1 LDA redram,X:BNE red2:JSR sendnull
1750.red2 JSR OSWRCH:INX:CPX datacount:BNE red1
1760.sendgreen LDX #0
1770.grn1 LDA greenram,X:BNE grn2:JSR sendnull
1790.grn2 JSR OSWRCH:INX:CPX datacount:BNE grn1
1800.sendblue LDX #0
1810.blu1 LDA blueram,X:BNE blu2:JSR sendnull
1830.blu2 JSR OSWRCH:INX:CPX datacount:BNE blu1
1840.nexty LDA ylow:SEC:SBC #pixelinc:STA ylow
1850 LDA yhi:SBC #0:STA yhi:JMP NWLIN
1870.sendnull TXA:PHA:LDA #6:LDX#7:JSR OSBYTE:LDA #0:JSR OSWRCH
1880 LDA #6:LDX#0:JSR OSBYTE:PLA:TAX:LDA #0:RTS
1890]:NEXT PASS
1900ENDPROC
```

Appendix I: Programme 10: Used to print out values of site temperature ratio for selected times after death

```
 1 REM PROGRAMME CALLED "LISTRAT"
 2 REM TO CALCULATE SITE TEMPERATURE RATIOS
10 @%=&2020F
20 DIM C$(4),T(4),P(4):C$(1)="E":C$(2)="B":C$(3)="L":C$(4)="R"
30 INPUT"Name of data file: "F$:F%=OPENUP(F$)
40 IF F%=0 PRINT"FILE NOT FOUND":END
50 INPUT"Time Interval: "DT,"Time Shift: "SHIFT:T=SHIFT-5:T0=SHIFT
60 PRINT
70 PRINT"        Time        Rectal/Brain    Rectal/Liver    Liver/Brain"
90 REPEAT PROCREAD:IF T=T0 PROCPRINT:T0=T0+DT
100 UNTIL EOF#F%
110 CLOSE#F%:END
120
130 DEF PROCREAD:T=T+5:FOR I=1 TO 4:TT=FNREADN(F%):IF TT>0 T(I)=TT
135 BR=T(2):LIV=T(3):RECT=T(4)
140 NEXT:ENDPROC
150
160 DEF PROCPRINT:PRINT T;:IF BR>0 PRINT RECT/BR; ELSE PRINT "                ";
170 IF LIV>0 PRINT RECT/LIV; ELSE PRINT "                ";
180 IF BR>0 PRINT LIV/BR; ELSE PRINT "  ";
185 PRINT:ENDPROC
190
200 DEF FNREADN(C%):LOCAL N$,C$:N$=""
210 REPEAT C$=CHR$(BGET#C%):UNTIL (C$)="0" AND C$<="9") OR C$="." OR EOF#C%
220 N$=C$
230 REPEAT C$=CHR$(BGET#C%):N$=N$+C$
240 UNTIL (C$)"9" OR C$<"0") AND C$<>"."
250 IF EOF#C% GOTO 280
260 REPEAT C$=CHR$(BGET#C%):UNTIL (C$)="0" AND C$<="9") OR C$="." OR EOF#C%
270 IF NOT EOF#C% THEN PTR#C%=PTR#C%-1
280 =VAL(N$)
```

Appendix I: Programme 11: Used to calculate steroid indices.

```
  1 REM PROGRAMME IS CALLED "LINCURU"
  2 REM TO DRAW X Y STREIGHT LINE PLOT FOR CARBON ATOM NUMBER VERSUS RETENTIOI
TIME IN THE STEROID INDEXES
  5 PRINT:PRINT"*  *  Line Graph and Interpolation Program  *  *":PRINT
 10 INPUT"How many data points",N:DIM X(N),Y(N)
 20 YMN=0:YMX=0:FOR I=1 TO N:INPUT"X,Y:"X(I),Y(I)
 30 IF Y(I)>YMX YMX=Y(I) ELSEIF Y(I)<YMN YMN=Y(I)
 40 NEXT:MODE4:PROCAXES(0,50,0,50):PROCPLOT
 44 PROCCOLDUMP:VDU26,0,7,39,0
 50 REPEAT PRINT"X or Y? ";:R$=GET$:PRINT"    ";R$;
 60 IF R$="X" PROCFINDY ELSE IF R$="Y" PROCFINDX ELSE VDU8,32,13
 70 UNTIL R$="Q" OR R$="N":PRINT:V=VPOS:VDU26:PRINT TAB(0,V);:END
 80
 90 DEF PROCFINDY:INPUT"="X
100 IF X<X(1) OR X>X(N) PRINT TAB(25,VPOS-1)"Out of range":ENDPROC
110 I=1:REPEAT I=I+1:UNTIL X(I)>=X
120 Y=Y(I-1)+(X-X(I-1))*(Y(I)-Y(I-1))/(X(I)-X(I-1))
130 PRINT TAB(25,VPOS-1);"Y=";STR$((INT(Y*100)/100))
131 *FX3,10
132 VDU2:PRINT"*X=";STR$(X)," Y="STR$(Y):VDU3
133 *FX3,0
140 VDU3:ENDPROC
150
160 DEF PROCFINDX:INPUT"="Y
170 IF Y<YMN OR Y>YMX PRINT TAB(25,VPOS-1)"Out of range":ENDPROC
180 I=1:REPEAT I=I+1:UNTIL (Y(I-1)<=Y AND Y<=Y(I)) OR (Y(I)<=Y AND Y<=Y(I-1))
190 X=X(I-1)+(Y-Y(I-1))*(X(I)-X(I-1))/(Y(I)-Y(I-1))
200 PRINT TAB(25,VPOS-1);"X=";STR$((INT(X*100)/100))
201 *FX3,10
202 VDU2:PRINT" X=";STR$(X),"*Y="STR$(Y):VDU3
203 *FX3,0
210 VDU3:ENDPROC
220
230 DEF PROCAXES(XX0,XX1,YY0,YY1):X0=XX0:X1=XX1:Y0=YY0:Y1=YY1:CLS:VDU5
240 OX=200:OY=99:RX=1000:RY=600:XF=RX/(X1-X0):YF=RY/(Y1-Y0)
250 PROCMV(X0,0):PROCDR(X1,0):D=-INT LOG X1:IF D<0 D=0
260 FOR X=0 TO X1 STEP FNSCALE(X1):PROCMV(X,0):PLOT 0,0,-30
270 VDU8,8,8,8,8:PROCPRINTF(X,6,D):PROCMV(X,0):PLOT 1,0,10:NEXT
280 PROCMV(0,Y0):PROCDR(0,Y1):D=-INT LOG Y1:IF D<0 D=0
290 FOR Y=0 TO Y1 STEP FNSCALE(Y1):PROCMV(0,Y):PLOT 0,0,20:VDU8,8,8,8,8,8
300 PROCPRINTF(Y,6,D):PROCMV(0,Y):PLOT 1,10,0:NEXT:VDU4:ENDPROC
310
320 DEF PROCPLOT:FOR I=1 TO N:PROCPT(X(I),Y(I)):NEXT
330 PROCMV(X(1),Y(1)):FOR I=1 TO N:PROCDR(X(I),Y(I)):NEXT:ENDPROC
340
350 DEF PROCMV(X,Y):MOVE OX+(X-X0)*XF,OY+(Y-Y0)*YF:ENDPROC
360 DEF PROCDR(X,Y):DRAW OX+(X-X0)*XF,OY+(Y-Y0)*YF:ENDPROC
370 DEF PROCPT(X,Y):PROCMV(X,Y):PLOT 0,4,4
380 PLOT 1,-8,0:PLOT 1,8,-8:PLOT 1,8,8:PLOT 1,0,8:ENDPROC
390
400 DEF FNSCALE(X):S=10^(INT LOG X)
410 IF X>6*S =S*2 ELSE IF X>3*S =S ELSE IF X>1.5*S =S/2 ELSE =S/5
420
430 DEF PROCPRINTF(X,W%,D%)
440 @%=&20000+&100*D%+W%:PRINT X;:@%=&A0A:ENDPROC
500 DEF FNGETN:LOCAL N$:N$="":REPEAT K$=GET$:N$=N$+K$:UNTIL K$=CHR$(13):=VAL N
900
```

```
 950 DEF PROCCOLDUMP
 999
1000REPEAT A$=GET$:UNTIL A$="L" OR A$="S" OR A$="Q":IF A$="Q" THEN ENDPROC
1010 IFA$="L"THENlineend=80:pixelinc=2 ELSE lineend=40:pixelinc=4
1020VDU29,0;0;:PROCcoldump:ENDPROC
1040REM COLOURJET BBC DUMP-MODES 0,1,2,4,5
1050DEF PROCcoldump
1060PROCassemble:REM ASSEMBLE CODE
1070REM THE FOLLOWING LINE DOES A BLACK/WHITE SWAP, REMOVE IF NOT REQUIRED
1080VDU19,0,7,0,0,0:VDU19,7,0,0,0,0
1090REM PRINTER IGNORE CHAR=0
1100*FX6,0
1110REM PRINTER ON SCREEN OFF
1120*FX5,1
1130VDU2
1140*FX3,10
1150CALL DUMP
1160REM SCREEN ONLY
1170*FX3,0
1175VDU3
1180*FX6,10
1185VDU19,0,0,0,0,0:VDU19,7,7,0,0,0
1190ENDPROC
1200DEF PROCassemble
12100SWORD=&FFF1
12200SWRCH=&FFEE
12300SBYTE=&FFF4
1240P%=&80
1250 xlow=P%
1260 xhi=P%+1
1270 ylow=P%+2
1280 yhi=P%+3
1290LGCOL=P%+4
1300DOTCTR=P%+10:REM CNTS DOTS TO PROCESS
1310buffptr=P%+11
1320datacount=P%+12
1330DIM COD% 700
1340FORPASS=0TO2STEP2
1350P%=COD%
1360redram=P%
1370greenram=P%+80
1380blueram=P%+160:P%=P%+240
1390[OPT PASS
1400.DUMP LDX #3:STX yhi \init y coordinate
1410 LDX #&FE:STX ylow
1420.NWLIN LDA yhi:BPL NWLIN1 \not end of picture
1430 LDA #&A:JMP OSWRCH \line feed &RTS
1440.NWLIN1 LDX #0:STX buffptr
1450 STX xlow:STX xhi:INX:STX datacount
1460.getbyte LDA #8:STA DOTCTR
1470.GTBYT DEC DOTCTR:BMI testwhite
1480 LDY #(xlow DIV256):LDX #(xlow MOD256)
1490 LDA #9:JSR OSWORD \read pixel
1500 LDA LGCOL:BPL GTPAL \not outside screen area
1510 LDA #0:BEQ putbuff
```

```
1520.GTPAL LDY#(LGCOL DIV 256):LDX#(LGCOL MOD 256)
1530 LDA #&B:JSR OSWORD \get physical colour
1540 LDA LGCOL+1
1550.putbuff LDX buffptr
1560 LSR A:ROL redram,X
1570 LSR A:ROL greenram,X
1580 LSR A:ROL blueram,X
1590 CLC:LDA xlow:ADC #pixelinc:STA xlow:LDA xhi:ADC #0:STA xhi:JMP GTBYT
1600.testwhite LDX buffptr:LDA redram,X:AND greenram,X:AND blueram,X
1610 INC buffptr:CMP #&FF:BEQ nextbyte
1620 INX:STX datacount
1630.nextbyte LDA buffptr:CMP #lineend:BNE getbyte
1640.sendrow LDA #27:JSR OSWRCH
1650 LDA #&59:JSR OSWRCH
1660 LDA datacount:CMP #1:BNE out
1670 JSR sendnull
1680 JMP nexty
1690.out JSR OSWRCH
1700. sendred LDX #0
1710.red1 LDA redram,X:BNE red2
1720 JSR sendnull
1730.red2 JSR OSWRCH:INX:CPX datacount:BNE red1
1740.sendgreen LDX #0
1750.grn1 LDA greenram,X:BNE grn2
1760 JSR sendnull
1770.grn2 JSR OSWRCH:INX:CPX datacount:BNE grn1
1780.sendblue LDX #0
1790.blu1 LDA blueram,X:BNE blu2
1800 JSR sendnull
1810.blu2 JSR OSWRCH:INX:CPX datacount:BNE blu1
1820.nexty LDA ylow:SEC:SBC #pixelinc:STA ylow
1830 LDA yhi:SBC #0:STA yhi \next Y-coordinate
1840 JMP NWLIN
1850.sendnull TXA:PHA:LDA #6:LDX#7:JSR OSBYTE:LDA #0:JSR OSWRCH
1860 LDA #6:LDX#0:JSR OSBYTE:PLA:TAX:LDA #0:RTS
1870]:NEXT PASS
1880ENDPROC
```

Table AII.1: Body variables of the cases. (continued on next page)

CASE NUMBER	Weight kg	Height cm	Surface Area m^2	Cooling Size Factor m^2/kg	Sex	Age Years	Circumference cm	
							Head	Hip
1	85	1.88	2.11	0.0248	M	67		
2	85	1.75	2.01	0.0237	M	55		
3	60	1.68	1.68	0.0280	M	72		
4	65	1.65	1.72	0.0265	F	65		
5	70	1.78	1.87	0.0267	M	70		
6	65	1.73	1.77	0.0272	F	67		
7	50	1.68	1.55	0.0310	M	70	54	92
8	65	1.68	1.74	0.0267	M	63		
9	76	1.70	1.88	0.0247	M	60		
10	-	-	-	-	M	70		
11	90	1.83	2.12	0.0236	M	60	58	106
12	85	1.83	2.07	0.0244	M	65	52	108
13	80	1.83	2.02	0.0253	M	44		
14	48	1.68	1.53	0.0319	M	38	53	76
15	93	1.9	2.21	0.0238	M	-		
16	45	1.68	1.48	0.0329	F	62		
17	85	1.68	1.95	0.0229	F	70		
18	75	1.78	1.93	0.0257	M	65	58	100
19	80	1.75	1.96	0.0245	M	68		
20	75	1.83	1.97	0.0263	M	62		
21	53	1.83	1.7	0.0321	M	75		
22	85	1.83	2.07	0.0244	M	57	56	100
23	65	1.68	1.74	0.0268	F	55	48	102
24	76	1.78	1.94	0.0255	M	45		
25	61	1.75	1.75	0.0287	M	83		
26	45	1.78	1.55	0.0344	M	-		
27	50	1.63	1.52	0.0304	F	60	52	100
28	75	1.78	1.96	0.0261	M	55	57	92
29	70	1.75	1.85	0.0264	M	62	53	100
30	70	1.6	1.73	0.0247	M	63		
31	100	1.88	2.27	0.0227	M	76		
32	70	1.73	1.83	0.0261	M	-		
33	70	1.75	1.83	0.0264	M	57	-	
34	82	1.52	1.79	0.0218	F	82	-	97
35	56	1.68	1.63	0.0291	M	-		
36	104	1.78	2.21	0.0213	M	-	66	110
37	55	1.75	1.67	0.0304	M	55		
38	85	1.91	2.13	0.0251	M	33		
39	75	1.68	1.84	0.0245	F	70		
40	90	1.96	2.23	0.0248	M	60		
41	53	1.73	1.63	0.0308	M	49		
42	70	1.68	1.79	0.0256	M	70	52	96
43	55	1.75	1.67	0.0304	M	60	60	86
44	75	1.83	1.97	0.0263	M	69	55	104
45	80	1.8	2.0	0.025	M	58		
46	70	1.63	1.75	0.025	F	59	56	106
47	117	1.83	2.37	0.0203	M	75		
48	45	1.6	1.44	0.032	F	-		

Table AII.1: Body variables of the cases. (continued on next page)

CASE NUMBER	Weight kg	Height cm	Surface Area m^2	Cooling Size Factor m^2/kg	Sex	Age Years	Circumference cm	
							Head	Hip
49	52	1.52	1.47	0.0283	F	-		
50	60	1.68	1.68	0.028	M	73		
51	60	1.52	1.57	0.0262	F	64		
52	102	1.91	2.31	0.0227	M			
53	63	1.73	1.75	0.0278	M	50		
54	67	1.73	1.80	0.0269	M	60	57	105
55	65	1.80	1.83	0.0282	M	57		
56	70	1.73	1.83	0.0261	M	50	55	96
57	42	1.88	1.57	0.0374	M	67		
58	65	1.6	1.68	0.0259	F	84		
59	85	1.78	2.03	0.0239	M	65		
60	-	1.75	-	-	M	63		
61	-	1.70	-	-	M	65		
62	70	1.78	1.87	0.0267	M	-		
63	50	1.68	1.55	0.031	M	70		
64	105	1.91	2.34	0.0223	M	76	60	110
65	55	1.73	1.65	0.03	F	30		
66	-	-	-	-	F	-		
67	71	1.73	1.84	0.0259	M	49		
68	69	1.78	1.86	0.0270	M	78		
69	45	1.65	1.47	0.0327	M	67		
70	78	1.83	2.0	0.0256	M	52		
71	115	1.73	2.34	0.0187	M	80		
72	67	1.75	1.81	0.0271	M	70		
73	-	-	-	-	F	-		
74	45	1.52	1.39	0.0308	F	66	54	88
75	85	1.75	2.01	0.0236	M	75	46	94
76	83	1.7	1.94	0.0235	M	-	66	117
77	55	1.52	1.51	0.0274	F	82	54	100
78	65	1.78	1.81	0.0279	M	-	48	80
79	60	1.83	1.79	0.0298	M	72	60	90
80	64	1.73	1.76	0.0277	M	-	-	-
81	-	1.85	-	-	M	73	57	100
82	65	1.83	1.85	0.0284	M	48	52	94
83	60	1.68	1.68	0.0280	F		50	104-
84	75	1.83	1.97	0.0262	M	62	52	100
85	60	1.75	1.73	0.0289	M	46	52	88
86	60	1.83	1.79	0.0298	M	70	56	102
87	76				M	74	61	107
88	70	1.68	1.79	0.0256	M	75	60	88
89	90	1.98	2.25	0.025	M	49	60	108
90	100	1.83	2.22	0.0222	M	70	48	112
91	83	1.68	1.93	0.0232	M	52	61	91
92	80	1.83	2.02	0.0252	M	-	64	100
93	45	1.6	1.44	0.0319	F	75	60	90
94	70	1.83	1.91	0.0273	M	62	66	97
95	67	1.53	1.68	0.0251	F	-	56	102
96	107	1.7	2.17	0.0203	M	60	71	119

Table AII.1: (continued from previous page) Body variables of the cases.

CASE NUMBER	Weight kg	Height cm	Surface Area m^2	Cooling Size Factor m^2/kg	Sex	Age Years	Circumference cm	
							Head	Hip
97	65	1.78	1.81	0.0279	M	80	46	94
98	87	1.75	2.03	0.0233	M	-	61	112
99	56	-	-	-	F	-	56	91
100	64	1.7	1.74	0.0272	M	77	61	97
101	65	1.75	1.79	0.0276	M	72	52	92
102	70	1.63	1.76	0.0251	F	85	61	107
104	55	1.68	1.62	0.0294	F	33	50	96
105	60	1.63	1.64	0.0274	F	57	56	96
106	70	1.65	1.77	0.0253	M	-	66	117
107	75	1.68	1.85	0.0246	F	76	54	104
108	60	1.70	1.7	0.0283	M	52	61	112
109	70	1.75	1.85	0.0264	M	63	56	96
110	70	1.68	1.79	0.0256	F	71	55	120
111	85	1.78	2.02	0.0241	M	55	60	102
112	70	1.8	1.89	0.0269	M	60	55	105
113	75	1.7	1.86	0.0249	F	65	56	112
114	55	1.7	1.63	0.03	M	55	50	96
115	-	-	-	-	-	-	-	
116	78	1.68	1.88	0.0241	M	61	54	102
117	64	1.52	1.61	0.0251	F	70	66	112

Table AII.2: Cause of death and length of monitoring period in the cases studied (continued on next page).
M = Male; F = Female; C = covered; U = uncovered;
Y = Autopsy was performed; N = Autopsy was not performed

CASE NUMBER	STATE OF INSULATION	AUTOPSY	MONITORING PERIOD HOURS	C A U S E (S) O F D E A T H
1	U	Y	20	Ischaemic Heart Disease Coronary Artery Atheroma
2	U	Y	22	Ischaemic Heart Disease
3	U	Y	33	Ischaemic Heart Disease
4	U	Y	44	Ischaemic Heart Disease
5	U	Y	20	Ischaemic Heart Disease
6	U	Y	60	Coronary Thrombosis
7	U	Y	27	Ischaemic Heart Disease Coronary Artery Atheroma
8	U	Y	40	Ischaemic Heart Disease
9	U	Y	53	Ischaemic Heart Disease Coronary Artery Atheroma
10	U	Y	50	Ischaemic Heart Disease
11	U	Y	21	Ischaemic Heart Disease Coronary Artery Atheroma
12	U	Y	20	Ischaemic Heart Disease Coronary Artery Athero-sclerosis
13	U	Y	44	Ischaemic Heart Disease
14	U	Y	21	Inhalation of Vomit
15	U	Y	15	Coronary Artery Disease
16	U	Y	36	Ischaemic Heart Disease
17	U	Y	17	Ischaemic Heart Disease
18	U	Y	17	Acute Myocardial Infarction Coronary Artery Throm-bosis & Atheroma
19	U	N	27	Unknown (natural)
20	U	Y	22	Coronary Thrombosis, Chronic Bronchitis and Emphysema
21	U	Y	27	Coronary Artery Thrombosis
22	U	Y	20	Ischaemic Heart Disease
23	U	Y	24	Ischaemic Heart Disease Coronary Artery Atheroma
24	U	Y	8	Myocardial Infarction
25	U	Y	27	Ischaemic Heart Disease Coronary Artery Atheroma
26	U	Y	20	Ischaemic Heart Disease
27	U	Y	22	Bronchopneumonia
28	U	Y	4	Ischaemic Heart Disease Coronary Artery Atheroma
29	U	Y	34	Ischaemic Heart Disease
30	U	Y	60	Ischaemic Heart Disease

Table AII.2: Cause of death and length of monitoring period in the cases studied (continued on next page).
M = Male; F = Female; C = covered; U = uncovered;
Y = Autopsy was performed; N = Autopsy was not performed

CASE NUMBER	STATE OF INSULATION	AUTOPSY	MONITORING PERIOD HOURS	C A U S E (S) O F D E A T H
31	U	Y	45	Ischaemic Heart Disease Coronary Artery Atheroma
32	U	Y	22	Acute Myocardial Infarction,Coronary Artery Thrombosis & Atheroma
33	U	N	14	Unknown (natural)
34	U	Y	47	Ischaemic Heart Disease Coronary Artery Atheroma
35	U	Y	18	Myocardial Infarction
36	U	Y	46.6	Ischaemic Heart Disease Coronary Artery Atheroma
37	U	Y	25	Ischaemic Heart Disease
38	U	Y	24	Myocardial Infarction
39	U	Y	24	Hypertensive Heart Disease
40	U	Y	28.5	Ischaemic Heart Disease Coronary Artery Atheroma
41	U	Y	24	Ischaemic Heart Disease Coronary Artery Atheroma
42	U	Y	24	Acute Asthma
43	U	Y	24	Ischaemic Heart Disease
44	U	N	30	Unknown (natural)
45	U	Y	35	Ischaemic Heart Disease
46	U	N	26	Unknown (natural)
47	U	Y	60	Inhalation of Gastric Contents, Fatty Degeneration of Myocardium
48	U	Y	46	Chronic Obstructive Airways Disease
49	U	N	2.5	Unknown (natural)
50	U	Y	19	Ischaemic Heart Disease
51	U	Y	21	Chronic Alcoholism
52	U	Y	23	Hypertrophic Cardiomyopathy
53	U	Y	28.75	Ischaemic Heart Disease Coronary Artery Atheroma
54	U	N	40	Unknown (Natural)
55	U	Y	20	Coronary Thrombosis
56	U	Y	24	Hypertensive Heart Disease
57	U	Y	37	Hypertensive Heart Disease
58	U	Y	47	Ischaemic Heart Disease
59	U	Y	28	Ischaemic Heart Disease
60	U	Y	33.75	Ischaemic Heart Disease
61	U	N	21	Unknown (Natural)

Table AII.2: Cause of death and length of monitoring period in the cases studied (continued on next page).
M = Male; F = Female; C = covered; U = uncovered;
Y = Autopsy was performed; N = Autopsy was not performed

CASE NUMBER	STATE OF INSULATION	AUTOPSY	MONITORING PERIOD HOURS	C A U S E (S) O F D E A T H
62	U	Y	26.5	Ischaemic Heart Disease
63	U	Y	21	Ischaemic Heart Disease
64	U	N	24	Unknown (Natural)
65	U	Y	20	Spontaneous Rupture of Cerebral Aneurysm
66	U		24	
67	U	Y	24	Ischaemic Heart Disease
68	U	Y	60	Haemoptesis Bronchial Carcinoma
69	U	Y	60	Ischaemic Heart Disease
70	U	Y	60	Ischaemic Heart Disease
71	U	Y	25	Ischaemic Heart Disease Coronary Artery Atheroma
72	U	Y	43	Congestive Cardiac Failure and Mitral Stenosis
73	U	Y	27	Cor pulmonale: Chronic Bronchitis & Emphysema
74	C	Y	28	Myocardial Infarction
75	C	Y	29	Ischaemic Heart Disease. Coronary Artery Atheroma
76	C	N	45	Unknown (Natural)
77	C	N	31.5	Unknown (Natural)
78	C	Y	46	Chronic Bronchitis
79	C	Y	30.5	Ischaemic Heart Disease
80	C	Y	32.5	Ischaemic Heart Disease Coronary Artery Atheroma
81	C	Y	26	Ischaemic Heart Disease
82	C	Y	24	Chronic Bronchitis
83	C	Y	24	Hypertensive Heart Disease. Hypothyroidism
84	C	Y	46	Ischaemic Heart Disease
85	C	Y	23	Ischaemic Heart Disease Coronary Artery Atheroma Chronic Bronchitis
86	C	N	50	Unknown (Natural)
87	C	Y	26	Ischaemic Heart Disease
88	C	N	24	Unknown (Natural)
89	C	Y	31	Hypertensive Heart Disease
90	C	Y	25	Ischaemic Heart Disease Coronary Artery Atheroma
91	C	Y	45	Ischaemic Heart Disease Coronary Artery Atheroma
92	C	Y	24	Ischaemic Heart Disease

Table AII.2: Cause of death and length of monitoring period in the cases studied
M = Male; F = Female; C = covered; U = uncovered;
Y = Autopsy was performed; N = Autopsy was not performed

CASE NUMBER	STATE OF INSULATION	AUTOPSY	MONITORING PERIOD HOURS	C A U S E (S) O F D E A T H
93	C	Y	44	Ischaemic Heart Disease Coronary Artery Atheroma
94	C	Y	42.5	Ischaemic Heart Disease
95	C	Y	32	Ischaemic Heart Disease Coronary Artery Atheroma
96	C	N	30	Unknown (Natural)
97	C	Y	25	Ischaemic Heart Disease
98	C	Y	27	Ischaemic Heart Disease Coronary Artery Atheroma
99	C	Y	60	Myocardial Infarction Arterial Sclerosis
100	C	N	22.5	Unknown (Natural)
101	C	Y	24	Ischaemic Heart Disease Coronary Artery Atheroma
102	C	Y	24	Ischaemic Heart Disease
103	C	Y	25	Coronary Artery Atheroma
104	C	Y	20	Bronchopneumonia
105	C	Y	20	Ischaemic Heart Disease Coronary Artery Atheroma
106	C	N	42	Unknown (Natural)
107	C	Y	24	Ischaemic Heart Disease Coronary Artery Atheroma
108	C	Y	21	Ischaemic Heart Disease
109	C	Y	18	Ischaemic Heart Disease Coronary Artery Atheroma Chronic Bronchitis and Emphysema.
110	C	Y	24	Hypertensive & Ischaemic Heart Disease
111	C	N	41	Unknown (Natural)
112	U	Y	29	Ischaemic Heart Disease Coronary Artery Atheroma
113	U	N	12	Unknown (Natural)
114	U		60	
115	U	Y	43	Haemopericardium.Rupture of Myocardial Infarction
116	C	Y	24	Ischaemic Heart Disease Coronary Artery Atheroma
117	C	Y	26	Ischaemic Heart Disease

Table AII.3: Normal temperature of the body site (^{o}C) at the moment of death as estimated from post-mortem data for covered bodies. Environmental temperature (^{o}C) at the commmencement of monitoring is also shown.

CASE NUMBER	Rectum	Liver	Brain	Environmental Temperature at Commencement of Monitoring ^{o}C
74	36.10	36.25	31.78	19.17
75	37.25	35.70	24.66	8.98
76	26.14	35.03	30.22	10.78
77	32.10	31.41	25.84	10.78
78	30.20	37.23	29.38	11.98
79	35.38	33.28	25.71	10.78
80	22.61	30.75	-	10.78
81	23.32	31.52	23.27	11.38
82	34.42	30.75	21.30	9.58
83	36.08	28.81	25.83	10.18
84	36.79	27.74	18.15	10.78
85	34.16	29.48	21.84	8.98
86	35.58	33.52	29.35	19.17
87	25.0	32.87	28.19	18.57
88	37.18	33.17	29.90	18.57
89	35.08	31.35	28.76	17.97
90	34.46	38.74	29.47	12.58
91	30.91	31.42	32.42	17.37
92	36.88	32.58	-	17.97
93	36.45	38.19	31.85	18.57
94	38.01	34.73	-	19.77
95	30.9	34.59	33.5	14.37
96	32.0	34.68	28.79	13.18
97	31.8	35.99	27.66	11.38
98	37.6	27.71	27.14	17.37
99	36.5	32.12	31.04	13.78
100	32.2	29.79	30.10	16.17
101	29.9	28.92	29.33	10.78
102	30.1	32.24	28.91	16.17
103	32.0	33.78	23.77	8.98
104	33.5	35.64	33.02	19.77
105	34.9	33.33	30.95	17.37
106	35.1	30.93	28.17	8.38

Table AII.4: Normal temperature of the body site (°C) at the moment of death as estimated from post-mortem data for naked bodies. Environmental temperature (°C) is also shown. (continued on next page)

CASE NUMBER	Rectum	Liver	Brain	Environmental Temperature at Commencement of Monitoring °C
1	36.29	25.94	25.05	14.37
2	37.48	25.91	24.70	17.17
3	36.72	27.97	24.30	14.70
4	36.61	31.40	32.15	16.77
5	36.95	-	27.05	8.38
6	37.13	28.03	24.22	16.50
7	36.12	24.08	28.34	8.3
8	37.87	30.52	30.00	18.82
9	30.37	30.17	27.24	16.17
10	30.92	27.32	28.44	15.57
11	36.21	25.53	29.35	15.57
12	35.74	24.08	24.98	19.17
13	35.15	26.28	22.71	8.98
14	32.12	27.25	28.31	14.97
15	-	26.55	-	16.87
16	36.74	32.5	26.84	16.77
17	36.02	29.56	24.47	13.18
18	36.38	19.32	27.99	15.57
19	37.19	22.53	16.91	9.58
20	37.28	29.96	25.94	20.32
21	36.91	31.33	26.64	15.57
22	36.77	25.1	26.95	17.97
23	36.07	25.79	-	18.57
24	40.71	24.70	21.65	16.50
25	35.40	33.87	32.55	13.78
26	39.99	32.07	29.69	18.32
27	39.59	29.77	-	18.57
28	37.71	26.16	24.95	14.97
29	36.86	27.91	24.50	17.37
30	38.87	31.61	28.69	17.64
31	38.19	28.52	31.10	14.97
32	37.82	27.63	26.06	15.31
33	-	28.94	26.03	15.81
34	31.47	26.47	24.81	17.37
35	27.76	21.92	19.22	15.0
36	36.86	27.33	33.21	17.37
37	-	30.01	22.60	15.54
38	37.12	27.56	22.16	11.98
39	36.44	25.68	25.11	10.18
40	37.64	23.33	28.53	15.57
41	38.9	33.22	24.75	18.29
42	35.95	24.20	30.64	13.78
43	36.97	30.48	34.33	17.37
44	34.10	25.40	-	16.77
45	-	26.86	26.18	15.59

Table AII.4: (Continued from previous page) Normal temperature of the body site (°C) at the moment of death as estimated from post-mortem data for naked bodies. Environmental temperature (°C) is also shown.

CASE NUMBER	Rectum	Liver	Brain	Environmental Temperature at Commencement of Monitoring °C
46	37.12	25.71	28.38	16.17
47	37.68	25.89	25.98	17.70
48	38.85	30.71	33.80	15.57
49	28.03	26.18	25.78	16.77
50	-	31.96	25.54	16.59
51	-	30.74	25.22	16.65
52	37.34	28.63	28.35	11.98
53	26.40	26.10	24.75	16.77
54	36.24	26.61	25.33	22.76
55	-	30.16	25.57	15.68
56	35.54	20.79	24.84	17.37
57	-	27.98	24.84	15.05
58	37.54	30.12	24.73	18.15
59	35.32	29.08	24.96	14.97
60	38.87	21.36	-	10.18
61	40.20	34.67	27.41	22.09
62	-	27.34	24.70	16.40
63	37.37	32.10	29.64	11.38
64	36.68	29.75	28.96	17.37
65	36.80	27.47	24.78	14.97
66	36.72	24.44	21.84	14.37
67	35.13	26.82	23.83	17.37

Table AII.5: Parameters used in the curve-fitting of double-exponential equation to brain data for naked bodies; P_1 - P_4 = Parameters, where time is in hours; RMS = residual Mean Square.

CASE NUMBER	P_1	P_2	P_3	P_4	RMS
1	-1.92	-0.525	2.92	-0.390	0.08
2	-2.5	-0.487	3.5	-0.381	0.03
3	-0.06	-1.529	0.94	-0.287	0.15
6	-0.8	-0.019	1.8	-0.075	0.07
7	-1.48	-0.368	2.48	-0.267	0.05
8	-0.8	-0.269	1.8	-0.235	0.06
10	0.8	-0.173	1.7	-0.008	0.07
11	-0.8	-0.084	1.8	-0.115	0.04
12	-1.9	-0.562	2.9	-0.420	0.31
13	-1.5	-0.432	2.5	-0.320	0.07
14	-1.6	-0.463	2.6	-0.351	0.08
17	-0.13	-1.353	1.13	-0.129	0.07
18	-0.5	-0.512	1.5	-0.231	0.07
19	-2.7	-0.556	3.7	-0.418	0.3
20	-0.18	-65.82	1.18	-0.221	0.13
21	-1.6	-0.386	2.6	-0.279	0.08
22	-1.2	-0.368	2.2	-0.267	0.16
24	-2.1	-0.659	3.1	-0.506	0.07
26	0.32	-0.497	6.79	-0.157	0.03
28	-0.003	1.54	10.003	-0.146	0.15
29	-1.6	-0.385	-2.6	-0.273	0.3
30	0.63	-0.346	0.37	-0.056	0.08
31	0.83	-0.204	0.17	-0.022	0.04
32	0.02	-0.009	0.98	-0.124	0.09
33	0.43	-0.299	0.57	-0.299	0.08
37	-0.88	-0.341	1.88	-0.256	0.04
38	-1.9	-0.169	2.9	-0.142	0.1
39	-0.95	-0.294	1.95	-0.214	0.05
40	-0.08	-0.362	0.92	-0.151	0.04
41	0.8	-0.104	1.8	-0.160	0.4
42	-1.2	-0.34	2.2	-0.247	0.04
43	-1.9	-0.172	2.9	-0.126	0.13
46	-2.3	-0.405	3.3	-0.299	0.15
47	-1.8	-0.523	2.8	-0.394	0.04
48	0.89	-0.156	0.11	-0.013	0.04
49	-1.7	-0.621	2.7	-0.371	0.05
50	-0.80	-0.301	1.8	-0.277	0.11
51	-1.55	-0.42	2.55	-0.309	0.04
53	-0.8	-0.069	1.8	-0.116	0.13
54	-0.39	-206.8	1.39	-0.319	4.04
55	-0.8	-0.22	1.8	-0.22	0.03
58	-0.55	-0.267	1.55	-0.199	0.04
59	-1.87	-0.387	2.87	-0.277	0.12
61	-0.8	-0.0820	1.8	-0.129	0.07
62	-0.8	-0.067	1.8	-0.109	0.2
63	0.79	-0.148	0.21	-0.009	0.03
64	0.48	-0.353	0.52	-0.072	0.06
65	-1.79	-0.402	2.79	-0.292	0.1
66	-0.22	-1.672	1.2	-0.344	0.15
67	0.72	-0.212	0.28	-0.054	0.2

Table AII.6: Parameters used in the curve-fitting of double-exponential equation to liver data for naked bodies; P_1 - P_4 = Parameters, when time is in hours; RMS = Residual Mean Square

CASE NUMBER	P_1	P_2	P_3	P_4	RMS
1	-0.5	-0.075	1.5	-0.108	0.1
2	0.06	-0.777	0.94	-0.099	0.01
3	0.45	0.253	-0.55	-0.051	0.1
4	0.53	-0.128	0.47	-0.008	0.05
6	0.61	0.151	o.39	-0.044	0.14
7	-1.0	-0.225	2.0	-0.152	0.1
9	0.5	-0.165	0.5	-0.043	0.05
11	0.19	-0.312	0.81	-0.065	0.07
12	-0.9	-0.255	1.9	-0.174	0.5
13	0.07	-0.662	0.93	-0.107	0.07
14	-0.4	-0.073	1.4	-0.1	-
15	-0.4	-0.138	1.4	-0.093	0.04
16	0.68	-0.128	0.32	-0.015	0.04
19	-0.8	-0.194	1.8	-0.13	0.1
20	0.78	-0.154	0.22	-0.033	0.03
21	-0.6	-0.198	1.6	-0.137	0.03
22	-2.07	-0.258	3.07	-0.174	0.3
23	-0.4	-0.033	1.4	-0.071	0.22
24	0.06	-0.340	0.94	-0.145	0.04
25	0.43	-0.122	0.57	-0.0119	0.04
26	0.07	-0.447	0.93	-0.082	0.02
29	-0.4	-0.046	1.4	-0.080	0.13
30	0.26	-0.295	0.74	-0.066	-
31	0.4	-0.196	0.6	-0.028	0.06
32	-0.6	-0.127	1.6	-0.068	0.05
33	-0.5	-0.231	0.5	-0.047	-
37	-0.04	-1.05	0.96	-0.995	0.04
38	-0.03	-0.131	1.03	-0.069	0.06
39	0.18	-0.206	0.82	-0.066	0.05
41	0.34	-0.32	0.66	-0.079	0.04
42	-0.4	-0.039	1.4	-0.082	0.08
43	0.92	-0.063	0.08	-0.007	0.06
44	-0.6	-0.095	1.6	-0.095	0.13
45	-0.4	-0.09	1.4	-0.09	0.02
46	-1.2	-0.241	2.2	-0.161	0.21
47	0.2	-0.484	0.8	-0.079	0.06
48	0.82	-0.114	0.18	-0.012	0.04
50	0.22	-0.289	0.78	-0.063	0.01
52	0.6	-0.082	0.4	-0.003	0.07
55	0.3	-0.208	0.7	-0.062	0.02
58	-0.1	-0.183	1.1	-0.099	0.09
59	0.25	-0.577	0.75	-0.108	0.07
61	-0.21	-0.164	1.21	-0.113	0.01
63	0.35	-0.078	0.65	-0.039	0.02
64	0.18	-0.236	0.82	-0.035	0.06
65	-0.38	-0.175	1.38	-0.118	0.07
67	0.39	-0.196	0.61	-0.043	0.1

Table AII.7: Parameters used in the curve-fitting of double-exponential equation to rectal data for naked bodies; P_1 - P_4 = Parameters, where time is in hours; RMS = Residual Mean Square

CASE NUMBER	P_1	P_2	P_3	P_4	RMS
2	-3.4	-0.149	4.4	-0.127	0.03
3	-3.4	-0.16	4.4	-0.139	0.0048
4	-3.3	-0.174	4.3	-1.512	0.04
5	-3.4	-0.125	4.4	-0.111	0.02
6	-2.2	-0.157	3.2	-0.111	0.088
7	-3.0	-0.156	4.0	-0.127	0.1
8	-4.2	-0.194	5.2	-0.156	0.2
9	-3.4	-0.149	4.4	-0.149	0.09
10	-3.4	-0.150	4.4	-0.149	0.09
11	-3.4	-0.075	4.4	-0.068	0.05
12	-3.4	-0.165	4.4	-0.132	0.09
13	-3.4	-0.093	4.4	-0.081	0.06
16	-3.4	-0.235	4.4	-0.197	0.05
17	-3.4	-0.134	4.4	-0.105	0.03
18	-3.4	-0.141	4.4	-0.108	0.08
19	-3.4	-0.111	4.4	-0.097	0.04
20	-3.4	0.241	4.4	-0.198	0.31
21	-3.4	-0.186	4.4	-0.155	0.06
23	-0.1	-0.284	0.9	-0.055	0.07
24	-3.24	-0.234	4.4	-0.182	0.04
25	-0.6	-0.022	1.6	-0.070	0.05
26	-3.4	-0.125	4.4	-0.116	0.02
27	-3.4	-0.066	4.4	-0.066	0.03
28	-3.4	-0.106	4.4	-0.087	0.02
29	-3.5	-0.167	4.5	-0.131	0.13
30	-3.4	-0.135	4.4	-0.122	0.03
31	-3.4	-0.143	4.4	-0.117	0.07
32	-3.4	-0.130	4.4	-0.108	0.03
34	-3.4	-0.086	4.4	-0.097	0.12
35	-3.4	-0.162	4.4	-0.147	0.07
36	-3.4	-0.122	4.4	-0.098	0.09
39	-0.6	-0.003	1.6	-0.029	0.03
41	-0.6	-0.109	1.6	-0.109	0.08
42	-0.6	-0.008	1.6	-0.040	0.05
47	-0.6	-0.022	1.6	-0.042	0.07
48	-0.6	-0.021	1.6	-0.071	0.04
50	0.22	-0.289	0.78	-0.063	0.01
53	-0.6	-0.01	1.6	-0.05	0.16
54	-3.4	-0.05	4.4	-0.054	0.3
55	0.3	-0.208	0.7	-0.062	0.02
58	-0.1	-0.183	1.1	-0.099	0.09
59	0.25	-0.577	0.75	-0.108	0.07
61	-0.21	-0.164	1.21	-0.113	0.01
63	0.35	-0.978	0.65	-0.039	0.02
64	0.18	-0.236	0.82	-0.035	0.06
65	-0.38	-0.175	1.38	-0.118	0.07

Table AII.8: Parameters used in the curve-fitting of three-exponential equation to the brain data for covered bodies; P_1 - P_6 = Parameters used for time in hours. RMS = Residual Mean Square

CASE NUMBER	P_1	P_2	P_3	P_4	P_5	P_6	RMS
74	-5.9	-0.151	4.5	-0.134	2.4	-0.153	0.09
75	-5.9	-0.004	4.5	-0.035	2.4	-0.017	0.32
76	-5.9	-0.034	4.5	-0.058	2.4	-0.016	0.06
77	-5.9	-0.006	4.5	-0.002	2.4	-0.049	0.08
78	-5.9	-0.142	4.5	-0.143	2.4	-0.143	0.04
79	-5.9	-0.143	4.5	-0.142	2.4	-0.138	0.05
81	-5.97	0.093	4.5	-0.105	2.47	-0.115	0.04
82	-6.59	-0.541	4.5	-0.588	3.09	-0.292	0.09
83	-5.9	-0.276	4.5	-0.248	2.4	-0.246	0.07
84	-5.9	-0.169	4.5	0.177	2.4	-0.188	0.04
85	-5.9	-0.034	4.5	-0.031	2.4	-0.073	0.06
86	-5.9	-0.068	4.5	-0.092	2.4	-0.046	0.13
87	-5.9	-0.190	4.5	-0.232	2.4	-0.115	0.09
88	-5.9	-0.034	4.5	-0.058	2.4	-0.016	0.06
89	-5.9	-0.019	4.5	-0.013	2.4	-0.05	0.15
90	-5.9	-0.039	4.5	-0.055	2.4	-0.016	0.05
91	-5.9	-0.081	4.5	0.106	2.4	-0.05	0.06
92	-5.9	-0.144	4.5	-0.121	2.4	-0.201	0.12
93	-5.9	-0.043	4.5	-0.067	2.4	-0.027	0.13
94	-5.9	-0.035	4.5	-0.058	2.4	-0.017	0.05
95	-5.9	-0.131	4.5	-0.169	2.4	-0.087	0.04
96	-5.9	-0.099	4.5	-0.090	2.4	-0.159	0.03
97	-5.9	-0.084	4.5	-0.083	2.4	-0.054	0.01
98	-5.9	-0.131	4.5	-0.134	2.4	-0.143	0.09
99	-5.9	-0.156	4.5	-0.180	2.4	-0.104	0.07
100	-5.9	-0.113	4.5	-0.142	2.4	-0.069	0.08
101	-6.15	-0.350	4.5	-0.409	2.4	-0.214	0.04
102	-5.9	-0.180	4.5	-0.175	2.4	-0.179	0.05
103	-5.9	-0.022	4.5	-0.013	2.4	-0.054	0.05
104	-5.9	-0.180	4.5	-0.180	2.4	-0.180	0.06
105	-5.9	-0.185	4.5	-0.185	2.4	-0.196	0.1
106	-5.9	-0.07	4.5	-0.099	2.4	-0.046	0.05
108	-5.9	-0.034	4.5	-0.058	2.4	-0.016	0.07
109	-5.9	-0.132	4.5	-0.135	2.4	-0.143	0.07
110	-6.2	-0.35	4.5	-0.41	2.7	0.214	0.03
111	-5.9	-0.004	4.5	-0.04	2.4	-0.02	0.1
112	-5.9	-0.14	4.5	-0.142	2.4	-0.143	0.04
113	-5.9	-0.169	4.5	-0.177	2.4	-0.186	0.03
114	-5.9	-0.19	4.5	-0.23	2.4	-0.115	0.09
115	-5.97	-0.116	4.5	-0.13	2.47	-0.125	0.09
116	-5.9	-0.10	4.5	-0.09	2.4	-0.16	0.03
117	-5.9	-0.16	4.5	-0.18	2.4	-0.11	0.07

Table AII.9: Parameters used in the curve-fitting of three-exponential equation to the liver data for covered bodies; P_1 - P_6 = Parameters used for time in hours. RMS = residual Mean Square

CASE NUMBER	P_1	P_2	P_3	P_4	P_5	P_6	RMS
74	-6.0	-0.117	5.1	-0.082	1.9	-0.157	0.06
75	-6.0	-0.066	5.1	-0.049	1.9	-0.08	0.03
76	-6.0	-0.054	5.1	-0.044	1.9	-0.055	-
78	-6.0	-0.064	5.1	-0.051	1.9	-0.065	0.02
79	-6.0	-0.077	5.1	-0.062	1.9	-0.077	0.02
80	-6.0	-0.064	5.1	-0.058	1.9	0.057	0.04
81	-6.0	0.074	5.1	-0.064	1.9	-0.074	0.05
82	-6.0	-0.111	5.1	-0.093	1.9	-0.113	0.02
83	-6.0	-0.055	5.1	-0.056	1.9	-0.05	0.03
84	-6.0	-0.069	5.1	0.069	1.9	-0.054	0.04
85	-6.0	-0.073	5.1	-0.071	1.9	-0.066	0.05
86	-6.0	-0.062	5.1	-0.063	1.9	-0.046	0.03
87	-6.0	-0.11	5.1	-0.07	1.9	-0.173	0.09
88	-6.0	-	5.1	-	1.9	-	0.04
89	-6.0	-0.103	5.1	-0.072	1.9	-0.149	0.06
90	-6.0	-0.094	5.1	-0.059	1.9	-0.144	0.01
91	-6.0	-0.062	5.1	0.051	1.9	-0.062	0.1
92	-6.0	-0.117	5.1	-0.075	1.9	-0.179	0.05
94	-6.0	-0.082	5.1	-0.068	1.9	-0.086	0.04
95	-6.0	-0.064	5.1	-0.058	1.9	-0.057	0.04
96	-6.0	-0.083	5.1	-0.064	1.9	-0.157	0.01
97	-6.0	-0.093	5.1	-0.093	1.9	-0.051	0.01
98	-6.0	-0.108	5.1	-0.088	1.9	-0.108	0.05
99	-6.0	-0.12	5.1	-0.083	1.9	-0.177	0.03
100	-6.0	-0.064	5.1	-0.065	1.9	-0.041	-
101	-6.0	0.103	5.1	-0.102	1.9	-0.061	0.06
102	-6.0	-0.027	5.1	-0.035	1.9	-0.019	0.02
103	-6.0	-0.05	5.1	-0.042	1.9	-0.063	0.02
104	-6.0	-0.076	5.1	-0.076	1.9	-0.055	0.07
105	-6.0	-0.127	5.1	-0.089	1.9	-0.158	0.15
106	-6.0	-0.064	5.1	-0.05	1.9	-0.065	0.02
107	-6.0	-0.1	5.1	-0.09	1.9	-0.11	0.02
109	-6.0	-0.07	5.1	-0.071	1.9	-0.066	-0.05
110	-6.2	-0.07	5.1	-0.05	1.9	0.08	0.03
111	-6.0	-0.09	5.1	-0.06	1.9	-0.14	0.01
112	-6.0	-0.093	5.1	-0.093	1.9	-0.05	0.01
113	-6.0	-0.05	5.1	-0.042	1.9	-0.06	0.02

Table AII.10: Parameters used in the curve-fitting of three-exponential equation to the rectal data of individual cases for the covered bodies; P_1 - P_6 = Parameters used for time in hours. RMS = Residual Mean Square.

CASE NUMBER	P_1	P_2	P_3	P_4	P_5	P_6	RMS
74	-6.01	-0.194	5.1	-0.138	1.91	-0.308	0.09
75	-6.00	-0.105	5.1	-0.077	1.9	-0.149	0.03
76	-6.00	-0.061	5.1	-0.055	1.9	-0.06	0.11
77	-6.00	-0.074	5.1	-0.068	1.9	-0.075	0.04
78	-6.00	-0.084	5.1	-0.073	1.9	-0.083	0.06
79	-6.00	-0.097	5.1	-0.079	1.9	-0.124	-
80	-6.00	-0.095	5.1	-0.091	1.9	-0.086	0.3
81	-6.01	0.194	5.1	-0.138	1.91	-0.307	0.07
82	-6.00	-0.066	5.1	-0.965	1.9	-0.054	0.2
83	-6.00	-0.061	5.1	-0.055	1.9	-0.06	0.1
84	-6.00	-0.061	5.1	0.059	1.9	-0.052	0.1
85	-6.00	-0.102	5.1	-0.10	1.9	-0.076	0.09
86	-6.00	-0.190	5.1	-0.135	1.9	-0.277	0.08
87	-6.00	-0.159	5.1	-0.124	1.9	-0.199	0.3
88	-6.00	-0.169	5.1	-0.119	1.9	-0.246	0.04
89	-6.15	-0.198	5.1	-0.131	2.05	-0.305	0.1
90	-6.00	-0.132	5.1	-0.093	1.9	-0.200	0.05
91	06.03	-0.195	5.1	0.145	1.93	-0.328	0.09
92	-6.00	-0.138	5.1	-0.102	1.9	-0.184	0.04
93	-6.00	-0.185	5.1	-0.124	1.9	-0.269	0.05
94	-6.00	-0.121	5.1	-0.111	1.9	-0.166	0.2
96	-6.00	-0.09	5.1	-0.074	1.9	-0.114	0.02
97	-6.00	-0.190	5.1	-0.135	1.9	-0.277	0.08
98	-6.01	-0.194	5.1	-0.138	1.91	-0.308	0.09
99	-6.00	-0.074	5.1	-0.068	1.9	-0.075	0.04
100	-6.00	-0.095	5.1	-0.91	1.9	-0.86	0.03
101	-6.00	0.121	5.1	-0.11	1.9	-0.167	0.1
102	-6.00	-0.174	5.1	-0.126	1.9	-0.124	0.01
103	-6.00	-0.061	5.1	-0.06	1.9	-0.075	0.03
105	-6.00	-0.066	5.1	-0.065	1.9	-0.055	0.19
106	-6.00	-0.10	5.1	-0.075	1.9	-0.16	0.08
107	-6.01	-0.194	5.1	-0.138	1.91	-0.31	0.09
108	-6.00	-0.1	5.1	-0.08	1.9	-0.16	0.08
110	-6.00	-0.132	5.1	-0.093	1.9	0.2	0.04
111	-6.00	-0.169	5.1	-0.119	1.9	-0.246	0.04
112	-6.00	-0.061	5.1	-0.06	1.9	-0.075	0.03
113	-6.00	-0.12	5.1	-0.11	1.9	-0.17	0.2
115	-6.00	-0.101	5.1	-0.1	1.9	-0.076	0.09
116	-6.00	-0.061	5.1	-0.06	1.9	-0.053	0.1
117	-6.00	-0.105	5.1	-0.08	1.9	-0.149	0.03

Table AII.11: Parameters used in the curve-fitting of three-exponential equation to the brain data for naked bodies; P_1 - P_6 = Parameters used for time in hours. . RMS = Residual Mean Square. (Continued on next page)

CASE NUMBER	P_1	P_2	P_3	P_4	P_5	P_6	RMS
1	-5.97	-0.093	4.5	-0.105	2.47	-0.115	0.09
2	-5.97	-0.856	4.5	-0.980	2.47	-0.388	0.01
3	-6.08	-0.678	4.5	-0.779	2.58	-4.021	0.07
4	-5.97	0.05	4.5	-0.073	2.47	-0.027	0.04
5	-5.97	-0.559	4.5	-0.301	2.47	-0.914	0.1
6	-5.97	-0.686	4.5	-0.804	2.47	-0.273	0.03
7	-6.00	-0.546	4.5	-0.606	2.5	-0.286	0.04
8	-6.09	-0.446	4.57	-0.325	2.52	-0.611	0.03
9	-5.97	-0.09	4.5	-0.118	2.47	-0.069	0.06
10	-5.97	-0.074	4.5	-0.101	2.47	-0.048	0.08
11	-5.97	-0.090	4.5	-0.089	2.47	-0.111	0.04
12	-6.03	-0.702	4.56	-0.49	2.47	-0.898	0.3
13	-5.97	-0.039	4.5	-0.087	2.47	-0.011	0.07
14	-5.97	-0.116	4.5	-0.13	2.47	-0.126	0.1
16	-5.97	-0.059	4.5	-0.083	2.47	-0.036	0.1
17	-5.97	-0.072	4.5	-0.076	2.47	-0.078	0.2
18	-4.73	-1.60	4.49	-1.68	1.25	-0.212	0.06
19	-5.97	-0.090	4.5	-0.110	2.47	-0.100	0.04
20	-6.00	-0.589	4.5	-0.639	2.5	0.305	0.09
21	-5.97	-0.068	4.5	-0.077	2.47	-0.083	0.01
22	-5.97	-0.083	4.5	-0.087	2.47	-0.110	0.015
24	-6.49	-0.942	4.5	-1.059	2.99	-0.538	0.06
25	-5.97	-0.072	4.5	-0.097	2.49	-0.039	0.025
26	5.97	-0.263	4.5	-0.22	2.47	-0.342	0.03
28	-5.97	-0.649	4.5	-0.417	2.47	-0.887	0.15
29	-5.97	-0.040	4.5	-0.053	2.47	-0.054	0.3
30	-5.97	-0.129	4.5	-0.106	2.47	-0.209	0.08
31	-5.97	-0.097	4.5	-0.128	2.47	-0.068	0.05
32	-5.97	-0.075	4.5	-0.093	2.47	-0.061	0.09
33	-5.97	-0.347	4.5	0.351	2.47	-0.321	0.08
34	-5.49	-1.276	4.5	-1.546	1.99	-0.354	0.11
35	-5.95	-0.824	4.5	-0.979	2.45	-0.387	0.2
36	-5.97	-0.090	4.5	-0.114	2.47	-0.063	0.11
37	-5.97	-0.365	4.5	-0.296	2.47-	-0.421	-
38	-5.97	-0.206	4.5	-0.161	2.47	-0.245	0.09
39	-5.97	-0.384	4.5	-0.416	2.47	-0.235	0.05
40	-5.97	-0.217	4.5	-0.231	2.47	-0.173	0.04
41	-6.12	-0.654	4.5	-0.769	2.62	-0.339	0.4
42	-5.97	-0.425	4.5	-0.454	2.47	-0.260	0.04
43	-5.97	-0.162	4.5	-0.179	2.47	-0.111	0.06
45	-6.01	-0.620	4.5	-0.695	2.51	-0.328	0.06
46	-5.93	-0.775	4.50	-0.909	2.43	-0.303	0.12
47	-6.00	-0.612	4.5	-0.636	2.5	-0.393	0.04
48	-5.97	-0.080	4.5	-0.104	2.47	-0.058	0.04
49	-5.97	-1.298	4.5	-1.495	2.47	-0.452	0.04
50	-5.97	-0.201	4.5	-0.222	-2.47	-0.185	0.12

Table AII.11: (Continued from previous page) Parameters used in the curve-fitting of three-exponential equation to the brain data for naked bodies; P_1 - P_6 = Parameters used for time in hours. . RMS = Residual Mean Square.

CASE NUMBER	P_1	P_2	P_3	P_4	P_5	P_6	RMS
51	-5.97	-0.092	4.5	-0.099	-2.47	-0.112	0.04
52	-5.97	-0.021	4.5	-0.013	2.47	-0.055	0.05
53	-5.97	-0.358	4.5	0.357	2.47	-0.257	0.1
54	-5.97	-1.621	3.6	-2.429	3.37	-0.595	3.3
55	-5.97	-0.147	4.5	-0.161	-2.47	-0.138	0.03
56	-5.97	-0.686	4.5	-0.422	2.47	-1.037	0.12
57	-5.97	-0.294	4.5	-0.293	-2.47	-0.237	0.05
58	-5.97	-0.332	4.5	-0.355	2.47	-0.224	0.04
59	-5.97	-0.651	4.5	-0.744	2.47	-0.288	0.11
61	-5.81	-0.614	4.5	-0.717	2.31	-0.278	0.04
62	-5.97	-0.015	4.5	-0.007	-2.47	-0.080	0.03
63	-5.97	-0.081	4.5	-0.104	2.47	-0.054	0.03
64	-5.97	-0.178	4.5	-0.216	2.47	-0.118	0.06
65	-5.97	-0.497	4.5	-0.341	2.47	-0.645	0.09
66	-5.97	-0.093	4.5	-0.108	2.47	-0.111	0.06
67	-5.97	-0.136	4.5	-0.161	2.47	-0.103	0.2
68	-6.01	-0.62	4.5	-0.695	2.51	-0.330	0.07
69	-5.97	-1.3	4.5	-1.50	2.47	-0.45	0.04
70	-5.97	-0.075	4.5	-0.093	2.47	-0.06	0.08
71	-5.97	-0.092	4.5	-0.105	2.47	-0.116	0.09
72	-6.00	-0.55	4.5	-0.61	2.5	-0.29	0.04
73	-5.97	-0.12	4.5	-0.13	2.47	-0.13	0.09

Table AII.12: Parameters used in the curve-fitting of three-exponential equation to the liver data for naked bodies; P_1 - P_6 = Parameters used for time in hours. RMS = Residual Mean Square. (Continue on next page)

CASE NUMBER	P_1	P_2	P_3	P_4	P_5	P_6	RMS
1	-6.00	-0.396	5.1	-0.183	1.9	-0.271	0.1
2	-6.00	-0.185	5.1	-0.149	1.9	-0.256	0.008
3	-6.00	-0.131	5.1	-0.152	1.9	-0.078	0.09
4	-6.00	0.056	5.1	-0.070	1.9	-0.026	0.05
6	-6.00	-0.10	5.1	-0.110	1.9	-0.067	0.14
7	-6.00	-0.215	5.1	-0.176	1.9	-0.251	0.11
8	-6.00	-0.248	5.1	-0.179	1.9	-0.417	0.038
9	-6.00	-0.093	5.1	-0.106	1.9	-0.061	0.053
10	-6.00	-0.068	5.1	-0.075	1.9	-0.05	0.09
11	-6.00	-0.127	5.1	-0.104	1.9	-0.181	0.073
12	-6.00	-0.296	5.1	-0.225	1.9	-0.40	0.51
13	-6.00	-0.208	5.1	-0.164	1.9	-0.301	0.06
14	-6.00	-0.08	5.1	-0.079	1.9	-0.097	0.10
15	-6.00	-0.013	5.1	-0.027	1.9	-0.002	0.23
16	-6.00	-0.069	5.1	-0.084	1.9	-0.041	0.04
17	-6.00	-0.10	5.1	-0.093	1.9	-0.101	0.065
18	-6.00	-0.244	5.1	-0.174	1.9	-0.324	0.6
19	-6.00	-0.198	5.1	0.159	1.9	-0.246	0.1
20	-6.00	-0.10	5.1	-0.113	1.9	-0.068	0.03
21	-6.00	-0.225	5.1	-0.177	1.9	-0.295	0.03
22	-6.01	-0.312	5.1	-0.209	1.91	-0.458	0.24
23	-6.00	-0.158	5.1	-0.136	1.9	-0.174	0.22
24	-6.00	-0.233	5.1	-0.20	1.9	-0.283	0.035
25	-6.00	-0.063	5.1	-0.074	1.9	-0.030	0.035
26	6.00	-0.157	5.1	-0.126	1.9	-0.216	0.017
27	-6.00	-0.199	5.1	-0.198	1.9	-0.076	0.66
28	-6.00	-1.251	5.1	-1.427	1.9	-0.252	0.12
29	-6.00	-0.203	5.1	-0.157	1.9	-0.289	0.11
30	-6.00	-0.148	5.1	-0.165	1.9	-0.089	0.02
31	-6.00	-0.082	5.1	-0.096	1.9	-0.044	0.06
32	-6.00	-0.108	5.1	-0.087	1.9	-0.126	0.052
33	-6.00	-0.111	5.1	-0.093	1.9	-0.171	0.02
34	-6.00	-0.367	5.1	-0.253	1.9	-0.565	0.2
35	-6.00	-0.323	5.1	-0.229	1.9	-0.496	0.12
36	-6.00	-0.033	5.1	-0.036	1.9	-0.025	0.17
37	-6.00	-0.193	5.1	-0.150	1.9	-0.278	0.02
38	-6.00	-0.142	5.1	-0.11	1.9	-0.196	0.05
39	-6.00	-0.134	5.1	-0.144	1.9	-0.085	0.05
40	-6.00	-0.082	5.1	-0.081	1.9	-0.062	0.09
41	-6.00	-0.141	5.1	-0.12	1.9	-0.207	0.04
42	-6.00	-0.167	5.1	-0.153	1.9	-0.162	0.08
43	-6.00	-0.018	5.1	-0.014	1.9	-0.048	0.088
44	-6.00	-0.142	5.1	-0.120	1.9	-0.178	0.12
45	-6.00	-0.166	5.1	-0.131	1.9	-0.234	0.08
46	-6.00	-0.260	5.1	-0.196	1.9	-0.339	0.2
47	-6.00	-0.150	5.1	-0.122	1.9	-0.229	0.05

Table AII.12: (Continued from previous page) Parameters used in the curve-fitting of three- exponential equation to the liver data for naked bodies; P_1 - P_6 = Parameters used for time in hours. RMS = Residual Mean Square.

CASE NUMBER	P_1	P_2	P_3	P_4	P_5	P_6	RMS
48	-6.00	-0.063	5.1	-0.078	1.9	-0.041	0.04
49	-6.00	-0.960	5.1	-0.559	1.9	-1.693	0.1
50	-6.00	-0.147	5.1	-0.162	1.9	-0.086	0.01
51	-6.00	-0.207	5.1	-0.158	1.9	-0.292	0.03
52	-6.00	-0.09	5.1	-0.099	1.9	-0.045	0.08
53	-6.00	-0.105	5.1	-0.107	1.9	-0.076	0.47
54	-6.00	-0.115	5.1	-0.106	1.9	-0.092	0.8
55	-6.00	-0.130	5.1	-0.143	1.9	-0.084	0.016
56	-6.00	-0.238	5.1	-0.188	1.9	-0.333	0.1
57	-6.00	-0.006	5.1	-0.003	1.9	-0.047	0.02
58	-6.00	-0.183	5.1	-0.143	1.9	-0.251	0.08
59	-6.00	-0.208	5.1	0.170	1.9	-0.318	0.06
60	-6.00	-0.189	5.1	-0.176	1.9	-0.146	0.4
61	-6.00	-0.182	5.1	-0.150	1.9	-0.228	0.013
62	-6.00	-0.149	5.1	-0.155	1.9	-0.108	0.03
63	-6.00	-0.121	5.1	-0.121	1.9	-0.072	0.2
64	-6.00	-0.087	5.1	-0.068	1.9	-0.132	0.01
65	-6.00	-0.174	5.1	-0.147	1.9	-0.208	0.07
66	-6.00	-0.045	5.1	-0.058	1.9	-0.030	0.1
67	-6.00	-0.112	5.1	-0.126	1.9	-0.066	0.09
68	-6.00	-0.087	5.1	-0.068	1.9	-0.132	0.01
69	-6.00	-0.07	5.1	0.084	1.9	-0.041	0.04
70	-6.00	-0.148	5.1	-0.165	1.9	-0.089	0.02
71	-6.00	0.248	5.1	0.179	1.9	-0.417	0.038
72	-6.00	-0.056	5.1	-0.07	1.9	-0.026	0.05
73	-6.00	-0.16	5.1	0.13	1.9	-0.22	0.017

Table AII.13: Parameters used in the curve-fitting of three-exponential equation to the rectal data for the naked bodies; P_1 - P_6 = Parameters used for time in hours. RMS = Residual Mean Square. (Continued on next page)

CASE NUMBER	P_1	P_2	P_3	P_4	P_5	P_6	RMS
1	-6.00	-0.159	5.1	-0.116	1.9	-0.216	0.03
2	-6.00	-0.212	5.1	-0.152	1.9	-0.308	0.009
3	-6.00	-0.222	5.1	-0.226	1.9	-0.127	0.02
4	-6.00	0.221	5.1	-0.171	1.9	-0.290	0.0044
5	-6.00	-0.123	5.1	-0.111	1.9	-0.124	0.02
6	-6.00	-0.174	5.1	-0.126	1.9	-0.224	0.005
7	-6.00	-0.216	5.1	-0.150	1.9	-0.324	0.06
8	-6.00	-0.282	5.1	-0.179	1.91	-0.477	0.007
9	-6.00	-0.240	5.1	-0.198	1.9	-0.319	0.08
10	-6.00	-0.285	5.1	-0.221	1.91	-0.426	0.08
11	-6.00	-0.136	5.1	-0.099	1.9	-0.198	0.04
12	-6.00	-0.260	5.1	-0.170	1.9	-0.412	0.06
13	-6.00	-0.130	5.1	-0.096	1.9	-0.186	0.05
14	-6.00	-0.124	5.1	-0.141	1.9	-0.094	0.07
16	-6.00	-0.289	5.1	-0.217	1.9	-0.378	0.04
17	-6.00	-0.132	5.1	-0.107	1.9	-0.133	0.03
18	-6.00	-0.207	5.1	-0.133	1.9	-0.321	0.05
19	-6.00	-0.154	5.1	0.114	1.9	-0.212	0.03
21	-6.00	-0.256	5.1	-0.181	1.9	-0.373	0.04
22	-6.01	-0.228	5.1	-0.146	1.91	-0.378	0.06
23	-6.00	-0.146	5.1	-0.156	1.9	-0.077	0.06
24	-6.00	-0.329	5.1	-0.329	1.9	-0.150	0.04
25	-6.00	-0.316	5.1	-0.220	1.9	-0.453	0.05
26	6.00	-0.188	5.1	-0.145	1.9	-0.256	0.01
27	-6.00	-0.02	5.1	-0.017	1.9	-0.053	0.009
28	-6.00	-0.244	5.1	-0.162	1.9	-0.353	0.02
29	-6.00	-0.228	5.1	-0.150	1.9	-0.358	0.08
30	-6.00	-0.157	5.1	-0.159	1.9	-0.109	0.03
31	-6.00	-0.201	5.1	-0.137	1.9	-0.312	0.03
32	-6.00	-0.124	5.1	-0.113	1.9	-0.109	0.03
34	-6.01	-0.331	5.1	-0.247	1.9	-0.492	0.1
35	-6.00	-0.244	5.1	-0.184	1.9	-0.344	0.06
36	-6.00	-0.170	5.1	-0.114	1.9	-0.263	0.07
38	-6.00	-0.212	5.1	-0.139	1.9	-0.318	0.03
39	-6.00	-0.146	5.1	-0.105	1.9	-0.215	0.02
40	-6.00	-0.139	5.1	-0.153	1.9	-0.063	0.03
41	-6.00	-0.193	5.1	-0.155	1.9	-0.266	0.07
42	-6.00	-0.169	5.1	-0.169	1.9	-0.104	0.03
43	-6.00	-0.220	5.1	-0.154	1.9	-0.322	0.1
44	-6.00	-0.204	5.1	-0.141	1.9	-0.305	0.05
46	-6.01	-0.273	5.1	-0.174	1.91	-0.450	0.1
47	-6.00	-0.141	5.1	-0.107	1.9	-0.186	0.05
48	-6.01	-0.336	5.1	-0.239	1.91	-0.478	0.03
49	-6.00	-0.612	5.1	-0.498	1.9	-0.640	0.2
52	-6.00	-0.121	5.1	-0.122	1.9	-0.071	0.03
54	-6.00	-0.154	5.1	-0.115	1.9	-0.238	0.23

Table AII.13: (Continued from previous page) Parameters used in the curve-fitting of three- exponential equation to the rectal data for the naked bodies; $P_1 - P_6$ = Parameters used for time in hours. RMS = Residual Mean Square.

CASE NUMBER	P_1	P_2	P_3	P_4	P_5	P_6	RMS
56	-6.01	-0.215	5.1	-0.141	1.91	-0.340	0.09
58	-6.00	-0.198	5.1	-0.152	1.9	-0.274	0.06
59	-6.00	-0.216	5.1	-0.149	1.9	-0.325	0.06
60	-6.00	-0.143	5.1	-0.143	1.9	-0.110	0.02
61	-6.01	-0.278	5.1	-0.188	1.91	-0.431	0.02
63	-6.00	-0.218	5.1	-0.171	1.9	-0.278	0.02
64	-6.00	-0.147	5.1	-0.099	1.9	-0.231	0.04
65	-6.00	-0.119	5.1	-0.105	1.9	-0.107	0.03
66	-6.00	-0.119	5.1	-0.102	1.9	-0.120	0.04
67	-6.00	-0.129	5.1	-0.121	1.9	-0.119	0.04
68	-6.00	-0.212	5.1	-0.152	1.9	-0.308	0.009
69	-6.00	-0.159	5.1	-0.116	1.9	-0.216	0.04
70	-6.00	-0.123	5.1	-0.110	1.9	-0.124	0.02
71	-6.00	-0.132	5.1	-0.107	1.9	-0.130	0.03
72	-6.00	-0.02	5.1	-0.02	1.9	-0.35	0.03
73	-6.00	-0.207	5.1	-0.133	1.9	-0.321	0.05

Table AII.14: Parameters used in the curve-fitting of four-exponential equation to brain(B), liver(L) and rectal(R) data of selected cases; P_1 - P_8 = Parameters for time in hours. RMS = Residual Mean Square.

CASE NUMBER	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	RMS
(1)B	-0.5	-0.62	1.5	-0.31	0.5	0.52	-0.5	0.52	0.07
(1)L	-0.32	-0.09	1.3	-0.12	-0.016	-51.0	0.0001	0.83	0.09
(1)R	-0.6	0.09	1.2	-0.03	0.4	0.11	-0.002	0.32	0.03
(43)L	0.66	-0.31	-0.39	-0.61	0.81	-0.02	-0.08	0.05	0.04
(22)B	-1.2	0.02	1.9	-0.06	0.53	-0.57	-0.3	-0.82	0.2
(64)B	0.57	-0.02	-0.04	-4.43	0.65	-0.33	-0.18	0.02	0.06
(22)L	-6.6	-0.34	5.0	-0.21	3.6	-0.55	-1.0	-0.79	0.24
(22)R	-5.7	-0.28	4.0	-0.14	3.7	-0.5	-1.0	-0.83	0.05
(64)R	-1.9	-0.27	2.2	-0.13	1.0	-0.6	-0.3	-1.4	0.1
(64)L	0.8	-0.42	-0.4	-0.8	0.9	-0.03	-0.2	0.02	0.04
(23)L	-6.6	-0.34	5.0	-0.21	3.6	-0.55	-1.0	-0.79	0.2
(65)B	-2.2	-0.17	2.5	-0.08	1.0	-0.38	-0.3	-0.53	0.04
(65)L	-5.7	-0.28	4.0	-0.14	-3.7	-0.50	-1.0	-0.83	0.05
(65)R	-1.9	-0.27	2.2	-0.23	1.0	-0.6	-0.3	-0.14	0.1