A QUANTIFICATION OF HEAT LOAD AS ASSESSED BY INDICATORS OF TISSUE DAMAGE IN RATS

by

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S U M M A R Y

Heatstroke is an illness that occurs when body temperature is grossly elevated, causing widespread tissue damage. The extent of tissue damage depends on the level of body temperature elevation and the duration. Despite the fact that the diagnosis of heatstroke is based on sound scientific principles, namely the elevation of serum enzyme levels as indicators of tissue damage, the sensitivity of these parameters of tissue damage in the prodromal period of heatstroke is less well established, especially for sub-lethal stress conditions. Furthermore, it is not known to what extent given elevations in serum enzyme levels reflect the nature of various combinations of hyperthermia and its duration as sustained during the prodromal period.

In an attempt to throw some light on the questions posed above anaesthetized rats were exposed to three different sets of thermal conditions. However, the amount of heat gained over and above baseline levels was controlled to a 20% rise irrespective of the experimental conditions. Above this increment animals did not survive thus indicating excessive stress. Plasma enzyme levels were assayed in each group of animals upon termination of stress, six hours post-stress and 24 hours post-stress in order to investigate the patterns of enzyme release as well as the sensitivity of the respective indicators of tissue damage.

On the basis of plasma enzyme assays, the tissue damage sustained during these particular experimental conditions was mild to moderate, completely reversible, not indicative of heatstroke but merely of

generalized tissue damage. The results suggest that in addition to the established positive relationship between the level and duration of hyperthermia and tissue damage, a third component,namely the rate of rise in body temperature, may constitute an important factor in the ultimate pathology. In this regard, i.e. sub-lethal stress, creatine kinase proved to be the most sensitive and, therefore, the most useful parameter of tissue damage.

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INTRODUCTION

Heat is a stress to which most people are exposed at one time or another. Its effects vary from slight discomfort to widespread tissue damage, sometimes severe enough to cause death. Heatstroke, the severest form of heat stress, is an affliction which is apparently sudden in onset, and since treatment takes priority over all else, its pathogenesis is often obscured.

A decrease in the incidence of heatstroke over the past few decades is a reflection, perhaps, of man's awareness of the potential hazards rather than an understanding of the problem. In the South African gold mining industry, research and effective acclimatization procedures have ensured that this scourge is kept at bay. No doubt, similar acclimatization programmes have been instituted in other industries where heat is a hazard. Despite this, one has to agree with Shapiro *et al* (1973) that the true incidence is probably higher than reported, mainly because many cases do not reach medical attention, while other patients presenting with high temperatures are often mistaken to have infectious diseases.

The true incidence is obscured further as a result of a lack of standardized diagnostic criteria. The cessation of sweating, disturbances in the central nervous system and arbitrary temperatures were proposed as diagnostic aids by Minard and Copman (1963) and Leithead and Lind (1964). However, heatstroke has also been diagnosed in the presence of normal sweating (Shibolet *et al*, 1967)

and consequently, the criterion which is of prime importance currently is tissue damage (Kew, 1976; Bynum *et al*, 1977; Hubbard *et al*, 1979). The origin of this can actually be traced back to Malamud *et al* (1946) who showed that heatstroke was associated with widespread tissue damage. This implies that the parameters should be those indicating tissue damage, namely, enzymes.

It is therefore not surprising that the clinical diagnosis of heatstroke in the South African gold mining industry rests squarely on serum enzyme assays and that the diagnosis thus derived has a medico-legal standing. However, while the diagnosis of overt cases of heatstroke is uncomplicated, the sensitivity of serum enzyme assays is less well established as a criterion of heat damage in the early or prodromal stage of heatstroke. Also the extent to which combinations of hyperthermia and exposure influence the outcome are mostly unknown.

OBJECTIVES

The objectives of this study are to investigate the early stage of heat stress which may lead to more serious consequences with specific reference to:

a) the assessment of the severity of heat stress in terms of combinations of different levels of hyperthermia and the duration of exposure (i.e. dose response);
b) the relationship between the reversibility of tissue damage and the magnitude and nature of the total heat load;
c) an evaluation of the sensitivity of the indicators of tissue damage under these experimental conditions.

CHAPTER 1

REVIEW OF THE LITERATURE

Heatstroke, the severest form of heat stress, is an enigma, largely because of its sudden onset and obscure pathogenesis. Varying diagnostic criteria have undoubtedly added to the confusion. Although this study places emphasis on the early stages of the affliction, it nevertheless remains important to consider the pathophysiology of heatstroke as widely as possible.

1.1 Historical Perspectives

Sun stroke or heatstroke, one of the oldest known afflictions, was associated by the ancients with Sirius, the dog star, because this star followed the sun and could be seen in the twilight (Wakefield and Hall, 1927). The oldest record of the affliction is in the Bible. In the fourth book of Kings (Chapter IV, verses 18 to 20) is an account of father and son going into the field to reap the harvest. The son complained of a headache, whereupon his mother set him on her lap, where he remained till his death at noon. In another account (book of Judith, Chapter VIII, verses 2 to 3) we are told of the death of Manasses (husband of Judith) when the "heat" came upon his head.

In 1743, due to the effects of heat during a heat-wave, 11 000 people are reported to have died in the streets of Peking (Levick, 1859).

The advent of steamships highlighted numerous heat injuries in the engine rooms. Towards the end of 1926 several people suffering from the injurious effects of heat were admitted to the Naval Hospital in Washington (Wakefield and Hall, 1927). The only fatality complained of mental confusion and delusions when discharged.

Of the oldest records of the affliction at sea is that of the British frigate, Liverpool. In 1841, while on a voyage from Muscat to Bushire, 30 men and 3 lieutenants died in one day, despite all precautions (Wakefield and Hall, 1927, in their citation of Wellstead in the latters "Travels to the City of Caliphs").

The first medical report on the effects of heat on men in the fire rooms of naval vessels of the U.S. Navy was made by Gihon (1873). In 1895, the same Gihon cited instances of heatstroke as a result of punishment in "sweatboxes". On release from these coffin-like boxes in which they had to stand upright, the men, in his words, "fell forward unconscious with burning skin and bounding pulse ....." . They were placed on the sicklist suffering from "adynamia" (a lack or loss of the normal or vital powers).

Andral (1838) was the first to correlate high atmospheric temperatures and heatstroke - in fact, he provided the first accurate account of post-mortem findings, namely, petechiae, "liquid blood" and venous engorgement. Apart from solar radiation, Condie (1858) emphasized the importance of humidity as an aetiological factor in the development of heatstroke. His patient, a Negro preacher, was overcome by

heat in a crowded church. His symptoms included convulsions alternating with tremors, dilated pupils, a thready pulse, and a skin covered by a cold, clammy sweat.

Modern warfare, especially under adverse climatic conditions such as encountered in tropical and semi-tropical regions, has focussed attention on heat injuries. British and American armies have encountered the problem, in training as well as in combat situations (Wakefield and Hall 1927). In the Iraq campaign the casualty rate among British troops was higher from heat (8%) than from all other causes (4,1% - Marsh, 1930).

Heat exhaustion is an occupational hazard in Grand Prix Racing. In the 1982 Brazilian Grand Prix, Nelson Picquet suffered the ill-effects of heat, and, in fact, collapsed on the winners rostrum. Under Grand Prix conditions, heat accumulation is due to the heat on the track, the heat generated by the engines, and most of all, the inability to dissipate heat because of the protective clothing worn by drivers.

In South Africa the incidence of heatstroke is confined to the Defence Force, the gold mining industry and sporting activities. In an article in the Sunday Tribune* (September 9, 1979) heatstroke in the Defence Force is highlighted and it is proposed that efforts will

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* *Sunday Tribune (September 9, 1979): 60 minutes that can maim or kill a soldier. Also, It's war - on heatstroke. Both articles on page 13.*

be made to eradicate at least 90% of the cases. The death of trainees, due to heatstroke, led to an investigation prompted by bereaved parents.

An article in the Sunday Express ** (November 22, 1981) highlights the possible causes, symptoms and treatment of heatstroke. The article also provides valuable information to those people who take part in sporting activities on hot, sunny days outlining several precautions. Reference is also made to the death (due to heatstroke) of a Johannesburg schoolgirl while on a nature walk.

In the South African gold mining industry heatstroke fatalities were first recorded in 1924 (Kielblock *et al*, 1982). Crude attempts at acclimatization were attempted and with the passage of time these techniques were refined. The acclimatization programme has been so successful that the incidence of heatstroke has actually decreased over the 12 year period 1969 to 1980 (both years inclusive) despite an increase in the underground labour force (Kielblock *et al*, 1982). Despite the decreased morbidity rate, the mortality rate has not decreased, this anomalous situation being attributed to poor recognition and treatment of heatstroke, advanced age of the victims and excessively high wet-bulb temperatures.

** *Sunday Express (November 22, 1981): How Heat can kill you.*

1.2 Heat Illnesses With Specific Reference To Heatstroke

The pathologic manifestations of heat accumulation in the body are generally grouped under the generic description of heat illnesses (Wilcox, 1920; Ladell, 1957; Barry and King, 1962. a).

Heatstroke is the most serious of the heat illnesses and has a mortality ranging from 10% to 80% (Wilcox, 1920, Kumar *et al*, 1964; Kew, 1976). It is apparently sudden in onset and in its advanced stage is characterized by a very high body temperature (usually in excess of 41,1^{o}C) and loss of consciousness (Malamud *et al*, 1946; Barry and King, 1962. a).

Heatstroke is common in tropical and desert regions, occurring also in hot occupational environments as mines, bakeries and boiler rooms of ships (Barry and King, 1962. b). In civilian populations the incidence of heatstroke increases in the hot, summer months as borne out by its increased incidence in May and June (being the hottest months with high humidity and low wind velocity) in India (Kumar *et al*, 1964). The affected individuals are usually over 50 years of age (Ferris *et al*, 1938) and in many instances even over 60 years of age (Austin and Berry 1956). Heatstroke is also common in the armed forces (Borden *et al*, 1945; Malamud *et al*, 1946; Sunday Tribune, 1979), being confined in this instance to basic trainees, most of whom are under 30 years of age (Fabricant, 1958).

Aetiological factors predisposing heatstroke are numerous and include environmental conditions, lack of heat acclimatization, physical exertion, age, dehydration and congenital susceptibility. Sustained physical activity in hot, humid conditions is a major contributing factor in the aetiology of heatstroke (Shibolet *et al*, 1967; Kew, 1976; Kielblock *et al*, 1982) amply illustrated in deep level mining in South African gold mines where the occurrence of heatstroke is directly related to exertion under these conditions. Heatstroke fatalities are likely to occur only at wet-bulb temperatures in excess of 28°C. Both morbidity and mortality rates increase dramatically at wet-bulb temperatures in excess of 33°C (Kielblock *et al*, 1982). These extreme conditions prevent the dissipation of heat by radiation, convection and evaporation of sweat (Kew, 1976). Lack of acclimatization has long been regarded as one of the chief factors leading to heatstroke (Minard and Copman, 1963). Of the 36 heatstroke cases of Shibolet *et al* (1967) only 14 had participated in a program of physical conditioning from one to several days. With the introduction of acclimatization programmes in the South African gold mines there has been a progressive decrease in the number of cases of fatal and non-fatal heatstroke (Kew, 1976).

Heatstroke in civilian populations is confined to the elderly (vide supra) whereas in military and industrial populations fit young men are afflicted. The vast majority of the congenitally susceptible cases (who could not be acclimatized) in the South African gold mines were over 40 years of age (Kew, 1976).

Despite the voluminous literature on heatstroke its pathogenesis is not defined, due mostly to the fact that the condition cannot be induced in man because of the high risk due to a high mortality (Kew, 1976; Hubbard *et al*, 1976). The most common contention is that heatstroke is due primarily to the noxious effect of excessive body temperature on tissues (Heilbrunn, 1954; Burger and Fuhrman, 1964 (a) and (b); Shapiro *et al*, 1973; Kew, 1976; Hubbard *et al*, 1976; Hubbard *et al*, 1977) accompanied by normal sweating (Shibolet *et al*, 1967) or a cessation of sweating before the onset of the condition (Barry and King, 1962. b). Treatment takes priority over all else and is usually aimed at reducing body temperature. Water, which is splashed on the subject and spread over his skin, is evaporated by blowing compressed air over it (Kielblock, 1982: personal communication). Cooling is continued until the patients rectal temperature has dropped to 38,4^{o}C or below. Medical attention is summoned and medical treatment instituted on site or in hospital.

In view of the fact that heatstroke strikes highly motivated young people under the discipline of work, military training, and sporting endeavour preventative measures include adequate rest periods and hydration (Shibolet *et al*, 1967). In tropical regions physical exertion is to be avoided during the hottest periods.

1.3 Heatstroke: Its definition and diagnosis

Heatstroke has often been defined as a heat disorder characterized by

a high body temperature (Malamud *et al*, 1946; Minard and Copman, 1963; Knochel, 1974) usually in excess of 41,1°C. It is characterized by disturbances to the central nervous system (Malamud *et al*, 1945; Leithead and Lind, 1964; Knochel, 1974) and an absence of sweating (Malamud *et al*, 1946; Minard and Copman, 1963; Leithead and Lind, 1964). A strict adherence to this definition can lead to underdiagnosis and unanticipated deaths (Hubbard *et al*, 1978). One of the many objections to this definition is the setting of an arbitrary temperature of 41,1°C in view of the fact that some of the victims of heatstroke die with rectal temperatures of 40,6°C (Leithead and Lind, 1964). A major objection is to the proposal that cessation of sweating is a cardinal sign in the diagnosis of heatstroke (Minard and Copman, 1963; Leithead and Lind, 1964). In fact, heatstroke has been diagnosed accompanied by normal sweating (Shibolet *et al*, 1967).

In view of these conflicting observations a more general concept of heatstroke has been developed, in which body temperature, itself, is a noxious agent (Hubbard, 1979). Heatstroke is now defined as a condition in which elevated body temperature causes tissue damage, the elevation in body temperature being due to an overloading or failure of the thermoregulatory mechanisms in the presence of high ambient temperatures (Kew, 1976). Tissue damage is widespread and characteristic. The organ which is most severly affected (apart from the brain) is the kidney (Kew, 1976) . Other tissues which may be damaged are the liver, skeletal muscle, the pancreas and the gastrointestinal tract (Kew *et al*, 1971; Kew, 1976). The successful management of the heat-stroke victim and his eventual recovery will depend on the extent and

severity of tissue damage. His outcome will depend to a very large extent on a rapid and accurate diagnosis of his condition. Consequently, a tried and tested laboratory diagnostic aid, which is both rapid and convenient would enhance considerably the heatstroke victim's chance of survival, particularly if the test were a reliable indicator of tissue damage.

A variety of tissue damage criteria have been employed, and in the case of heatstroke fatalities, tissue damage has been confirmed at post-mortem examination.

Initially, most investigators (Wilcox, 1920; Wilson, 1940; Borden *et al*, 1945; Malamud *et al*, 1946; Haseeb *et al*, 1958) relied almost entirely on post-mortem examinations to determine the extent and severity of tissue damage. Gradually other criteria were introduced to detect tissue damage. Laboratory investigations included haematological examinations as well as biochemical tests on blood and urine.

Increased haematocrit values were found by Ferris *et al* (1938) and Barry and King (1962. b) and were considered indicative of haemoconcentration. A decrease in the haemoglobin concentration was noted by Fabricant (1958) and ascribed to a haemodilution. Neutrophilic leucocytosis was found to occur consistently (Malamud *et al*, 1945;

Leithead and Lind, 1964; Barry and King 1962. b; Fabricant, 1958). Thrombocytopaenia, which is indicative of an impaired clotting mechanism, was found by Malamud *et al* (1946), Fabricant (1958), Leithead and Lind (1964) and Shibolet *et al* (1967). A decrease in serum prothrombin and fibrinogen levels were regarded as being responsible for the widespread haemorrhages seen at post-mortem examinations (Shibolet *et al*, 1962; Shibolet *et al*, 1967).

The most common biochemical change in blood was an increase in blood urea (Leithead and Lind, 1964; Kew *et al*, 1969; Shibolet *et al*, 1967; Kew *et al*, 1970) signifying kidney damage. A simultaneous increase in urinary protein strengthened the suspicion (Kew *et al*, 1967; Shibolet *et al*, 1967; Kew *et al*, 1970).

Electrocardiographic changes (Malamud, *et al*, 1946; Fabricant, 1958; Baxter *et al*, 1958) accompanied by changes in serum enzyme levels (Kew *et al*, 1969; Shapiro *et al*, 1973; Kew, 1976; Bynum, 1977) were also used as indicators of tissue damage.

In all the investigations outlined so far findings indicative of tissue damage were confirmed by findings at post-mortem examinations.

Other investigators have depended entirely on some of the criteria mentioned above but without confirmation at post-mortem because all patients under observation survived, or because no deaths occurred in their experimental (animal) model or because post-mortem examinations were not performed. Thus, electrocardiographic changes were employed as indicators of tissue damage by Goldberg *et al* (1952), Barry and

King (1962. a), Williams *et al* (1962), Winsor (1968) and Knochel (1974), supported in each case by some biochemical change. The electrocardiographic changes were a widening of the Q R S complex and a variety of other non-specific changes (Goldberg *et al*, 1952; Fabricant, 1958; Kew *et al*, 1969). Hyperkalaemia was a consistent finding in some cases (Goldberg *et al*, 1952; Shibolet *et al*, 1962) whereas in others hypokalaemia was common (Shibolet *et al*, 1967; Kew *et al*, 1967; Sohar *et al*, 1968). These conflicting findings cast doubt on the contention of some researchers (Goldberg *et al*, 1952) that the electrocardiographic changes are due to hyperkalaemia.

1.4 Serum Enzyme Assays As A Diagnostic Aid

1.4.1 Basic Mechanisms

There are two categories of enzymes in the serum (Harper, 1971). "Functional" serum enzymes are actively secreted into the circulation and serve a physiological function therein (pseudocholinesterase, lipases and the enzymes involved in coagulation). These enzymes are normally present in high quantities in serum. "Non-functional" serum enzymes whose substrates and cofactors may be absent from serum apparently have no function there. Their normal levels in serum may be about a million times lower than in the tissues.

The presence of enzymes in serum is a reflection of the differential permeabilities of different organs and cells, and is not influenced primarily by cellular concentrations (Bernstein, 1975). Membrane

permeability is affected by many factors some of which are trauma, cell necrosis, action of chemicals, stress, metabolic factors and in particular, anoxia.

Serum enzyme levels are homeostatically regulated (Bernstein, 1975). The range of the various enzymes are held within fairly narrow limits and the influence of age, sex, nutrition and pathophysiological factors do affect such levels. The manner in which the pathophysiological serum enzyme level is maintained remains for the most part obscure (Schmidt and Schmidt, 1970). Input to the serum is from various cells (Schmidt and Schmidt, 1970; Bernstein, 1975). The output from the serum are the processes of excretion (bile, urine) and inactivation (degradation of the enzyme and inhibition by various serum factors) (Bernstein, 1975). Maintenance of the normal difference between the enzyme concentrations in the cells and serum is a process closely linked to the energy metabolism of the cell (Schmidt and Schmidt, 1970). Impairment of energy transformation, as in injury to the cell, results in a movement of enzymes out of the cell, the rate depending on the concentration gradient, molecular mass and location of the particular enzyme. The importance of these factors varies relative to the severity of injury to the cell (Schmidt and Schmidt, 1970).

A comparison of the effect on cell enzymes as a result of slight damage (increased permeability from inflammation) with that resulting from more serious damage (loss of cells or cell constituents following tissue necrosis) reveals that the increased serum enzyme activity in the latter case is a reflection of enzymes coming from intracellular

organelles in contrast to the former which come from the cytosol (Bernstein, 1975).

Within the cell the enzymes are located in various compartments (cytoplasm, mitochondria, lysosomes, microsomes and nucleus) and are classified into Types I and II (Schmidt and Schmidt, 1970). Type I enzymes are easily extractable and are probably only loosely bound to the hyaloplasm (LD and ALT), whereas type II enzymes are released only when the cell is severely disrupted, like the mitochondrial enzyme glutamate dehydrogenase. AST, malate dehydrogenase and isocitrate dehydrogenase probably belong to a further class (Type III) since their cytoplasmic isozymes are very soluble and their mitochondrial isozymes rather insoluble.

The level of any one enzyme may not be informative about its tissue of origin, but, a comparison of the levels of several enzymes may be useful, since the individual enzymes are present in different tissues in different ratios (Kachmar and Moss, 1976). If they are derived from a single cell type, their ratio in the plasma will approximate their tissue of origin. The normal myocardium contains 125 I.U. per gram of LD and 50 I.U. per gram of AST, a ratio of LD to AST of 2,5. After myocardial infarction, the ratio of LD to AST in the serum is still 2,5 (500 I.U. per litre of LD and 200 I.U. per litre of AST) (Schmidt and Schmidt, 1967). On the other hand, if a group of enzymes in the serum are derived from several organs their concentrations in these organs would have to be considered. AST is equally represented in the liver and heart muscle whereas ALT has a concentration in liver

which is ten times its concentration in the myocardium (Schmidt and Schmidt, 1967; Bernstein, 1975). One would expect, therefore, increased serum levels of AST during myocardial infarction and normal or moderately elevated levels of ALT in the serum. In the case of liver diseases both AST and ALT levels in serum would be expected to increase considerably. The envisaged changes in the serum due to myocardial infarction and liver disease do, in fact, occur (Kachmar and Moss, 1976). ALT levels in serum during severe liver diseases are characteristically higher than AST levels and the De Ritis ratio (ALT/AST) which is normally less than 1,0 becomes greater than unity. In myocardial infarctions the ratio of ALT to AST is significantly decreased (Kachmar and Moss, 1976).

1.4.2 Serum Enzymes In Heatstroke

The diagnosis of heatstroke, based on certain clinical findings, is complicated during the presentation of atypical symptoms. Despite the fact that one or more of the classical heatstroke symptoms (coma, anhidrosis, and fever over 41,1^{o}C) could be absent a person could still sustain severe heat-induced injury (Hubbard *et al*, 1978). Widespread tissue damage occurs (Kew, 1976) and an elevation of serum enzyme levels might be anticipated in heatstroke victims. Changes in serum enzyme levels were employed as indicators of tissue damage by Bedrak (1965), Kew *et al* (1967), Shibolet *et al* (1967) and Schrier *et al* (1967). Since 1967 changes in serum enzyme levels in heatstroke cases have been reported with increasing frequency (Kew *et al*, 1969; Kew *et al*, 1970; Kew *et al*, 1971; Spurr (1972); Shapiro *et al*, 1973; Knochel *et al*, 1974; Wyndham *et al*, 1974; Kew 1976; Francesconi *et al*, 1977; Hubbard *et al*, 1978; Hubbard *et al*, 1979). Although elevated serum enzyme

levels in heatstroke have been reported by many researchers and clinicians, the significance thereof has apparently been largely underrated.

Diagnostic and prognostic significance was first attached to the rise in serum enzymes noted in heatstroke by Kew *et al* (1967) and Shibolet *et al* (1967), who agreed that elevations in AST in excess of 1000 I.U. per litre of serum indicated severe heatstroke. The degree of elevation of enzyme levels is a reliable index of the severity of tissue damage in heatstroke, as demonstrated by Kew *et al* (1971). Fifteen of their 53 patients who had serum AST levels over 1000 I.U. per litre in the first 24 hours had severe renal, hepatic and cerebral damage. Death or permanent sequelae were common. Twenty patients with AST values less than 1000 I.U. per litre showed signs of mild or moderate tissue injury. Only 1 of these 20 patients died. Similar findings, which confirmed an unfavourable outcome for patients with AST levels exceeding 1000 I.U. per litre, were made by Knochel (1974) whose 3 patients died.

The high levels of AST in the serum of dogs used as an animal heatstroke model (Shapiro *et al*, 1973) support the contention that heatstroke is caused by the noxious effects of heat accumulated in the body and that the severity of heatstroke is directly and closely related to the duration of the high temperature phase. Their results and contentions were supported by post-mortem findings.

Serum AST, ALT and LD were elevated in the dog heatstroke model of Bynum *et al* (1977), suggesting generalized organ damage. Computation of the De Ritis ratio from their results (ALT/AST = 483/526 = 0,90) would suggest generalized tissue damage, a contention supported by post-mortem findings.

Since widespread tissue damage is characteristic of heatstroke (Kew, 1976) elevation of serum enzymes might be anticipated. In documented cases of heatstroke serum AST, ALT, CK and LD levels are all markedly elevated and continue to rise for the first 48 hours then fall, but still remain elevated for 96 hours (Kew, 1976).

All the cases cited thus far supported the assumptions of Kew *et al* (1971) and Shibolet *et al* (1976) that:

i) heatstroke is accompanied or preceded by widespread cellular injury, and

ii) heat injury will result in the release into the circulation of the transaminase enzymes found in high concentrations in heart, skeletal muscle, brain, liver and kidneys.

Exertion-induced hyperthermia produces a significantly higher incidence of cellular injury and heatstroke death than hyperthermia alone at lower core temperatures (Hubbard *et al*, 1978). Furthermore, an elevation in AST provides a more sensitive measure of generalized cell damage in exertion-induced heatstroke than does ALT. According to Hubbard *et al* (1979), there are no reports of CK activity exceeding 1000 I.U./litre in heatstroke cases without physical effort. Exertion-induced heatstroke,

on the other hand, is accompanied by extreme elevations in serum CK, ALT and AST levels. Serum AST and ALT levels exceeding 1000 I.U./litre confirmed the hypothesis (Hubbard *et al*, 1978) that in their rat heatstroke model the incidence of heatstroke mortality (induced with or without physical effort) was accompanied by evidence of cellular injury.

A clear distinction exists between enzyme changes which follow exercise in hot conditions and those seen in heatstroke (Wyndham *et al*, 1974). Their results indicate that some injury occurs in tissues as a result of hyperthermia due to exercise in the heat. These increases in serum enzymes after exercise in the heat are neither as consistent nor as high as those described in heatstroke; in fact the rapid return of these increased enzyme levels to resting values indicate that the injury was rapidly reversible.

1.4.2.1 Plasma/Serum CK

Creatine Kinase or CK (ATP: creatine phosphotransferase, EC 2.7.3.2) catalyses the reversible transfer of a phosphate group from magnesium dependent adenosine triphosphate to creatine (King, 1965; Rosalki, 1967; Ogunro *et al*, 1977. a). The reaction from left to right is regarded as being the forward reaction.

$$ATP\text{-}Mg^{++} + Creatine \rightleftharpoons ADP\text{-}Mg^{++} + Creatine\ Phosphate + H^{+}$$

CK is found in high concentrations in skeletal muscle, cardiac muscle and brain (Tsung, 1976; Ogunro *et al*, 1977. b; Hubbard *et al*, 1979). Elevated levels of CK activity are found in diseases involving skeletal muscle and cardiac muscle (especially during the first 72 hours following infarction) (Griffiths, 1966).

In confirmed cases of heatstroke CK is, more often than not, elevated. Serum CK levels were elevated in all cases examined by Shibolet *et al* (1967) peak levels being reached between the 2nd and 5th day. Of 26 patients in whom CK was measured (Kew *et al*, 1971) elevated values were found within 24 hours or on admission. Serum CK values exceeded 1000 I.U./litre in 2 of the 3 fatal cases of Knochel (1974). In the South African gold mines serum CK values are very often in excess of 1000 I.U./litre in confirmed cases of heatstroke (Kielblock, 1982: personal communication).

1.4.2.2 Plasma/Serum LD

Lactate dehydrogenase or LD (L-lactate: NAD oxidoreductase, EC 1.1.1.27) is a hydrogen transferring enzyme which catalyses the oxidation of L-lactate to pyruvate with the mediation of NAD as hydrogen acceptor (Kachmar and Moss, 1976).

$$\text{L-lactate} + NAD^+ \rightleftharpoons \text{Pyruvate} + NADH + H^+$$

LD activity is present in almost all tissue (Wroblewski and La Due, 1955; King, 1965; Kachmar and Moss, 1976) and is found only in the

cytoplasm of the cell (Kachmar and Moss, 1976).

In clinically confirmed cases of heatstroke LD levels are always elevated. In 10 patients in whom serum LD was measured by Shibolet *et al* (1967) elevations were observed in all, with peak values being noted on the 3rd day. In 2 of 3 cases displaying elevations in LD levels (Schrier *et al*, 1967) the LD isoenzyme most markedly elevated was the skeletal muscle/liver fraction. Their contentions were supported by biochemical and autopsy findings.

In a study of 25 cases of heatstroke (Kew *et al*, 1967) elevations in serum LD occurred in all, the increases being attributed to widespread thermal injury associated with heatstroke. Serum LD elevations occurred in all cases within 24 hours of the onset of heatstroke (Kew *et al*, 1969) with peak values being noted in 72 hours. These high values persisted for 4 to 17 days. In another study (Kew *et al*, 1970) serum LD was elevated in 33 of 39 cases (5 having died underground in the mine) on admission. Peak values were reached in 48 hours and elevations persisted for up to 28 days, with a mean of 14 days. Of another 41 cases (Kew *et al*, 1971) LD was elevated in 38 on admission with elevations occurring subsequently in the remaining 3. Maximum values were reached in 48 hours with return to normal in 2 to 28 days.

In a dog heatstroke model (Bynum *et al*, 1977) increased LD values were noted at death, suggesting widespread cell damage including the liver, cardiac and skeletal muscle, and the brain, the suspected damage

being confirmed at autopsy.

1.4.2.3 Serum/Plasma AST and ALT

Aspartate aminotransferase or aspartate transaminase (AST) and alanine aminotransferase or alanine transaminase (ALT) constitute a group of enzymes which catalyze the interconversion of amino acids and alpha oxoacids by transfer of amino groups (Kachmar and Moss, 1976). AST (L-Aspartate: 2 - oxoglutarate aminotransferase, EC 2.6.1.1) catalyses the reaction.

$$\text{L-aspartate} + \alpha\text{ - oxoglutarate} \underset{\text{AST}}{\overset{\text{P-5-P}}{\rightleftharpoons}} \text{oxalacetate} + \text{L - glutamate}$$

whilst ALT (L-alanine: 2 - oxoglutarate aminotransferase, EC 2.6.1.2) catalyses the reaction

$$\text{L - alanine} + \alpha\text{ - oxoglutarate} \underset{\text{P-5-P}}{\overset{\text{ALT}}{\rightleftharpoons}} \text{pyruvate} + \text{L - glutamate}$$

Despite being reversible, reaction equilibria favour the formation of aspartate and alanine respectively.

Both enzymes are widely distributed in animal tissues (Kachmar and Moss, 1976). In most disease conditions AST levels are higher than those of ALT except in the case of liver disease, when ALT levels generally exceed AST, indicating that ALT is the more liver - specific enzyme (Schmidt and Schmidt, 1967; Bernstein, 1975; Kachmar and Moss, 1976).

The De Ritis ratio (ALT/AST) which is normally less than unity exceeds 1,0 in liver diseases (Schmidt and Schmidt, 1967; Kachmar and Moss, 1976).

Of 36 heatstroke cases examined by Shibolet *et al* (1967) 28 had elevated AST levels. In the ten severe cases AST levels were elevated on admission and peak values were noted between the third and fourth day. Based on correlations with other signs of hepatic damage it was concluded that these raised AST levels were indicative of liver damage.

In 25 cases of heatstroke (Kew *et al*, 1967) elevated serum levels of AST and ALT was a consistent finding , the increases being attributed to widespread tissue damage. In another study (Kew *et al*, 1970) AST levels were elevated in all 34 cases studied, with ALT elevations occurring in the 31 cases in whom it was measured. Serum levels of both enzymes remained elevated for well over 20 days. It was suggested, on the basis of the elevated enzyme levels and supporting biopsy findings, that transient hepatic damage had occurred in all the cases. Of a group of 84 miners provisionally diagnosed as heatstroke cases, serum AST and ALT levels (as well as CK and LD) were elevated within 24 hours of admission in 75 in whom diagnosis was confirmed (Kew *et al*, 1974). In 15 of them AST levels exceeded 1000 I.U./litre in the first 24 hours. Tissue damage (renal, hepatic and cerebral) was severe and death or permanent sequelae was common. In 20 cases, in whom AST values were below 1000 I.U. tissue injury was mild or moderate and completely reversible. There was only a single fatality in the latter group.

Also, in animal heatstroke models the elevation of serum enzymes is a consistent finding. The artificial heating of dogs to a rectal temperature of 41,5°C on 4 consecutive days (Spurr, 1972) caused an increase in AST and ALT levels. Considered with increased levels of isocitrate dehydrogenase (liver specific) the increased AST and ALT levels were indicative of acute hepatic injury.

In a group of 34 dogs subjected to effort at an environmental temperature of 43°C to 45°C (relative humidity 50%) serum AST levels rose in 14 in which rectal temperatures were maintained between 43°C and 43,9°C (Shapiro *et al*, 1973). The mean serum AST levels rose from a normal value of 23 I.U./litre to well over 1000 I.U./litre. In 3 of them mean levels exceeded 2000 I.U./litre. These results were indicative of widespread tissue damage, confirmed at post-mortem examination.

Serum AST and ALT levels of anaesthetized dogs (maintained at an ambient temperature of 42°C to 46°C), whose rectal temperatures rose to 43°C to 44,5°C, were elevated significantly (Bynum *et al*, 1977). These findings suggested generalized tissue damage, as indicated by a De Ritis ratio of 0,90 (vide supra).

Substantial evidence was provided by Hubbard *et al* (1978) to support the widely held concept that in heatstroke cases elevations in serum AST levels reflect generalized tissue damage. Serum AST and ALT levels were elevated in excess of 1000 I.U./litre and reached peak levels in 24 hours, remaining elevated for 72 to 96 hours. An interesting finding was that in those rats in which hyperthermia was

induced without the animals being subjected to exertion, the AST and ALT curves (which reflected survivors with AST and ALT levels in excess of 1000 I.U. per litre) were identical indicating primarily liver damage. When work and hyperthermia were combined, AST levels were higher than ALT indicating that AST is a better indicator of generalized tissue damage. These results suggest that, diagnostically, the extent of tissue injury due to exertion-induced hyperthermia is different from hyperthermia in the absence of effort (Hubbard *et al*, 1979). In exertion-induced hyperthermia, injury occurred at lower thermal loads and the rate of mortality was higher than in the passively heated animals. In view of these findings it was concluded that although the extent of heat- or work - induced tissue damage can be assessed by quantitative changes in the levels of selected serum enzymes, the distinction can be better made by assessing both the level and pattern of enzyme release.

1.5 Summary

Heatstroke is still an enigma despite sound scientific principles of diagnosis. Furthermore, despite the wealth of information acquired over the last decade from serum enzyme changes in overt heatstroke cases, it is clear from this review that there is still a dearth of knowledge in respect of the early changes in tissue which eventually give rise to more serious sequelae.

CHAPTER 2

MATERIALS AND METHODS

The general objective of this study is to determine the nature and extent of serum enzyme changes during the initial stages of experimental hyperthermia. The techniques and procedures applied are outlined in this chapter, and where applicable, their choice is motivated.

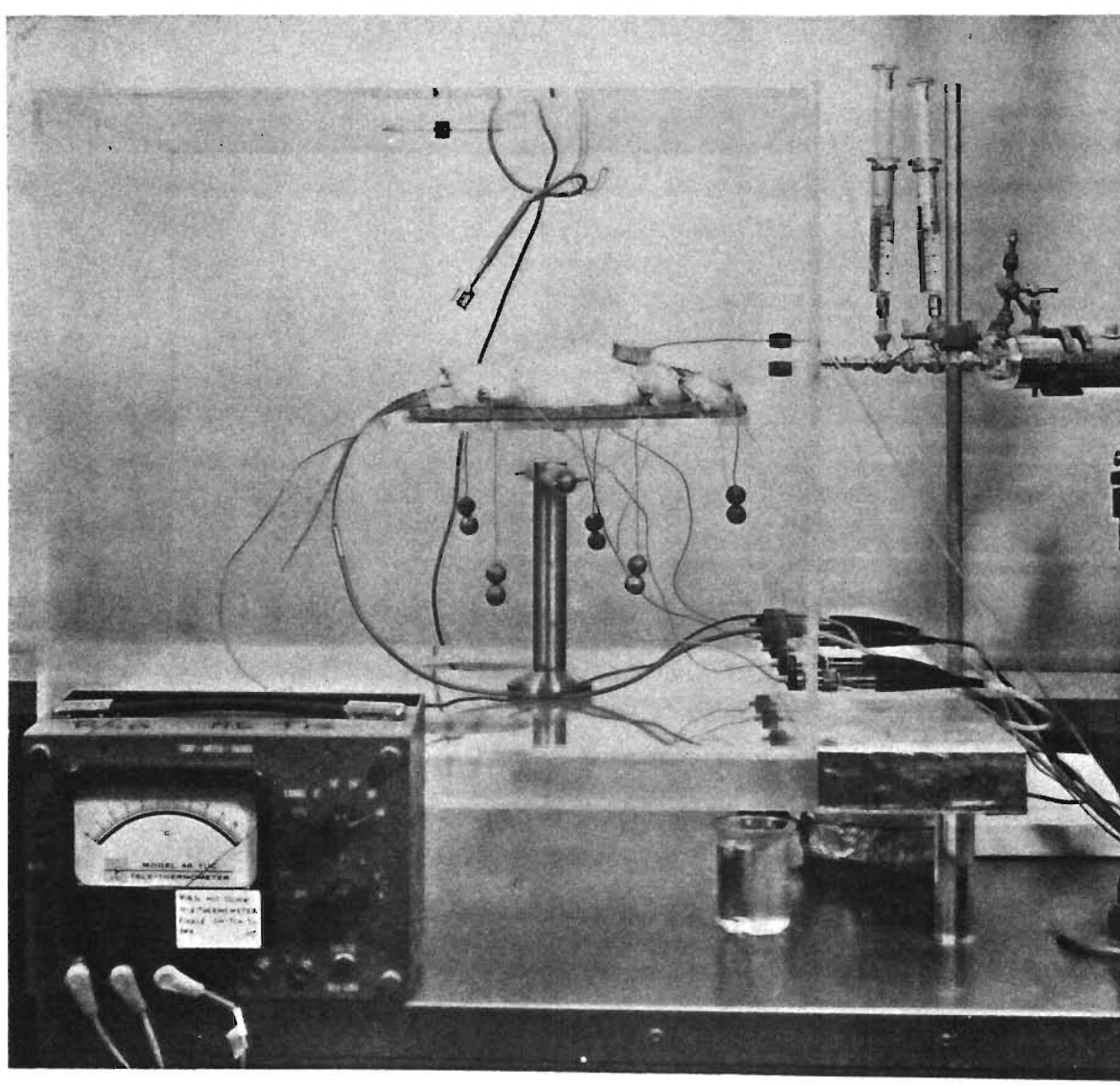
2.1 Experimental Animals

Highly inbred Wistar strains of male, *Rattus norvegicus albinus*, 200 to 300 grams in weight, corresponding to an age of 12 to 14 weeks and generally regarded as young and healthy, were used throughout. The animals were housed in a special room (maintained at 20°C and 60% relative humidity) so as to minimize fluctuations in environmental conditions. Food (commercially available rodent pellets by EPOL) and water were available *ad libitum*, except on "experimental" days when animals were fasted for 12 hours. Food and water were witheld for the duration of the experiment, and those animals which were sacrificed 6 and 24 hours after the termination of stress received only water during their survival period.

2.2 Anaesthesia

Sodium pentobarbitone ("Sagatal", Maybaker, S.A.) was the anaesthetic of choice mainly because of its extensive use in similar investigations.

FIGURE 2.1 THE INDUCTION OF HEAT STRESS



The animals were anaesthetized by an intra-peritoneal injection of sodium pentobarbitone, a dose of 40mg/kg body mass being employed. The stock anaesthetic (60mg/ml) was diluted in 0,85% saline to give a final concentration of 10mg/ml. This represents the lower limit of the recommended dosage for rats.

Despite the fact that the animals were rendered passive thus precluding any behavioral responses to heat stress (saliva spreading to achieve evaporative heat loss), enhanced salivation was not suppressed, resulting in a condition akin to the wasteful dripping of sweat in hot, humid environments with low windspeed, ultimately leading to dehydration.

2.3 Environmental Stress/Heat Load

Heat stress was induced in a specially designed "Perspex" cabinet (Figure 2,1), heat being generated by a high capacity "Shandon" heater element drying oven. Manipulation of the outlet valve of the cabinet enabled one to control the temperature within the cabinet to within 0,1^{o}C of the desired setting. The temperature within the cabinet was uniform and no appreciable gradients could be detected between different localities. The temperature was monitored by means of a YSI "405" thermistor (Yellow Springs Instrument) suspended in the centre of the cabinet. The probe was connected by means of a jack plug to a thermometer unit (YSI "Thermistemp Tele-thermometer" Model 46-TUC), temperature readings being obtained directly on a linear scale in degrees Celsius. The thermistor was accurate to within 0,1^{o}C when checked against a certified thermometer.

Humidity levels within the cabinet were maintained by means of thermostatically controlled water bath, and measured using a HM 111 "Relative Humidity Indicator" (Weather Measure, Sacramento, California) connected to a HMP 11 probe. By careful manipulation of the inlet to the cabinet the relative humidity within the cabinet could be held constant at the two experimental levels of 10% and 50%, both at a dry-bulb temperature of 45^{o}C.

2.4 Basic Measurements And Instrumentation

2.4.1 Heart Rate

The heart rate was calculated from electrocardiographic RR' intervals. For this purpose sub-cutaneous needle electrodes were placed in the axillae and lower abdomen, the electrocardiogram being recorded on an Elema-Schonander "Mingograf 81" 8-channel polygraph.

2.4.2 Body Temperature

Core temperature (T_c) and skin temperature (T_s) were measured using an electrical thermometer (See Section 2.3). T_c was measured following insertion of a YSI "402" rectal probe to a depth of about 6cm into the colon via the rectal orifice. This temperature measurement was a reflection of core, rather than rectal, temperature. Skin temperature (T_s) was measured on the base of the tail on its anterior surface using a YSI "409" skin temperature probe. This probe was insulated from the environment by wrapping in cotton wool. Both probes were kept in

position by adhesive tape.

2.4.3 Tissue Enzyme Assays In Plasma

The discovery of very high control LD activities in rat serum by Papadopoulos *et al* (1967) instigated an in-depth investigation into this discrepancy. They found that several factors had contributed to these high control values as well as the variability between determinations. Allowing blood to stand before centrifuging for serum resulted in the disruption of platelets and the haemolysis of red cells, both events resulting in a release of LD into the serum. Furthermore, allowing clotted blood to stand before centrifuging resulted in a progressive elevation of serum LD, probably as a result of haemolysis and platelet disruption. In fact, a year earlier, Cohen and Larson (1966) had shown similar findings on human blood and had established, conclusively, the role of platelets in the elevation of serum LD.

As a consequence of the above findings all enzymes were assayed in plasma. Blood was drawn from the animals, under ether anaesthesia, by insertion of an hypodermic syringe needle into the dorsal aorta after the abdomen had been opened. The blood was allowed to flow through a PVC tube (attached to the needle) into a graduated centrifuge tube, the syringe, PVC tube and centrifuge tube being heparinized prior to use. Depending on the size of the animal, 4 to 8 ml of blood could be collected in this manner, the animal being sacrificed immediately thereafter. The blood was centrifuged immediately in a bench centrifuge at 2000 rpm for 10 minutes and three-fourths ($\frac{3}{4}$) of the supernatant

plasma removed for analysis.

In view of the fact that plasma levels of CK, LD, AST and ALT are all elevated within 24 hours of a positive diagnosis of heatstroke, the levels of these enzymes were assayed at the termination of stress, 6 hours later and 24 hours later, and if necessary, at 24 hour intervals thereafter.

Because of the small quantities of blood obtained, analytical methods which required small samples and which permitted duplicate determinations were chosen. Consequently, diagnostic kits from Boehringer Mannheim (GmbH) were used in the assay of plasma enzyme levels. "Monotest" kits were preferred, thus ensuring that fresh reagents were available for each assay. Furthermore, the activities of each kit were routinely checked against control sera from Boehringer Mannheim (Precinorm E). All reagents for enzyme assay were kept in a water bath at 30°C for at least 30 minutes prior to use.

All enzyme assays were performed in a PYE-UNICAM SP 8-100 Split Beam ultra-violet spectrophotometer with a temperature-controlled cell-housing (maintained at 30°C). Grade A pipettes were employed throughout. Assays were performed at 30°C in keeping with the recommendations of the Commission of Enzymes of the International Union of Biochemistry.

Plasma CK was assayed with the Boehringer NAC - activated Monotest Kit and plasma AST, ALT, and LD were assayed with the respective Boehringer optimized Monotest Kits. One International Unit of activity

is defined as the oxidation of 1 micromole of NADH per minute at 30°C for AST, ALT and LD. For CK, one International Unit of activity is defined as the reduction of 1 micromole of NADP per minute at 30°C. In those cases in which the absorbance change per minute was too great the plasma was diluted ten times in 0,9% NaCl and the final result multiplied by 10.

2.5 Assessment Of Heat Stress

The consequence of exposing man or experimental animals to a heat load is heat stress (Hatch, 1963), reflected as a change in the parameters of heat stress, namely, body temperature, body heat storage and heart rate. In this study, the assessment of heat stress is confined to the measurement of heart rate (as an index of the total physiological strain) and body heat storage.

2.5.1 Heart Rate

In an effort to circulate large quantities of blood rapidly through the periphery for cooling purposes the heart rate increases, this response being one of the most striking changes which occur during heat exposure (Gold, 1960; Williams *et al*, 1962). The heart rate was computed from the RR' intervals of the electrocardiogram.

2.5.2 Heat Storage

Heat storage by body tissues provides a good index of heat stress for a given, standardized stress (Gold, 1961). A low heat storage reflects good heat dissipation whereas a high heat storage reflects poor heat dissipation. Body heat storage was computed by use of the formula:

$$S = 3,4742m.\ T_m$$

where S is body heat storage in kilojoules, 3,4742 is the specific heat of all body tissues in kilojoules per kg per ^{o}C, m is the mass in kilograms, and T_m is the mean body temperature in ^{o}C. The mean body temperature was derived by use of the equation:

$$T_m = 0,80\ T_c + 0,20\ T_s$$

where T_c is core temperature in ^{o}C and T_s is skin temperature in ^{o}C. The mean body temperature computed in this manner is in accord with Stolwijk's recommendation for warm conditions (Stolwijk and Hardy, 1966). Body heat storage is dependent on the site and number of skin temperature measurements (as well as the weighting of T_c and T_s). Since T_s was measured at one site only body heat storage is used as an index and not in its absolute sense.

2.6 Heat Stress Levels

The severity of heat stress is proportional to the environmental heat load and to the duration of exposure (Shapiro *et al*, 1973; Shibolet *et al*, 1976; Hubbard *et al*, 1977). This implies that in heatstroke the severity of tissue damage would be proportional to the elevation in core temperature and the exposure time. However, during the prodromal period of heatstroke there is uncertainty as to whether this relationship pertains, and if so, to what degree. How high does the body temperature have to be before heatstroke develops? How long does one have to be exposed to the environmental heat load before heatstroke develops? Furthermore, which combination of environmental heat load and exposure time will be most deleterious? These questions are largely unanswered. This study is an attempt to answer some, or all, of these questions. To do this, experimental simulation is necessary.

To make conditions comparable, the experimental procedure was manipulated to give, for identical values of body heat storage, three different experimental situations, as depicted in Table 2.2 and Figure 2.3 . A body heat storage gain of 20% above baseline was chosen because increments above this value resulted in death.

Table 2.2: Experimental Heat Stress Conditions

Stress level	Heat load	Average core temperature ^{o}C	Duration (minutes)
I	45^{o}C Dry-bulb 10% Relative humidity	T_c = 42,0^{o}C	46,7
II	45^{o}C Dry-bulb 10% Relative humidity	T_c = 42,5^{o}C	56,9
III	45^{o}C Dry-bulb 50% Relative humidity	T_c = 42,3^{o}C	39,4

T_c : mean core temperature

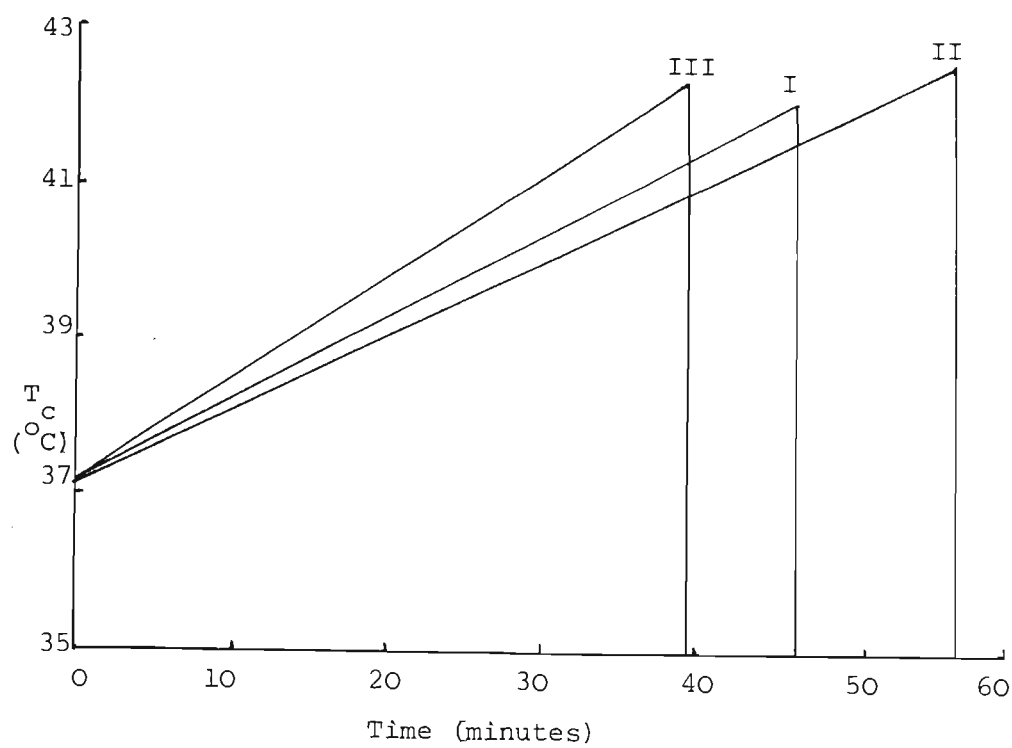
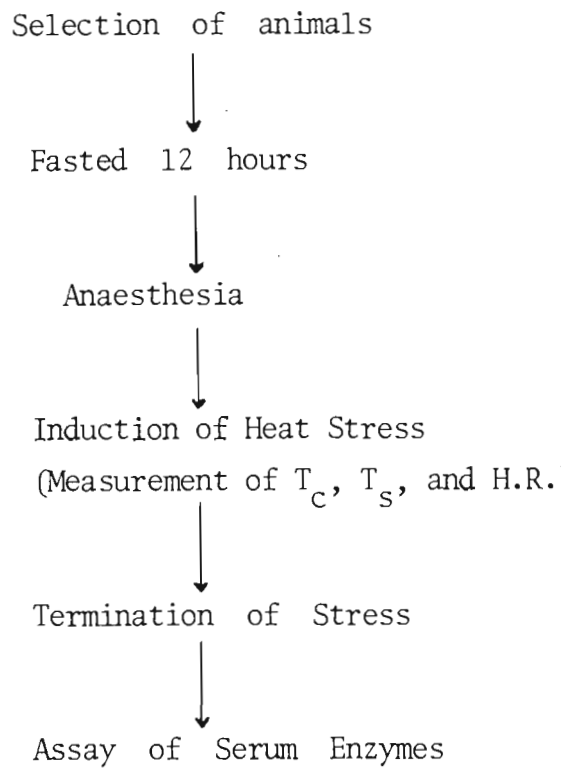


Figure 2.3: Schematic Representation Of Experimental Heat Stress Conditions

2.7 Experimental Protocol



Thirty animals were exposed to each environmental heat load. Each animal was anaesthetized and placed supine on a special platform in the cabinet. Once the electrocardiograph leads and temperature probes were in position, core temperature (T_c), skin temperature (T_s) were recorded and the mean body temperature (T_m), body heat storage (S) and heart rate (HR) calculated. These initial (pre-stress) measurements were regarded as baseline, each rat being its own (anaesthetized) control. The lid of the cabinet was then slid into position and once closed the desired temperature and relative humidity within the cabinet was obtained within a minute of lid closure. The physiological responses of each animal (T_c, T_s, T_m, S and HR) were determined at 10-minute intervals for the duration of the stress.

When the stress was terminated the animals were removed from the cabinet. Ten of the thirty animals in each group were exsanguinated immediately and their plasma assayed for CK, LD, AST and ALT. The remaining 20 animals in each group were allowed to recover at room temperature and their physiological responses (T_c, T_s, T_m, S and HR) were monitored at 10-minute intervals during the recovery phase. All measurements were terminated once the animals showed signs of being awake. Ten of these 20 animals in each group were exsanguinated 6 hours after termination of stress and the remaining 10 animals in each group were exsanguinated 24 hours after the stress was terminated. Plasma levels of CK, LD, AST and ALT were determined in both cases.

In addition to these 90 experimental animals (3 groups of 30 animals per group) 10 animals were selected as controls and their plasma was assayed to establish base-line values for CK, LD, AST and ALT.

2.8 Statistical Analysis

Heat stress was assessed by employing two indices, namely heart rate and body heat storage (See Section 2.5). The latter index was calculated using mean body temperature as a major component. Means and standard deviations were calculated for each of the above.

Changes in plasma enzyme levels were related to the different stress levels at the respective response times. In view of the tremendous individual variation in plasma enzyme levels within each group of

animals, all enzyme changes were subjected to an analysis of variance with a two-way interaction i.e. plasma enzyme levels were first related to stress levels and then to response times and finally these two relationships were interacted.

CHAPTER 3

RESULTS

The physiological responses to heat exposure measured were heart rate, body temperature and changes in plasma enzyme levels at all levels of stress. Furthermore, body temperature measurements (T_c and T_s) were used to compute body heat storage and this parameter was employed in setting the upper limit of each stress level (Section 2.6).

All the raw data have been appended.

3.1 Heart Rate

An analysis of the heart rate data in Table 3.1 indicates that there is a significant increase in this parameter of heat stress above base-line at each level of stress employed in this study.

3.2 Mean Body Temperature

Mean body temperature increases significantly above resting values at each level of stress. This increase is readily apparent in Table 3.1 and Figure 3.2.

3.3 Body Heat Storage

A perusal of Table 3.3 indicates that irrespective of size, the heat

Table 3.1: Changes in Heart Rate and Mean Body Temperature During Heat Stress

Stress level	Mean Mass (g)	Tmi	S.D.	Tmt	S.D.	HRi	S.D.	HRt	S.D.	Time (min)	S.D.	$\frac{dTm}{dt}$	n
I	272,4	35,1	0,70	42,0	0,11	371	35	444	35	46,7	5,9	0,15	15
II	239,9	35,5	0,59	42,5	0,06	373	45	513	37	56,9	6,5	0,13	15
III	255,3	35,2	0,53	42,3	0,63	377	41	490	46	39,4	6,5	0,18	30

Tmi : Mean initial mean body temperature, ^{o}C

Tmt : Mean end-of-stress mean body temperature, ^{o}C

HRi : Mean initial heart rate, beats per minute

HRt : Mean end-of-stress heart rate, beats per minute

S.D.: Standard deviation

n : Number of observations (animals)

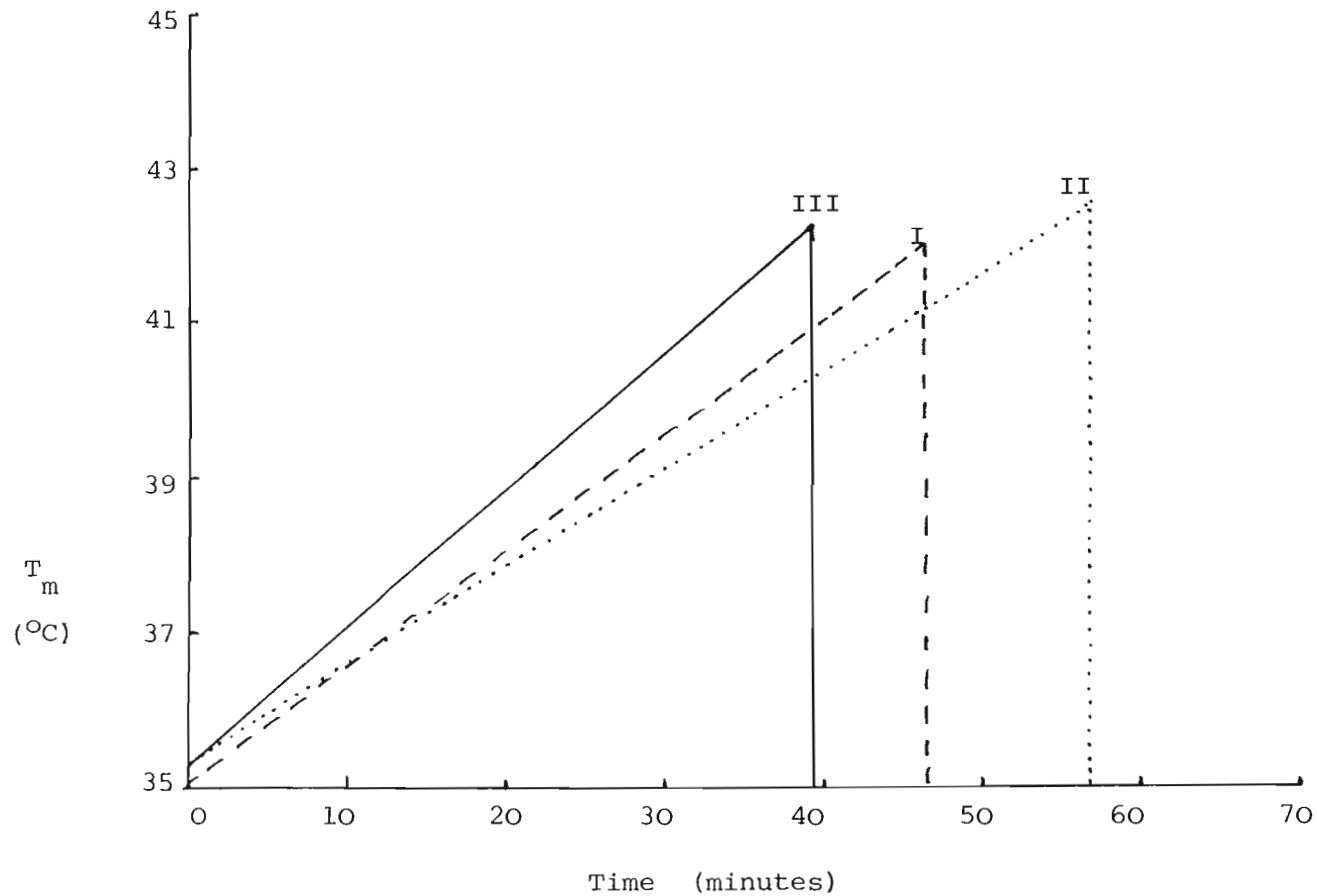


Figure 3.2: Changes In Mean Body Temperature During Heat Stress

Table 3.3: Changes in Body Heat Storage During Heat Stress

Stress level	Mean Mass (g)	Si	S.D.	St	S.D.	Time (min)	S.D.	% ΔS	n
I	272,4	33,19	3,31	39,77	3,60	46,7	5,9	19,8	15
II	239,9	29,51	1,59	35,49	2,09	56,9	6,5	20,3	15
III	255,3	31,10	2,97	37,38	3,60	39,4	6,5	20,2	30

Si : Mean initial body heat storage in kilojoules

St : Mean end-of-stress body heat storage in kilojoules

%ΔS: Percentage change in mean body heat storage

n : Number of observations (animals)

S.D.: Standard deviation

Table 3.4: Analysis of Variance with two - way interaction. F - and P - values

Enzyme	F value for stress levels 0, I, II and III	P	F value for response times 0,6 and 24 hrs	P	F value for interaction between stress levels and response times	P
CK	16,386	<0,05	26,918	<0,05	13,693	<0,05
LD	14,564	<0,05	24,597	<0,05	16,725	<0,05
AST	3,971	<0,05	9,647	<0,05	3,765	<0,05
ALT	4,820	<0,05	7,980	<0,05	3,078	<0,05

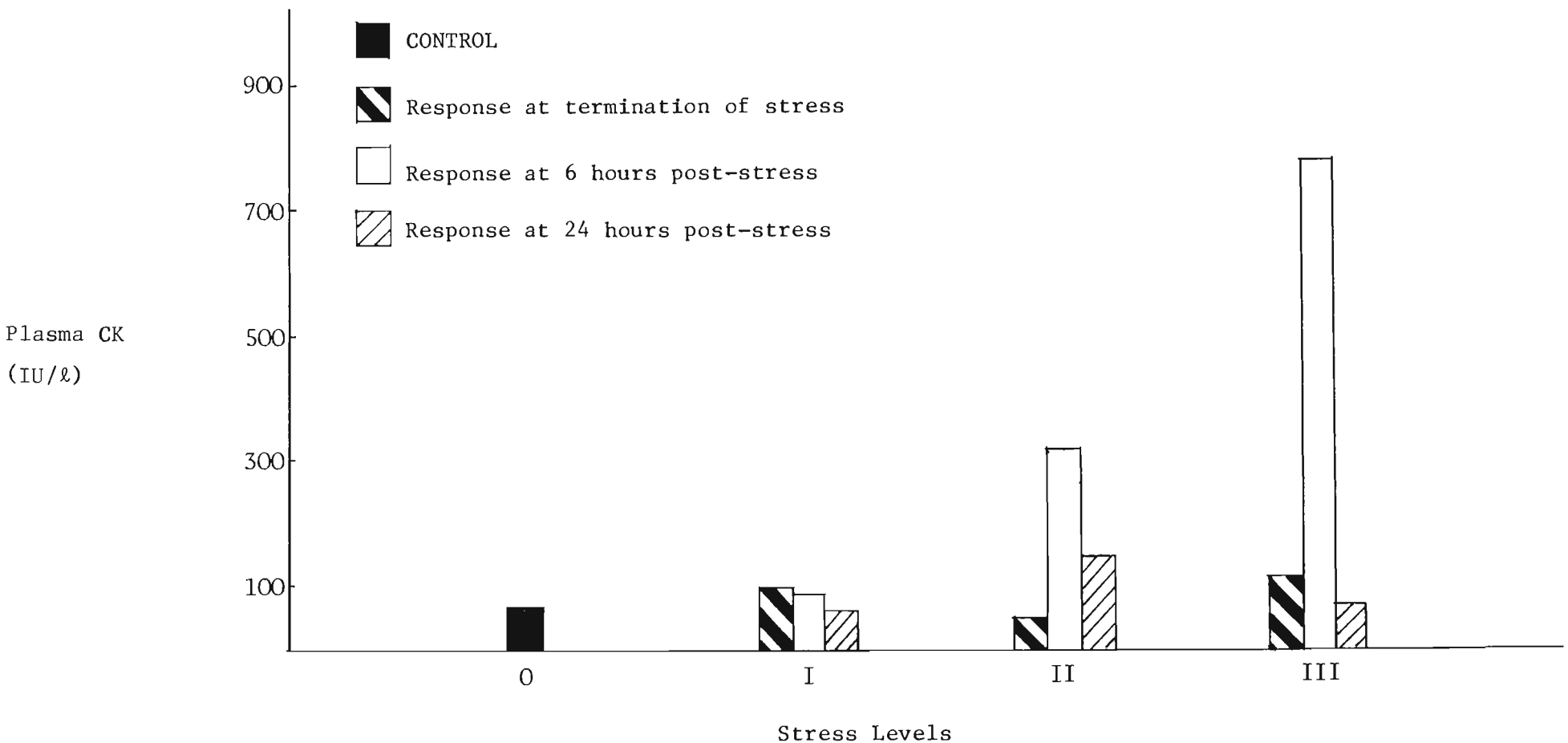


Figure 3.5: Plasma CK profiles of animals subjected to heat stress

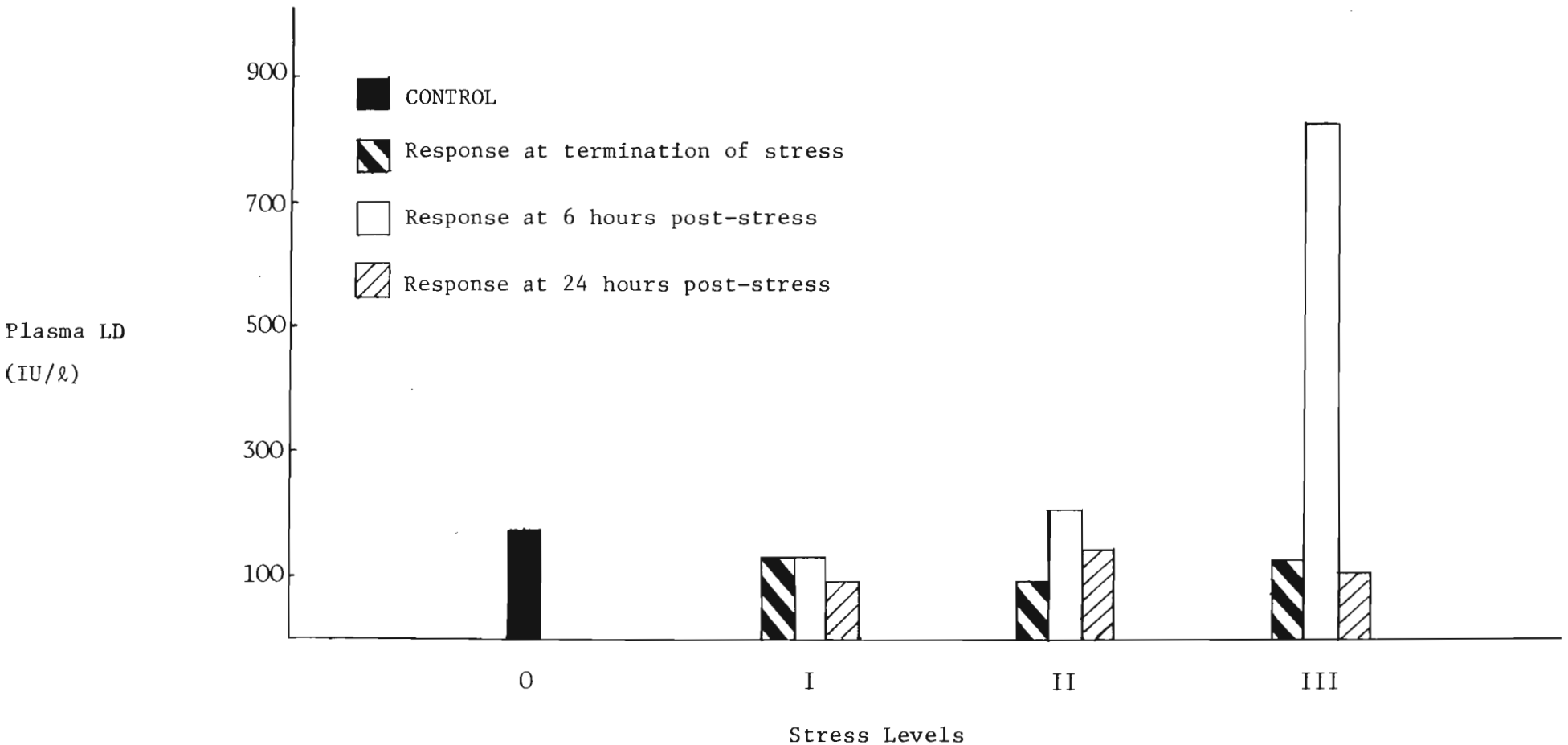


Figure 3.6: Plasma LD profiles of animals subjected to heat stress

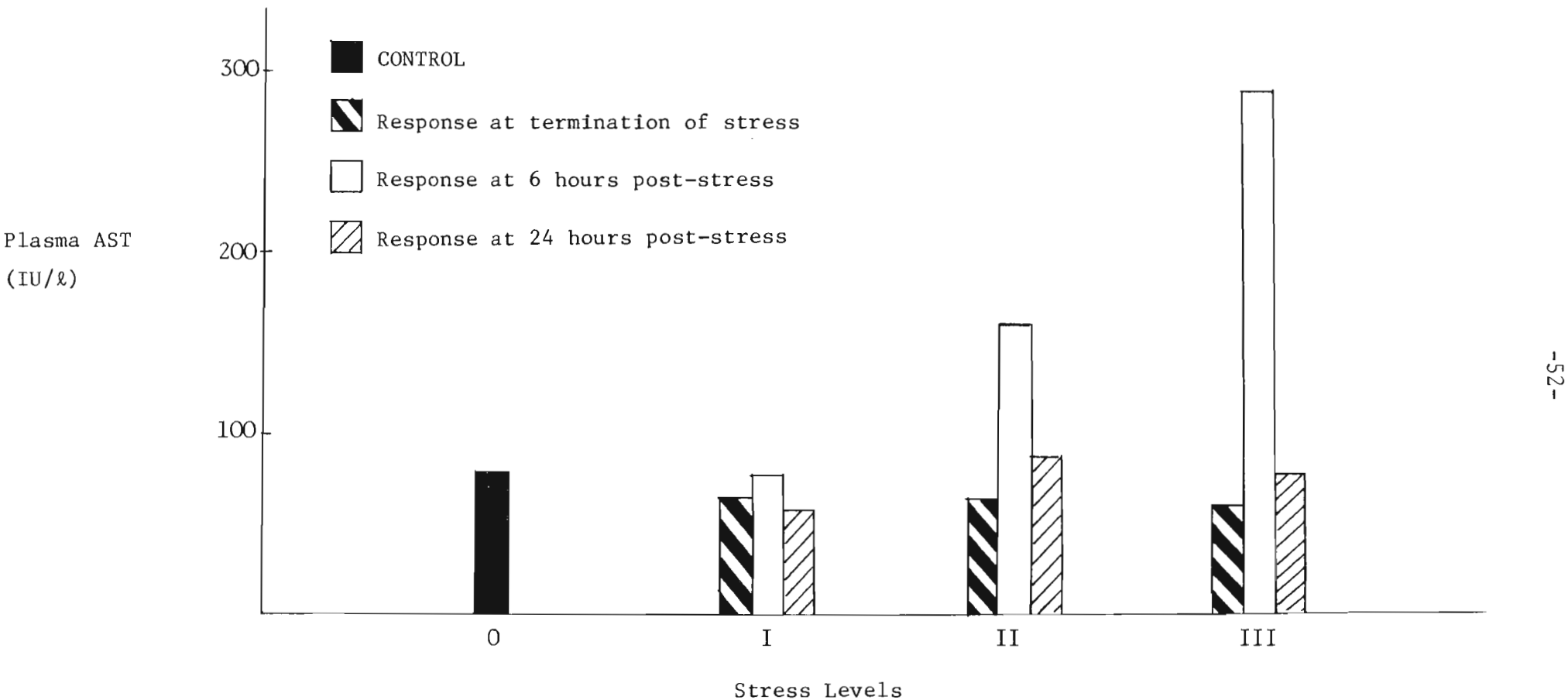


Figure 3.7: Plasma AST profiles of animals subjected to heat stress

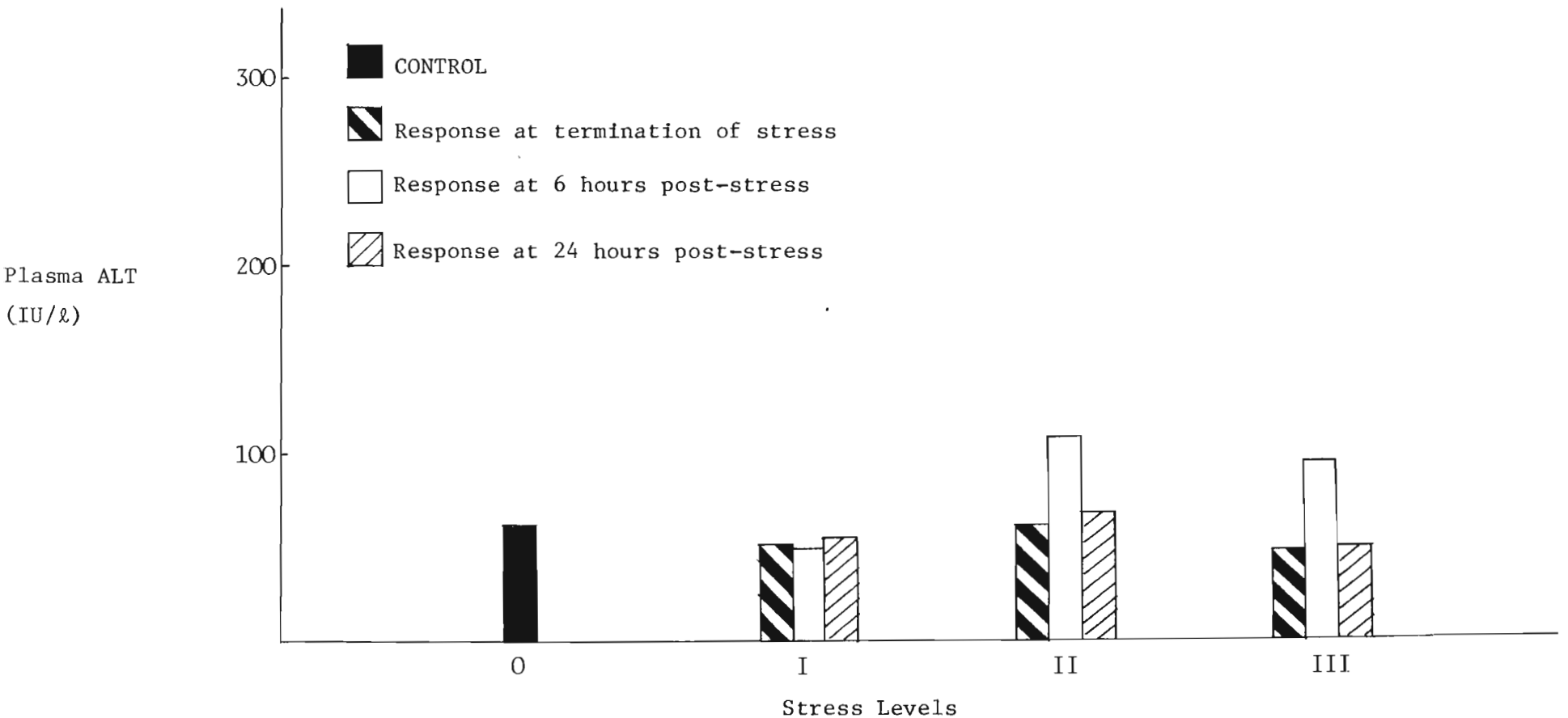


Figure 3.8: Plasma ALT profiles of animals subjected to heat stress

gain in all cases was identical in terms of unit body mass, i.e. heat storage per kilogram body mass.

3.4 Plasma Enzyme Profiles

Plasma levels of CK, LD, AST and ALT were measured at three response times at all levels of stress (See Section 2.6). Tables 1.10 through to 1.12 inclusive in the appendix contain the means of two plasma enzyme level determinations made on each animal at each level of stress. It is noted that there is tremendous individual variation in each group. Consequently, these data (Tables 1.10 to 1.12 inclusive) as well as the data in Table 1.14 of the Appendix (Plasma enzyme levels of control group of animals) were subjected to an Analysis of Variance with interaction between enzyme levels and stress levels and enzyme levels and response times. The results of the analysis (F - values) are presented in Table 3.4. It is noted that all the enzyme changes are significant at a level of 95% ($P < 0,05$).

Mean values were calculated for the enzyme levels at each stress level and response time. The mean enzyme levels for each response time were plotted against the different levels of stress, illustrated graphically in Figure 3.5 to 3.8 inclusive. These figures illustrate the fact that the most obvious change in plasma enzyme levels occurs 6 hours after the termination of stress for the second and third levels of stress. Also, except for ALT levels, the greatest change in plasma enzyme levels occurred during the third level of stress. Furthermore, all elevated plasma enzyme levels had reverted to normal 24 hours post-stress.

CHAPTER 4

DISCUSSION

In order to investigate the early signs of abnormal function (which initiate more serious consequences) this study was designed so that the severity of stress could be assessed in terms of the level of hyperthermia and the duration of exposure. A logical consequence would be the establishment of a relationship between the reversibility of tissue damage and the magnitude and nature of the heat load. It follows that an evaluation of the sensitivity of the indicators of tissue damage would be of fundamental importance.

4.1 Temporal Changes

In this study plasma enzyme levels were assayed immediately after the stress was terminated, six hours post-stress and twenty four hours post-stress for all three levels of stress. There were no changes in plasma enzyme levels at the termination of stress or at 24 hours post-stress. Furthermore, the plasma levels of all four enzymes (AST, ALT, CK and LD) had returned to normal twenty four hours post-stress, suggesting that no permanent tissue damage had occurred and that the animals in this study had not suffered heatstroke. In documented heatstroke cases, the serum levels of all four enzymes continue to rise during the first forty eight hours of diagnosis and remain elevated for ninety six hours or more (Kew, 1976).

The most sensitive assay is that done at six hours post-stress. In suspected heatstroke cases a zero-hour (on admission) sample is sometimes omitted as treatment is of utmost importance and takes priority. By the time the patient is admitted to hospital and the first assay performed four to six hours may have elapsed. This sample, taken after admission is, in fact, the equivalent of the six hour assay of this study. The next sample should be taken eighteen hours post-admission, being the equivalent of the twenty four hour post-stress sample of this study.

4.2 Sensitivity Of The Parameters Of Tissue Damage

The degree of elevation of serum enzyme levels is a reliable index of tissue damage (Kew *et al*, 1971). Slight or moderate elevations in enzyme levels suggest slight or moderate tissue damage whereas large increases in enzyme levels suggest severe tissue damage. All four enzymes recommended for suspected heatstroke cases (AST, ALT, CK and LD) have been employed in this study.

An analysis of the results (Figures 3.5 to 3.8 inclusive) indicate that the most sensitive of the four enzymes employed in this study is CK, followed closely by LD. AST is intermediate in sensitivity and ALT is the least sensitive. The sensitivity thus derived is based on the magnitude of the increase in plasma enzyme levels for any given stress situation. These findings contrast sharply with those of Hubbard *et al* (1979) who found that in those animals subjected to hyperthermia in the absence of physical effort CK was the least sensitive, the greatest sensitivity being shown by AST

and ALT. However, it must be pointed out that the animals in the study of Hubbard *et al* (1979) had suffered from heatstroke, and this would account for the sustained high levels of AST, ALT and CK twenty four hours post-stress. Also, in the dog heatstroke model of Bynum *et al* (1977) the most sensitive enzyme was LD, followed by AST and ALT (CK not being assayed). It appears, therefore, that under different conditions the sensitivity of enzymes changes. In heatstroke, where tissue damage is widespread, severe and very often permanent, the degree of elevation of serum enzymes is very great whereas in this study, where heatstroke was not induced, the degree of elevation of serum enzymes is moderate and transient.

The pattern of plasma enzyme increase in this study indicates that the tissue damage which had occurred was of a generalized nature. This finding is augmented by:

a) a high CK level which is mostly indicative of skeletal muscle and cardiac muscle damage and possibly also the gastrointestinal tract (Ogunro *et al*, 1977);

b) a high LD level, which is indicative of generalized tissue damage (Kachmar and Moss, 1976);

c) a comparatively high AST level (higher than ALT) which is also indicative of generalized tissue damage (Hubbard *et al*, 1979);

d) a De Ritis ratio of 0,65 for the second level of stress, indicating generalized tissue damage (Kachmar and Moss, 1976); and,

e) a De Ritis ratio of 0,32 for the third level of stress indicating cardiac damage predominating over liver damage (Kachmar and Moss, 1976).

Collectively, these findings support the view that a generalized transient, moderate or mild tissue damage had occurred during this study.

4.3 Plasma Enzyme Levels As Indicators Of The Nature Or Severity Of Heat Stress

Three different levels of heat stress were devised in this study (Section 2.6). Since the degree of serum enzyme elevation is a function of tissue damage (Kew *et al*, 1971) it follows that the greater the degree of stress, the greater is the degree of tissue damage, reflected as a greater increase in serum enzyme levels. On the basis of the magnitude of serum enzyme elevations as a reflection of the severity of stress it is evident in this study that the severity of stress increases, firstly as a result of the rate of rise in body temperature and then in terms of an absolute rise in body temperature. The greatest elevations in plasma enzyme levels occurred in those animals subjected to stress level three (Figure 3.5 to 3.8 inclusive). Increases in plasma enzyme levels of a comparatively lower degree had occurred in animals exposed to the second level of stress. The first heat stress level was not severe enough to cause any measurable tissue damage.

The most sensitive enzyme during the third level of stress was CK, followed closely by LD and then AST. There was a slight elevation in ALT levels. This pattern of enzyme elevation (CK > LD > AST >> ALT) is indicative of generalized tissue damage, with the possibility that cardiac damage was considerable (on the basis of a high plasma CK and a De Ritis ratio of 0,32).

CK was still the most sensitive enzyme during the second level of stress but it was followed in this instance by AST, ALT and LD. This pattern of enzyme elevation (CK > AST > ALT > LD) is indicative of a generalized tissue damage, supported by a De Ritis ratio of 0,65.

The findings, on the basis of plasma enzyme elevations, namely, that the third level of stress is the most severe, with the second level of stress being of intermediate severity and the first level of stress being least severe, are supported only in part by changes in mean body temperature (Figure 3.2 and Table 3.1). It is evident from the results that in attaining the equivalent quantity of heat storage above base-line (20%) different combinations of exposure time and mean body temperature (T_m) were obtained. Reference to Figure 3.2 indicates that:

a) for the third level of stress, a short exposure induced a relatively high final T_m;

b) for the second level of stress, a long exposure induced a relatively high final T_m; and

c) for the first level of stress, an intermediate exposure induced a relatively low final T_m.

It is evident that a similar T_m has been attained in the second and third levels of stress, albeit using different exposure times. If one then considers the respective plasma enzyme changes, it is clear that the third level of stress is the more severe and that the severity is related primarily to a greater rate of change in T_m (Table 3.1) rather than the absolute level in T_m. Thus the severity of stress, as has been well established, is related to the absolute body temperature elevation in the first instance, and, secondly, exposure time. The results of this study suggest that a third determinant may be added, namely rate of change in body temperature.

4.4 Conclusions

The following conclusions are drawn on the basis of the experimental findings of this study and the ensuing discussion.

1. The tissue damage which occurred during this study was of a mild to moderate nature, widespread and completely reversible. The animals in this study had not, therefore, suffered heatstroke and the experimental conditions are therefore representative of sub-acute stress. In this respect, they also represent the early or prodromal stage of heatstroke.

2. It was concluded that enzyme release patterns following heat stress may change in accordance with the nature of stress imposed. Indirect support for this view exists when considering patterns associated with overt experimental heat stroke.

3. The severity of stress is related to the absolute body temperature elevation and exposure time. When the final absolute body temperatures are similar, rate of change of body temperature becomes the deciding factor.

4. The implication of a direct relationship between the level of stress experienced and the rate of change in mean body temperature suggests that permissible upper limit body temperatures (e.g. WBGT limit of 38^{o}C) (Dukes-Dobos and Henschel, 1973) be reduced to cater for conditions likely to induce rapid elevations in body temperature. Further research is, however, necessary.

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A P P E N D I X

Table 1.1: Responses of animals subjected to Stress I. Blood sampled at termination of stress

Rat No. & Mass	1 (292)					2 (289)					3 (249)					4 (252)					5 (300)				
	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR
t_0	36,9	25,4	34,6	8,39	375	37,6	26,2	35,3	8,47	390	37,8	22,6	34,8	7,18	357	36,4	23,5	33,8	7,07	333	36,7	23,5	34,1	8,48	364
t_{10}	37,3	38,2	37,5	9,08	345	38,1	37,7	38,0	9,12	380	38,2	36,8	37,9	7,83	373	36,6	36,4	36,6	7,65	343	37,2	38,6	37,5	9,33	333
t_{20}	38,2	40,7	38,7	9,37	318	39,1	40,8	39,4	9,46	380	39,2	40,4	39,4	8,15	387	37,8	39,0	38,0	7,95	368	38,4	40,8	38,9	9,68	337
t_{30}	39,2	40,6	39,5	9,56	347	40,5	41,0	40,6	9,73	390	40,6	40,6	40,6	8,39	405	39,0	40,3	39,3	8,21	405	40,0	40,6	40,1	9,99	370
t_{40}	40,4	41,2	40,6	9,83	364	41,4	42,0	41,5	9,95	408	41,5	41,5	41,5	8,57	411	40,5	41,3	40,7	8,50	429	40,9	41,3	41,0	10,20	395
t_{50}	41,2	41,8	41,3	10,01	375	42,0	42,2	42,0	10,08	414	42,0	42,2	42,0	8,68	426	41,6	41,7	41,6	8,70	458	42,0	42,2	42,0	10,47	438
t_{60}	42,0	42,4	42,1	10,20	405						42,0	41,8	42,0	8,77	476										
Time to 42,0°C (minutes)	59					46					44					55					50				

t_0; t_{10} : commencement of stress; 10 minutes later

T + 10; T + 20: 10 and 20 minutes after removal of stress

Tc : core temperature, °C

Ts : skin temperature, °C

Tm : mean body temperature, °C

S : heat stored, Calories

HR : heart rate, beats per minute

Table 1.2: Responses of animals subjected to Stress I. Blood sampled 6 hours post - stress

Rat No & Mass	1 (307)					2 (311)					3 (280)					4 (277)					5 (249)				
	Tc	Ts	Tm	S	HR	Tc	Tc	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR
t_0	37,8	28,3	35,9	9,15	335	37,6	28,0	35,7	9,21	408	37,7	28,0	35,8	8,31	405	38,2	27,0	36,0	8,27	455	36,6	28,0	34,9	7,21	330
t_{10}	38,2	38,0	38,2	9,72	339	38,2	37,8	38,1	9,83	377	38,1	37,2	37,9	8,81	357	39,0	37,6	38,7	8,90	469	37,4	38,3	37,6	7,77	357
t_{20}	39,0	38,9	39,0	9,93	349	39,2	39,7	39,3	10,14	387	39,2	39,8	39,3	9,14	366	40,4	40,4	40,4	9,28	484	38,8	40,8	39,2	8,10	395
t_{30}	41,2	41,3	41,2	10,50	361	40,4	41,0	40,5	10,55	405	40,6	40,9	40,7	9,44	387	41,4	41,2	41,4	9,50	509	40,4	41,2	40,6	8,38	405
t_{40}	41,8	41,6	41,8	10,64	380	41,6	41,9	41,7	10,75	417	41,6	41,5	41,6	9,66	405	42,0	41,8	42,0	9,64	522	41,2	41,2	41,2	8,51	435
t_{50}	42,0	42,2	42,0	10,71	392	42,0	42,1	42,0	10,85	441	42,0	41,8	42,0	9,75	414						42,0	42,1	42,0	8,68	462
T+10	40,2	35,4	39,2	9,98	385	41,0	36,0	40,0	10,32	448	40,8	34,9	39,6	9,21	405	40,8	34,8	39,6	9,10	504	40,6	34,0	39,3	8,12	435
T+20	39,5	34,5	38,5	9,81	375	40,0	35,0	39,0	10,06	426	39,6	33,8	38,4	8,93	414	39,7	33,6	38,5	8,84	488	39,5	32,0	38,0	7,85	395
T+30	39,0	34,0	38,0	9,68	335	39,0	34,2	38,0	9,81	426	38,6	32,6	37,4	8,69	405	38,6	32,6	37,4	8,60	472	38,6	29,8	36,8	7,61	341
T+40	38,4	27,4	36,2	9,22	323	38,6	32,3	37,3	9,64	397	38,4	29,0	36,5	8,48	400	38,2	29,6	36,5	8,38	448					
T+50						38,2	29,2	36,4	9,39	375						37,6	28,0	35,7	8,20	435					
Time to 42°C (min	43					44					45					35					48				

t_0; t_{10} : commencement of stress; 10 minutes later

T+10; T+20 : 10 and 20 minutes after removal of stress

Tc : core temperature, °C

Ts : skin temperature, °C

Tm : mean body temperature, °C

S : heat stored; Calories

HR : heart rate, beats per minute

Table 1.3: Responses of animals subjected to Stress I. Blood sampled at 24 hours post - stress

Rat No. & Mass	1 (259)					2 (263)					3 (271)					4 (224)					5 (263)				
	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR
t_o	37,2	27,2	35,2	7,56	361	37,6	28,2	35,7	7,79	375	37,6	26,6	35,4	7,96	382	36,8	24,8	34,4	6,40	370	36,6	25,6	34,4	7,50	324
t_{10}	38,1	36,8	37,8	8,13	355	38,0	36,2	37,6	8,21	375	38,4	37,4	38,2	8,59	382	37,2	35,4	36,8	6,84	355	37,4	38,0	37,5	8,19	314
t_{20}	39,3	40,3	39,5	8,49	368	39,1	37,4	38,8	8,46	429	39,6	40,0	39,7	8,92	411	38,2	39,6	38,5	7,15	351	38,4	39,8	38,3	8,36	345
t_{30}	40,8	41,3	40,9	8,79	390	40,6	39,8	40,4	8,83	435	40,8	40,8	40,8	9,17	417	39,4	41,0	39,7	7,38	357	39,4	40,5	39,6	8,65	375
t_{40}	41,8	41,8	41,8	8,99	411	41,7	41,4	41,6	9,09	462	41,7	41,8	41,7	9,38	429	40,6	41,5	40,8	7,58	382	40,8	41,4	40,9	8,93	417
t_{50}	42,0	42,0	42,0	9,02	420	42,0	41,8	42,0	9,15	492	42,0	42,2	42,0	9,45	438	41,8	41,8	41,8	7,77	432	42,0	42,3	42,1	9,18	448
t_{60}																42,0	41,8	42,0	7,80	435					
T+10	40,6	34,8	39,4	8,47	414	40,9	34,7	39,7	8,65	441	40,6	33,7	39,2	8,82	441	40,2	33,6	38,9	7,23	444	40,4	34,2	39,2	8,54	395
T+20	39,6	33,8	38,4	8,26	392	39,8	33,8	38,6	8,42	411	39,3	32,3	37,9	8,53	432	39,0	32,8	37,8	7,02	423	39,0	32,7	37,7	8,23	370
T+30	38,6	32,9	37,5	8,05	359	38,9	33,0	37,7	8,23	382	38,3	30,6	36,8	8,26	431	38,2	28,8	36,3	6,75	414	37,8	31,4	36,5	7,97	361
T+40	37,8	32,0	36,6	7,87	359	38,0	32,0	36,8	8,03	368	38,0	28,0	36,0	8,10	444	37,4	26,0	35,1	6,52	408	37,0	29,0	35,4	7,72	339
T+50	37,5	28,4	35,7	7,67	359	37,8	29,4	36,1	7,88	408															
Time to 42°C (min)	42					44					43					53					50				

t_0; t_{10} : commencement of stress; 10 minutes later

T+10; T+20 : 10 and 20 minutes after removal of stress

Tc : core temperature, °C

Ts : skin temperature, °C

Tm : mean body temperature, °C

S : heat stored; Calories

HR : heart rate, beats per minute

Table 1.4: Responses of animals subjected to Stress II. Blood sampled at termination of stress. (Abbreviations and symbols as for Tables 1.1; 1.2 and 1.3)

Rat No. & Mass	1 (234)					2 (222)					3 (239)					4 (222)					5 (223)				
	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR
t_0	37,6	27,6	35,6	6,91	337	36,9	28,4	35,2	6,49	351	36,6	28,6	35,0	6,94	328	37,8	27,7	35,8	6,59	353	38,3	30,0	36,6	7,09	387
t_{10}	38,5	38,8	38,6	7,49	349	37,3	37,0	37,2	6,86	349	38,1	39,0	38,3	7,59	328	38,3	38,9	38,4	7,07	366	38,7	38,0	38,6	7,45	375
t_{20}	39,6	40,6	39,8	7,73	370	38,1	39,6	38,4	7,08	351	39,0	40,1	39,2	7,78	353	39,4	39,9	39,5	7,27	385	39,5	40,4	39,7	7,67	411
t_{30}	40,5	41,5	40,7	7,90	387	39,0	40,7	39,3	7,25	380	40,1	41,2	40,3	7,80	366	40,4	41,2	40,6	7,47	392	40,6	41,5	40,8	7,88	458
t_{40}	41,4	41,9	41,5	8,06	414	40,1	41,1	40,3	7,43	420	41,0	41,7	41,1	8,16	385	41,5	41,9	41,6	7,66	432	41,6	41,8	41,6	8,05	484
t_{50}	42,2	42,2	42,2	8,19	455	40,9	41,8	41,1	7,57	429	41,8	42,2	41,9	8,31	411	42,3	42,3	42,3	7,79	545	42,4	42,3	42,4	8,20	536
t_{60}	42,5	42,4	42,5	8,25	492	41,8	42,3	41,9	7,72	455	42,5	42,5	42,5	8,43	476	42,5	42,5	42,5	7,83	561	42,5	42,4	42,5	8,21	541
t_{70}						42,5	42,7	42,5	7,83	492															
Time to Tc = 42,5° C (minutes)	54					69					58					52					52				

Table 1.5: Responses of animals subjected to Stress II. Blood sampled at 6 hours post - stress. (Abbreviations and symbols as for Tables 1.1; 1.2 and 1.3)

Rat No. & Mass	1 (230)					2 (265)					3 (242)					4 (230)					5 (239)				
	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR
t_0	37,4	27,2	35,4	6,75	316	37,4	27,2	35,4	7,78	303	37,8	29,4	36,1	7,25	425	37,9	26,4	35,6	6,79	444	38,4	24,4	35,6	7,06	400
t_{10}	38,4	37,3	38,2	7,29	347	38,0	36,8	37,8	8,30	304	38,7	39,3	38,8	7,80	438	38,5	36,8	38,2	7,28	451	38,7	35,2	38,0	7,53	403
t_{20}	39,7	38,9	39,5	7,54	392	39,1	38,1	38,9	8,56	337	40,1	40,9	40,3	8,09	434	39,4	39,7	39,5	7,53	454	39,7	38,3	39,4	7,81	404
t_{30}	40,7	39,8	40,5	7,73	423	40,0	39,4	39,9	8,77	364	41,2	41,5	41,3	8,28	428	41,1	41,5	41,2	7,86	469	40,3	39,8	40,2	7,97	392
t_{40}	41,7	41,3	41,6	7,94	438	41,0	41,0	41,0	9,02	395	42,0	42,0	42,0	8,43	526	41,7	42,0	41,8	7,98	472	41,3	41,3	41,3	8,19	410
t_{50}	42,4	41,7	42,3	8,06	504	42,0	42,0	42,0	9,23	429	42,5	42,2	42,4	8,52	571	42,4	41,8	42,3	8,07	469	41,8	41,5	41,7	8,28	433
t_{60}	42,5	41,8	42,4	8,08	508	42,5	42,5	42,5	9,35	465						42,5	42,0	42,4	8,09	465	42,5	42,2	42,4	8,42	484
t_{70}																									
T+10	41,4	36,3	40,4	7,70	496	41,7	35,4	40,4	8,89	465	41,8	35,2	40,5	8,13	571	41,5	33,6	39,9	7,62	508	41,6	34,6	40,2	7,97	465
T+20	40,2	35,0	39,2	7,47	458	40,6	33,8	39,2	8,63	423	40,7	33,8	39,3	7,90	531	40,0	32,0	38,4	7,33	483	39,2	33,2	38,0	7,53	405
T+30	39,0	33,7	37,9	7,24	406	39,5	33,0	38,2	8,40	392	39,8	32,8	38,4	7,71	496	38,7	29,0	36,8	7,01	454	38,2	29,4	36,4	7,22	423
T+40	38,3	32,8	37,2	7,10	390	38,8	31,8	37,4	8,22	373	39,4	31,2	37,8	7,58	484	38,2	26,6	35,9	6,84	422					
T+50	38,0	29,6	36,3	6,93	375	38,5	28,8	36,6	8,04	357	39,0	28,5	36,9	7,41	476	37,7	25,2	35,2	6,72	417					
T+60	37,5	27,7	35,6	6,78	355	38,0	29,4	36,3	7,98	313	38,6	27,3	36,3	7,29	451	37,4	24,4	34,8	6,64	447					
T+70	37,2	26,8	35,1	6,70	345	37,7	28,8	35,9	7,90	324	38,2	26,8	35,9	7,21	303	37,4	24,2	34,8	6,68	461					
Time to Tc = 42,5° C (minutes)	52					56					46					52					56				

Table 1.6: Responses of animals subjected to Stress II. Blood sampled at 24 hours post - stress. (Abbreviations and symbols as for Tables 1.1; 1.2 and 1.3)

Rat No. & Mass	1 (260)					2 (258)					3 (242)					4 (260)					5 (233)				
	Tc	Ts	Tm	S	HR	Tc	Ts	Tc	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR
t_0	37,5	24,4	34,9	7,52	451	37,0	27,4	35,1	7,51	392	37,4	23,5	34,6	6,95	382	36,8	25,4	34,5	7,44	347	37,3	23,5	34,5	6,68	380
t_{10}	37,8	36,6	37,6	8,10	411	37,1	37,7	37,2	7,97	405	38,0	38,1	38,0	7,64	375	37,0	35,6	36,7	7,92	347	37,5	35,4	37,1	7,17	380
t_{20}	38,7	39,7	38,9	8,39	390	38,1	40,2	38,5	8,25	387	39,1	40,7	39,4	7,91	380	38,0	39,6	38,3	8,26	375	38,4	38,6	38,4	7,43	405
t_{30}	39,6	40,3	39,7	8,58	392	39,7	41,4	40,0	8,57	403	40,4	41,4	40,6	8,15	403	39,2	40,8	39,5	8,52	405	40,1	40,3	40,1	7,76	411
t_{40}	40,5	40,9	40,6	8,75	395	40,7	41,6	40,9	8,75	417	41,3	41,6	41,4	8,31	411	40,6	41,2	40,7	8,78	435	40,8	40,9	40,8	7,89	435
t_{50}	41,4	41,7	41,5	8,94	405	41,4	41,7	41,5	8,87	429	42,0	42,3	42,1	8,44	438	41,6	41,9	41,7	8,99	432	41,8	42,0	41,8	8,09	480
t_{60}	41,9	42,0	41,9	9,04	480	42,1	42,5	42,2	9,03	472	42,5	43,0	42,6	8,56	522	42,5	42,2	42,4	9,16	480	42,5	42,3	42,5	8,21	536
t_{70}	42,5	42,5	42,5	9,17	556	42,5	42,3	42,6	9,13	545															
T+10	41,3	33,4	39,7	8,57	522	40,8	32,0	39,1	8,36	545	40,9	34,2	39,6	7,94	488	41,2	34,6	39,9	8,60	448	41,5	35,4	40,3	7,79	522
T+20	39,6	32,1	38,1	8,22	480	39,3	30,0	37,4	8,01	500	39,6	33,0	38,3	7,69	454	39,4	33,2	38,2	8,23	411	39,8	34,1	38,7	7,47	476
T+30	38,8	29,2	36,9	7,96	476	38,1	27,5	36,0	7,70	417	38,3	32,0	37,0	7,44	429	38,2	32,0	37,0	7,98	400	38,5	33,2	37,4	7,24	435
T+40	38,3	25,6	35,8	7,71	458	37,6	25,0	35,1	7,51	351	37,8	29,0	36,0	7,24	411	37,6	27,6	35,6	7,68	380	38,0	30,2	36,4	7,04	455
T+50	37,6	24,7	35,0	7,55	429	37,0	23,5	34,3	7,34	339	37,3	26,0	35,0	7,03	405	37,2	25,4	34,8	7,51	357	37,4	26,0	35,0	6,79	435
T+60																					37,0	24,4	34,5	6,67	390
Time to Tc = 42,5° C (minutes)	69					64					55					60					58				

Table 1.7: Responses of animals subjected to Stress III. Blood sampled at termination of stress. (Abbreviations and symbols as for Tables 1.1; 1.2 and 1.3)

Rat No. & Mass	1 (277)					2 (260)					3 (246)					4 (284)					5 (284)				
	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR
t_0	36,8	30,0	35,4	8,15	387	36,4	27,6	34,6	7,47	375	36,0	28,2	34,4	7,03	385	37,0	28,0	35,2	8,30	480	37,0	27,6	35,1	8,28	444
t_{10}	38,0	43,0	39,0	8,97	429	37,2	36,4	37,0	7,99	444	37,0	37,5	37,0	7,58	405	38,5	39,8	38,8	9,14	509	37,4	35,8	37,1	8,74	438
t_{20}	41,6	44,2	42,1	9,68	500	39,2	40,4	39,4	8,51	444	38,2	39,0	38,4	7,83	423	41,0	41,8	41,2	9,70	509	38,5	39,5	38,7	9,12	472
t_{30}	42,2	44,6	42,7	9,81	508	41,2	41,6	41,3	8,91	429	40,6	40,8	40,6	8,30	441	42,3	42,3	42,3	9,97	510	41,0	41,8	41,2	9,70	508
t_{40}						41,8	42,0	41,8	9,02	444	41,3	41,3	41,3	8,44	462						42,1	42,4	42,2	9,94	526
t_{50}																									
Time to termination of stress (minutes)	29					34					36					30					40				

Table 1.7: (Continued)

Rat No. & Mass	6 (263)					7 (236)					8 (241)					9 (214)					10 (221)				
	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR
t_0	37,2	27,2	35,2	7,68	359	37,4	28,0	35,5	6,96	324	37,2	29,0	35,6	7,11	385	37,8	29,0	36,0	6,40	422	36,8	28,8	35,2	6,46	364
t_{10}	38,3	38,4	38,3	8,36	405	38,5	38,7	38,5	7,55	395	38,5	38,8	38,6	7,71	387	39,0	39,2	39,0	6,93	414	38,0	38,5	38,1	6,99	375
t_{20}	39,8	40,2	39,9	8,71	414	40,2	40,6	40,3	7,89	435	39,8	40,5	39,4	7,99	405	41,0	41,6	41,1	7,30	435	39,3	39,8	39,4	7,23	408
t_{30}	41,2	41,4	41,2	9,00	414	41,6	41,6	41,6	8,15	435	41,5	41,6	41,5	8,31	435	42,4	42,6	42,4	7,54	476	40,8	41,0	40,8	7,49	435
t_{40}	42,3	41,8	42,2	9,21	455	42,6	42,2	42,5	8,34	480	42,7	42,4	42,6	8,53	500	43,2	43,3	43,2	7,68	541	42,0	41,8	42,0	7,70	444
t_{50}						42,7	42,3	42,6	8,35	488											42,4	42,1	42,3	7,76	472
Time to termination of stress (minutes)	40					41					40					36					43				

Table 1.8: Responses of animals subjected to Stress III. Blood sampled at 6 hours post - stress. (Abbreviations and symbols as for Tables 1.1; 1.2 and 1.3)

Rat No. & Mass	1 (263)					2 (250)					3 (235)					4 (260)					5 (300)				
	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR
t_0	37,8	29,0	36,0	7,06	420	37,0	28,0	35,2	7,30	400	37,0	28,6	35,3	6,89	349	38,0	29,0	36,2	7,81	400	37,8	28,4	35,9	8,94	375
t_{10}	38,8	39,4	38,9	7,62	435	37,8	37,4	37,7	7,83	395	37,6	36,4	37,4	7,29	349	39,0	38,8	39,0	8,41	411	38,6	37,6	38,4	9,56	380
t_{20}	40,3	41,0	40,4	7,92	476	39,2	38,8	39,1	8,13	408	38,4	38,0	38,3	7,47	332	41,0	41,0	41,0	8,85	420	40,2	39,6	40,1	9,98	403
t_{30}	41,8	42,1	41,9	8,20	545	40,5	40,2	40,4	8,39	414	39,3	39,4	39,3	7,67	349	42,0	42,2	42,0	9,07	448	41,2	41,4	41,2	10,27	438
t_{40}	43,0	43,0	43,0	8,42	600	41,8	41,4	41,7	8,66	420	40,6	40,8	40,6	7,93	370	43,3	43,2	43,3	9,34	531	42,4	42,4	42,4	10,56	472
t_{50}	43,2	43,3	43,2	8,47	612	42,4	42,0	42,3	8,78	500	41,7	42,3	41,8	8,16	387	43,5	43,3	43,5	9,38	556	43,4	43,2	43,4	10,79	536
t_{60}											42,3	42,6	42,4	8,26	405										
T+10	42,1	35,4	40,8	7,98	588	40,8	34,6	39,6	8,21	489	40,6	35,4	39,6	7,72	385	42,4	36,4	41,2	8,89	571	42,4	36,0	41,1	10,24	545
T+20	40,6	34,2	39,3	7,70	556	39,6	33,8	38,4	7,98	469	39,6	34,0	38,5	7,51	368	41,0	35,0	39,8	8,59	526	40,8	35,0	39,6	9,87	527
T+30	39,0	31,0	37,4	7,33	469	38,2	33,0	37,2	7,71	414	38,0	32,4	36,9	7,19	387	39,4	34,0	38,3	8,27	476	39,4	33,0	38,1	9,49	472
T+40	38,0	28,0	36,0	7,05	395	38,0	30,0	36,4	7,55	435						38,8	30,8	37,2	8,03	368	39,0	31,6	37,5	9,34	448
T+50						37,6	27,6	35,6	7,39	400						38,4	29,2	36,6	7,89	324	38,4	28,8	36,5	9,08	400
T+60																									
Duration of stress (minutes)	44					46					55					43					50				

Table 1.8: (Continued)

Rat No. & Mass	6 (264)					7 (244)					8 (250)					9 (225)					10 (289)				
	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR
t_0	37,5	25,4	35,1	7,69	400	37,3	25,5	34,9	7,08	377	36,4	25,2	34,2	7,09	400	36,6	24,0	34,1	6,36	387	36,6	25,0	34,3	8,22	359
t_{10}	37,6	38,8	37,8	8,29	411	37,8	36,8	37,6	7,61	405	37,0	36,2	36,8	7,64	414	36,8	34,0	36,2	6,77	429	37,0	36,4	36,9	8,85	405
t_{20}	40,8	40,6	40,8	8,93	451	39,5	40,0	39,6	8,02	480	38,2	38,5	38,3	7,94	403	37,4	37,2	37,4	6,98	423	38,4	39,2	38,6	9,25	429
t_{30}	42,0	41,4	41,9	9,18	472	41,2	41,0	41,0	8,33	508	39,2	39,4	39,2	8,14	435	39,0	39,2	39,0	7,29	458	40,0	40,2	40,0	9,60	444
t_{40}	42,4	41,8	42,3	9,26	541	42,4	41,6	42,2	8,55	517	40,8	40,6	40,8	8,46	441	40,9	40,7	40,9	7,63	462	41,2	41,0	41,2	9,87	462
t_{50}											41,1	40,8	41,0	8,52	448										
t_{60}																									
T+10	41,5	35,3	40,3	8,82	513	41,0	34,0	39,6	8,02	487	39,4	33,0	38,1	7,91	423	39,4	32,0	37,9	7,08	444	40,0	33,6	38,7	9,29	448
T+20	39,6	33,8	38,4	8,42	448	39,0	32,2	37,6	7,63	411	38,2	32,2	37,0	7,68	411	38,2	29,6	36,5	6,81	429	38,6	32,6	37,4	8,97	403
T+30	38,2	31,7	36,9	8,09	431	38,0	31,0	36,6	7,41	382	37,3	30,0	35,8	7,44	344	37,6	26,8	35,4	6,62	411	37,2	30,0	35,8	8,58	387
T+40	37,8	28,8	36,0	7,89	413	37,2	28,6	35,5	7,19	375	36,6	27,6	34,8	7,22	375	37,0	24,8	34,6	6,45	400					
T+50	37,4	27,0	35,3	7,74	400																				
T+60																									
Duration of stress (minutes)	36					37					42					40					40				

Table 1.9: Responses of animals subjected to Stress III. Blood sampled at 24 hours post - stress. (Abbreviations and symbols as for Tables 1.1; 1.2 and 1.3)

Rat No. & Mass	1 (294)					2 (268)					3 (263)					4 (281)					5 (248)				
	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR
t_0	36,8	28,8	35,2	8,59	286	36,8	29,5	35,3	7,86	332	37,0	30,0	35,6	7,77	395	37,0	29,6	35,5	8,28	364	37,4	25,6	35,0	7,21	333
t_{10}	38,1	39,2	38,3	9,35	343	37,6	38,8	37,8	8,42	357	37,6	38,8	37,8	8,26	403	38,0	38,8	38,2	8,90	400	38,4	39,3	38,6	7,94	370
t_{20}	39,3	39,7	39,4	9,61	366	38,8	40,0	39,0	8,86	364	38,8	40,0	39,0	8,52	438	39,2	40,4	39,4	9,20	417	40,4	40,0	40,3	8,30	385
t_{30}	41,0	41,2	41,0	10,01	377	40,8	41,5	40,9	9,11	397	40,8	41,6	41,0	8,94	451	41,0	41,6	41,1	9,59	429	42,0	42,0	42,0	8,64	451
t_{40}	41,6	42,2	41,7	10,18	395	42,0	42,4	42,0	9,36	438	41,8	42,2	41,9	9,14	451	42,0	42,2	42,0	9,80	462	42,2	42,1	42,2	8,68	451
t_{50}	42,2	42,6	42,3	10,32	414	42,3	42,5	42,3	9,42	444	42,7	42,7	42,7	9,32	488	42,6	42,6	42,6	9,94	536					
T+10	41,8	36,2	40,7	9,93	403	41,6	35,4	40,4	8,98	429	41,6	36,2	40,5	8,85	472	41,0	34,5	39,7	9,26	500	40,8	33,7	39,4	8,11	420
T+20	40,4	35,2	39,4	9,60	390	40,0	34,6	38,9	8,66	392	39,8	35,2	38,9	8,49	448	39,2	33,4	38,0	8,87	455	39,0	32,6	37,7	7,76	380
T+30	39,5	34,0	38,2	9,31	400	39,5	33,0	38,2	8,50	361	38,8	34,2	37,9	8,27	435	38,6	32,4	37,4	8,71	438	37,8	31,8	36,6	7,53	326
T+40	38,8	33,4	37,7	9,20	385	38,8	31,8	37,4	8,32	375	38,2	32,4	37,0	8,09	411	38,2	31,4	36,8	8,59	400	37,4	29,4	35,8	7,37	308
T+50	38,5	31,6	37,1	9,06	375	38,5	30,0	36,8	8,17	351	38,0	31,4	36,7	8,01	403	37,8	29,3	36,1	8,42	390					
T+60	38,0	30,0	36,4	8,88	349	38,0	29,6	36,3	8,08	328	37,8	30,0	36,2	7,91	385	37,6	28,7	35,8	8,35	364					
Duration of stress (minutes)	40					44					50					45					32				

Table 1.9: (Continued)

Rat No. & Mass	6 (221)					7 (278)					8 (241)					9 (234)					10 (226)				
	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR	Tc	Ts	Tm	S	HR
t_0	37,2	27,4	35,2	6,46	366	37,6	26,4	35,4	8,16	390	36,4	25,8	34,3	6,86	348	36,8	27,2	34,9	6,77	293	37,2	27,2	35,2	6,60	414
t_{10}	38,2	37,2	38,0	6,97	390	38,8	39,6	39,0	8,99	392	37,7	39,2	38,0	7,60	319	38,2	39,0	38,4	7,45	328	38,6	38,8	38,6	7,25	422
t_{20}	39,4	39,2	39,4	7,22	423	41,0	40,8	41,0	9,45	423	40,4	41,2	40,6	8,11	387	40,2	40,4	40,2	7,82	382	40,8	41,0	40,8	7,66	462
t_{30}	41,2	40,4	41,0	7,53	462	42,0	40,6	41,7	9,63	465	41,6	41,4	41,6	8,31	451	41,9	41,9	41,9	8,14	438	42,2	40,8	41,9	7,86	500
t_{40}	42,3	41,0	42,0	7,71	488	42,7	41,5	42,5	9,80	519											42,5	41,6	42,3	7,93	513
t_{50}	42,6	41,0	42,3	7,76	508																				
T+10	41,4	34,0	39,9	7,32	492	41,2	33,5	39,7	9,15	496	39,8	32,8	38,4	7,68	377	40,2	34,2	39,0	7,57	387	41,0	34,0	39,6	7,43	500
T+20	39,8	33,4	38,5	7,07	423	39,8	32,8	38,4	8,86	444	38,8	32,0	37,4	7,49	363	39,0	33,4	37,9	7,36	366	39,6	33,0	38,3	7,18	429
T+30	38,6	32,0	37,3	6,84	395	38,6	32,0	37,3	8,60	417	37,6	30,0	36,1	7,22	333	37,6	31,6	36,4	7,07	316	38,6	32,0	37,3	6,99	414
T+40	37,8	29,8	36,2	6,64	390	38,2	29,8	36,5	8,43	414											38,0	28,6	36,1	6,78	420
T+50																									
T+60																									
Duration of stress (minutes)	42					36					28					30					33				

Table 1.10: Plasma enzyme levels (U/ℓ) of animals subjected to Stress I

Number of animals	Response at termination										Response at 6 hours post-stress										Response at 24 hours post-stress									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
CK	95	99	66	351	98	33	74	58	62	45	66	70	62	169	58	54	120	54	144	87	70	70	83	41	70	54	66	54	50	62
LD	98	84	84	285	100	128	123	103	197	103	118	261	118	158	69	69	157	84	192	84	74	79	89	84	74	59	197	59	59	79
AST	76	66	84	78	76	43	59	48	63	34	82	69	62	112	86	33	94	37	131	42	62	75	67	59	71	41	49	46	49	32
ALT	54	39	51	52	86	51	57	39	50	32	57	60	36	38	69	33	37	52	60	41	59	72	82	65	53	36	39	29	40	42

Table 1.11: Plasma enzyme levels (U/ℓ) of animals subjected to Stress II

Number of animals	Response at termination										Response at 6 hours post-stress										Response at 24 hours post-stress									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
CK	25	25	83	33	37	50	33	33	99	74	425	338	177	165	277	367	495	338	290	379	450	438	78	227	66	62	50	33	45	41
LD	75	100	140	60	55	108	79	98	74	148	210	225	185	155	320	157	295	221	108	236	195	200	215	205	85	103	103	64	138	138
AST	72	82	78	73	69	52	42	39	40	82	177	170	300	212	243	94	158	44	45	167	101	112	146	160	94	32	42	46	42	74
ALT	73	73	61	62	61	36	34	46	51	106	114	82	165	217	125	38	50	45	30	185	88	91	139	87	94	24	27	23	46	39

Table 1.12: Plasma enzyme levels (U/ℓ) of animals subjected to Stress III

Number of animals	Response at termination										Response at 6 hours post-stress										Response at 24 hours post-stress									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
CK	83	83	99	66	227	58	330	74	50	83	867	1184	344	1552	1703	468	322	385	406	718	83	95	78	87	50	62	54	83	66	70
LD	143	113	42	113	123	107	174	95	110	226	1445	837	410	1778	1155	738	796	500	207	430	118	148	98	118	113	103	93	133	59	79
AST	60	53	42	48	56	73	72	60	57	73	1100	163	97	433	376	231	80	70	87	263	105	80	130	130	53	47	92	51	43	40
ALT	48	48	31	38	30	76	60	46	46	41	157	107	87	128	93	80	56	67	56	98	62	56	44	52	31	60	41	53	42	47

Table 1.13: Plasma enzyme levels (U/ℓ) (mean values) of animals at all levels of stress and all sampling (response) times

Response Times (hours)

Stress Levels		0		6		24
0	CK	58	CK	58	CK	58
	LD	172	LD	172	LD	172
	AST	75	AST	75	AST	75
	ALT	61	ALT	61	ALT	61
I	CK	98	CK	88	CK	62
	LD	131	LD	131	LD	85
	AST	63	AST	75	AST	55
	ALT	51	ALT	48	ALT	52
II	CK	49	CK	325	CK	149
	LD	94	LD	211	LD	145
	AST	63	AST	161	AST	85
	ALT	60	ALT	105	ALT	66
III	CK	115	CK	795	CK	73
	LD	125	LD	830	LD	106
	AST	59	AST	290	AST	77
	ALT	46	ALT	93	ALT	49

Table 1.14: Plasma enzyme levels (U/ℓ) of control group of animals

	Number of Animals									
Enzyme	1	2	3	4	5	6	7	8	9	10
CK	66	50	66	54	66	62	45	62	66	42
LD	150	145	155	105	205	150	285	271	148	108
AST	57	61	56	52	89	75	88	81	92	96
ALT	54	40	39	62	67	67	64	71	72	78