THE ANALYSIS OF ISOTOPE CLEARANCE DATA IN BIOLOGICAL SYSTEMS

by

H. I. GLASS, B.A.

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CONTENTS

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Abstract: Clearance curves resulting from biological studies using radioactive isotopes are frequently described mathematically in terms of the summation of a number of exponential terms. This allows the curves to be interpreted by reference to the physical characteristics of a model of the biological system. Numerous exponential curve fitting methods are now available which make use of digital computers. Despite the very widespread application of exponential curve analysis, a systematic study of the relative importance of the factors which affect the parameter errors has not yet been described.

A quantitative statistical study of the problem is described in this thesis with particular reference to the special limitations encountered in biological investigations. These limitations are firstly, the limited number of samples and, secondly, the relatively poor accuracy normally associated with such studies. The accuracy would not normally be better than ± 2% nor would the number of samples exceed sixty. Of the ten principal factors which affect the errors in the estimated parameters, two of these, the exponent and amplitude ratios, are intrinsic factors dependent on the system under study. The principal factors under the control of the investigator are the number of samples, the data accuracy, the sampling frequency, and the duration of sampling, which determines the extent to which the data define the function under study. Other factors of lesser importance were not investigated in the same detail as those mentioned above.

Artificial data, on which a controlled random error was superimposed, were generated by a computer programme and recorded on magnetic tape in a format suitable for exponential analysis by the Berman SAAM-22 computer programme. The parameter errors were estimated by a statistical analysis of twenty curve fitting operations carried out on twenty different sets of data with a constant controlled random error. Twice the coefficient of variation, expressed as a percentage, was taken to be the parameter error. A range of exponential and amplitude ratios was investigated for two exponential and three exponential functions with data errors from 2 - 10%.

The study has indicated, in quantitative terms, the effects of the various factors on the errors associated with the estimated parameters, and also the relative importance of these factors. The results indicate the conditions which must be fulfilled if reliable results are to be obtained by exponential analysis. The information is also of value in designing investigations which will subsequently involve exponential analysis of the data. In view of the parameter errors encountered in the study of two and three exponential functions, it appears unlikely that analysis of biological data in terms of a greater number of exponentials will be helpful unless further independent information is available concerning the biological system under study.

Two clinical applications of exponential curve fitting procedures are described. In a study of uric acid metabolism, two different computer programmes were used to examine the same data. A mathematical significance test was used to indicate which sets of data were better fitted by a double rather than a single exponential function. It was found that with one of

these programmes only, when using a particular weighting factor on the data, a strong correlation exists between the indication of a double exponential function in the data and the clinical diagnosis of gout. This is interpreted as evidence of the existence of uric acid in two different physiological forms in gouty patients.

In the second study, the detailed investigation of a depth focusing radioisotope collimator, and its use in the measurement of local cortical blood flow in the brain, is described. By using this collimator, clearance curves of radioactivity from a very small volume of brain tissue in the cortex were obtained. The curves were analysed empirically by means of a double exponential curve fitting procedure, in order to determine the initial slope. No biological significance is assigned to the individual exponential terms. Since the collimator is designed to accept radiation originating specifically in the cortex, the detector is particularly sensitive to changes of flow in this tissue. The results obtained for cortical tissue in normals agree with the values of grey matter flow determined by other workers on much larger regions of the brain containing both grey and white tissues.

PREFACE

Chapter 1 presents a brief historical review of the nature of the problem and examines previous work. The work presented in Chapters 2 to 6 is an original study of the limitations of exponential curve fitting. No such systematic study has been reported previously. The investigation has been made possible by the availability of large capacity, high speed, digital computers which allowed the data generation, data analysis and statistical analysis to be performed entirely by computer. Assistance with the data generation programme was obtained from Mrs. A. C. de Garreta and this is gratefully acknowledged. Two clinical examples of the application of exponential curve fitting are presented. Standard methods have been used for the assay of the stable uric acid and the radioactive uric acid. The assays were performed by Miss V. Holloway and Miss R. N. Arnot whose assistance is also gratefully acknowledged. Although the method of assessing the optimum function which fits the data and also the use of compartmental analysis are not themselves original, there has been no previously reported study combining these procedures in the field of uric acid metabolism such as described in Chapter 7. A brief review of methods of estimating cerebral blood flow is presented in Chapter 8. This is followed by the derivation of a theoretical expression which enables the magnitude of the correction factor which must be applied if a brain collimator giving an enhanced response to cortical tissue is used. A detailed investigation of the characteristics of a unique depth focusing collimator, by assessing its response to an actual brain containing non-uniformly distributed activity is

presented. Both the type of collimator and the detailed investigation procedure have not been previously described. The application of this collimator allows the assessment of cortical blood flow in the human brain; an investigation which has not been possible previously without craniotomy. I am grateful to my clinical colleagues Dr. F. Scott, Mr. J. Sloan Robertson and Dr. A. Murray Harper for their generous collaboration.

I N D E X

CHAPTER 1

Introduction

"... you can fit curves with exponentials all day long ..."
(Quotation from Compartments, Pools and Spaces in Medical Physiology, p.486, 1967, U.S. Atomic Energy Commission, Oak Ridge, Tennessee.)

Summary: The origin of exponential curves frequently encountered in descriptions of biological investigations is traced to the use of the compartmental analysis, or tracer kinetics, method. The experimental curves are assumed to be the solution of a set of equations associated with a model of a particular biological system. By analysis of these curves, which it is further assumed can be represented mathematically by the sum of a number of exponential terms, the physical characteristics of the model may be calculated. Attention is drawn to the numerous exponential curve fitting methods available, and is contrasted with the great paucity of quantitative information concerning the limitations of analysing data in terms of the sum of exponential terms. The purpose of this thesis is to present some basic quantitative information concerning exponential curve fitting when the data have the large errors and small sample size frequently encountered in biological investigations. Two clinical applications are presented. In one of these, two different curve fitting methods are used in a study of uric acid metabolism, and the results obtained with only one of these methods show a strong correlation with the clinical findings. In

the second application, the empirical use of exponential curve fitting to determine the blood flow in the superficial region of the brain using a depth focusing radioisotope collimator is presented.

1.1 Historical Aspects and Statement of the Problem

In the discussion which followed the presentation of an erudite mathematical paper to the Institution of Electrical Engineers in 1959, the adjective 'serendipitous' was introduced. The word serendipity was first used in the English language by Horace Walpole in 1754. He used the word to describe the faculty of making ... "unexpected discoveries by accidents and sagacity, of things they were **not** in quest of ...". The etymology of this word is traced by Prof. L. A. Goodman (1) to a tale of the Three Princes of Serendip reputed to have been written by the Italian author Armenp in the year 1557. Although the work described in this thesis was planned as an attempt to obtain information about a well defined problem, the results, and the application of these results, recalled to mind the rather unusual word of Horace Walpole's.

Fitting exponential functions to data derived from biological systems is a common practice in medical research work. That such procedures are potentially hazardous and subject to severe practical limitations has been the subject of numerous comments at scientific meetings, and in published papers. For example, Bergner (2) describes how he investigated a theoretical seven compartment system in which the rate constants were given some arbitrary values. The corresponding set of differential equations was

solved on a digital computer, and the numerical solution data thus produced were treated as experimental data. Curves were fitted to the data points and he tried to obtain the original system. In no case was this possible. The resulting models had no resemblance to the original seven compartment model. Results presented in the subsequent chapters of this thesis would suggest that is is unlikely that such a possibility ever existed.

Comments on exponential curve fitting, with few exceptions, have one thing in common, namely that they are usually qualitative comments. Berson (3) indicated that by manual analysis of data representative of those found in protein metabolism studies, on which a 1% random error had been introduced in the sum of three exponentials, errors of at least 10% were obtained in some of the rate constants, and occasionally some of the errors were "prohibitive". Despite the widespread use of exponential curve fitting procedures, and despite the publication of the proceedings of three major conferences on the subject of compartmental analysis and dynamic studies (5, 8, 11), there is remarkably little quantitative information available on the relative importance of the various factors which govern exponential curve fitting, and particularly when the data possess large errors and small sample size as frequently encountered in biological investigations. Ackerman (9) points out "... if there are 'n' exponential terms, a best solution can be found for all 2n constants if at least 'n + 2^n points are measured ... since the over-used idea of degrees of freedom loses meaning when applied to sums of exponentials. The various derived parameters in the explicit solution to a model are not "independent", or in mathematical terms, the various exponentials are not mutually orthogonal. Thus a small

error in setting one of these terms may result in large compensating errors in several other parameters". Matthews (4) suggests that the minimum number of plasma samples required for an analysis of a three compartment non-steady state model to simulate protein metabolism is 24, with more frequent sampling during the early part of the study. Even this minimum would require other information to becollected, such as whole body counts.

In many cases the exponential curves are the result of biological investigations carried out using radioactive isotopes. Natural radioisotopes have been used for many years to study biochemical and physiological processes, but when artificial radioisotopes produced in nuclear reactors and cyclotrons became freely available, their use became widespread, particularly in biology and medicine. The principal uses of radioisotopes in medicine has been in the estimation of "miscible pool" sizes, in the study of transport characteristics of certain elements and compounds within the body, and in the study of metabolic pathways, both qualitatively and quantitatively.

A biological system may be represented, or theoretically simulated, by means of a model, consisting of various compartments or pools of different sizes, between which material may transfer at different rates. The quantitative analysis of the transport characteristics and pool sizes is often referred to as compartmental, or kinetic, analysis. The term "compartmental" is a slightly emotive one, since the existence, or connotation of a particular physiological "compartment" is often questionable. Recently, the more general term "tracer kinetics" has become more widely

used. This allows investigations such as cardiovascular blood flow studies to be included under such a heading. In view of the closely related mathematical methods which are often used in these studies, this is an attractive proposal. It is not the purpose of this introduction to discuss the validity of the concepts of tracer kinetics. These concepts and definitions have recently been the subject of a report to the International Commission on Radiological Units and Measurements (12). If the formulation of some suitable model, however idealised, allows one to obtain a better understanding of the way in which a particular biological or physiological system functions, and to predict how the system is likely to react to a particular stimulus or to a change in the physical characteristics in the system, then such a model is probably useful. It is a matter of lesser importance whether or not the individual compartments of the model are anatomically identifiable, or whether each compartment represents an "average" of several smaller compartments, each with slightly different characteristics, as long as the models add to the general understanding of the system under investigation. These remarks also apply although the system may in fact be in a non-steady state, and the compartments are non-uniform.

The assumptions and basic equations of such kinetic systems are presented in several reviews of this topic (13, 14, 15, 16, 17). If a compartmentalised model of a system is assumed, then it is possible to describe the system mathematically by a set of first order linear differential equations. The observed activity versus time curves in one or more of the compartments represent the solution of the set of differential

equations which characterise the system and which are themselves determined by a particular model or set of models. The mathematical solution of the set of first order linear differential equations takes the form of the sum of several exponential terms in which the total number of exponents is equal to the number of compartments in the system (if the system is an open one). The relative sizes of the compartments, and the transfer rates between the compartments determine the amplitudes and exponents of the various exponent terms. For a particular model, the number of exponential terms contained in the data is known. An exponential function containing the specified number of terms may then be fitted to the data by any one of several methods available (see below). The amplitude and exponent values so obtained are used to calculate the constants of the model according to the analytical solution of the model system. It will be appreciated that the number of exponential terms is imposed on the data and a solution is obtained to fit this condition. This method of utilizing the data has been described by Berman (6, 18, 19) and forms the basis of a mathematical formulation, based on which an elaborate computer programme for the simulation and analysis of model systems has been written. This computer programme has been used in the theoretical investigation of exponential curve fitting presented in this thesis. The same data may possibly be fitted by a smaller or larger number of terms. Some method of estimating the goodness of fit of the various possible solutions to the data is obviously desirable. Rossing (20) evaluated nitrogen washout curves from dogs, which were assumed to be multi-exponential. He fitted increasing numbers of exponential terms to the data until no significant improvement in fit was obtained.

A similar approach has been made in the uric acid metabolism study described in Chapter 7.

An alternative approach to the method of imposing successive solutions of increasing number of terms on the data is to try and establish the number of terms contained in the data and then postulate the model to conform with this number of exponential terms. An integral transform method has been proposed in order to determine the number of components in the data analytically (7, 19). The errors in this procedure have been discussed in detail by Callahan and Pizer (22) and they have suggested that the method can only be used in practice to determine the number of terms in the data. The actual magnitude of the exponents and the amplitudes would then have to be found by conventional curve fitting procedures. Since the method requires certain smoothing and extrapolation procedures to be carried out on the original data prior to analysis, there seems to be little advantage in the method in its present form over the alternative procedure of fitting the reasing numbers of exponential terms to the same data and testing for a significant improvement in fit. Callahan and Pizer investigated the effect of data errors, and the resolving power of the method was tested using three triple exponential functions on which 5% and 10% random errors were imposed. The ratio of the amplitude to exponential exponent was kept constant for each term in the summed exponential functions. The paper is significant in that it presents data which relate the number of exponents which can be extracted from the data to the amplitude and exponent ratio, to the data error, and to the number of data points, in a small number of exponential functions. The results will be

discussed in greater detail in Chapter 6.

Myhill (23) has recently presented some preliminary results on the effect of errors and incomplete data on the definition of some mathematical models. However, he examined a model which was analysed in terms of a power function and which simulated the clearance of alkaline earths from the body. 15 and 30 point data with 2% and 5% random error were examined, in which the last point was 15% and 40% of the value of the first point. However, it is not easy to compare these results with those described subsequently in later chapters owing to the fact that an exponential function was not examined. The factors which effect convergence were the principal interest in that study, but the paper does present another limited quantitative study.

Many exponential curve fitting procedures have been described in the literature. The most elementary method is the graphical "peeling off" technique (24). Smith and Morales (25) reported a high degree of accuracy by the use of this method, but this has been questioned by Perl (26). He has described a modification of the "peeling off" method, by which he achieved results which were comparable to those obtained by more elaborate methods. Bell and Garcia (27) have also described an automated fitting procedure based on the "peeling off" technique, but found that the parameter estimates were inferior in accuracy to those obtained by another method described by them which was based on a combination of a Gauss-Newton and gradient procedure (27). Danford (28) has discussed the relative merits of various procedures including Cornell's method (29), which works well for well defined data, and Prony's method (30). Lanczos (31) has also

examined Prony's method in a single study of three exponentials, in which rounding off errors were imposed on the data. He was unable to recover the original function used to generate the data and concluded that the method was unsatisfactory dup to the sensitivity of the exponents and amplitudes to the accuracy of the data. Marquardt's method of 'the path of steepest descent' (32) obtains rapid convergence, but the computer programme based on it (33), like Prony's method, does not permit the application of weighting factors. Nearly all the methods described above use a least-squares minimizing procedure to obtain a best fit to the data, and some, but not all, also provide estimates of the parameter errors. Although the exponential curve fitting method of Gilles and Ben Hameid (34) allows the use of weighting factors, (as described in two studies in chapters 2 and 7) no parameter error estimates are obtained using their method. With Berman's method (18) one obtains parameter error estimates, and weighting factors can be applied whose magnitudes are dependent on the data accuracy and sampling procedure used in obtaining the data. Because of these points, and for other logistic reasons discussed in subsequent chapters, the Berman method was used for the theoretical investigation described in this thesis. A more detailed description of this method is given in chapter 3. Of the more elaborate methods, no detailed study of their relative merits has been reported. Attention has been paid by some authors to the problem of acceleration of the convergence procedure without particular attention being paid to the parameter error estimates. Many of these minimizing procedures have been reviewed by Powell (35). The relative merits of those fitting methods which have the same facilities available

are probably marginal, although, as will be demonstrated in chapter 7, more biologically meaningful results have been obtained with one method than with another. However, the methods have not yet been compared mathematically, which may be a more acceptable mode of comparison than the biological criterion used in this work.

As has been pointed out by Hart (10), the theory of multi-compartmental analysis of both steady state and unsteady state systems is still being developed. Moreover, the meaningful application of biological data to even a very simple steady state model, with a good appreciation of the numerical limitations of the curve fitting methods being used, is still relatively rare. Many of the non-compartmental analyses of biological systems, although perhaps more intellectually acceptable, do not always provide estimates of the parameter errors calculated by these methods and are therefore possibly less valuable than an alternative "compartmental" approach, which does produce error estimates. It will be apparent from this introductory review that despite the multiplicity of exponential curve fitting methods available, and the wide range of application of these methods, there still remains a significant lack of quantitative information regarding the limigations of these methods when applied to data derived from biological investigations.

It is the purpose of this thesis to provide some of the basic quantitative information which is required to assess the value of the results obtained using exponential curve fitting procedures. This information was obtained using a curve fitting procedure which is widely used and is

readily available in the form of a computer programme. Aside from the intrinsic interest of such information, it was felt that it may also permit the possibility of predicting whether an investigation, which will subsequently involve some curve fitting procedures as part of the data analysis, is likely in fact to produce meaningful results. For reasons which are presented later, it will be apparent that obtaining a complete solution to this problem is economically prohibitive, and an attempt has been made to acquire what is probably the minimum necessary data in order to make a realistic assessment of the important factors of the problem. A preliminary study of two exponentials (Chapter 2) was most useful in designing the more detailed study (Chapter 3), which was carried out subsequently (Chapter 4). In the more complex case of three exponentials, it was not possible to perform a preliminary study and this is reflected to some extent in the more limited information which was produced by this study (Chapter 5). However, both of the principal theoretical investigations produced several unexpected results and both yielded potentially useful information (Chapter 6).

The application of exponential curve fitting to two different clinical fields is presented. In chapter 7, uric acid metabolism, a field in which the application of tracer kinetics is not common, is studied using two different curve-fitting methods. From the results obtained using one of these methods, it appears possible to associate a pathological condition with compartmental model parameters which are different from those of a normal person. This differentiation was less clearcut when the alternative curve fitting procedure was used. The selection of the curve fitting

method and the implications of the findings with the method used are discussed. Although exponential curve fitting is now commonly used in the determination of regional cerebral blood flow using inert gases, the application described in chapter 8 is an empirical use of curve fitting in order to obtain a mathematical estimate of the initial slope of a clearance curve. An unusual collimator design, which allows blood flow in the superficial regions of the brain to be measured, is also described in detail. The results obtained using this collimator in normal and pathological cases are presented.

CHAPTER 2

A Preliminary Study

Summary: A preliminary quantitative study of some of the factors which affect the parameter errors resulting from fitting a two exponential function using two different computer programmes is described. The factors investigated were 1) Exponent ratio, 2) Data error, 3) Amplitude ratio, 4) Number of data points, 5) Frequency of sampling and 6) Weighting factor. The effect of the initial guess of the parameters used to initiate the iterative procedure was not investigated since it was found that, when using one of the curve fitting methods, the choice of the initial guess did not affect the result. The correct parameter values were always used as the initial guesses. The randomised error test data was generated manually, and only a limited statistical study, using samples of ten per investigation, was attempted. A statistical method of estimating the parameter errors was used, and was considered to be more reliable than the error estimates produced by one of the programmes, although the errors estimated by the latter were found to be comparable, on some occasions. Two examples were used to assess the practical significance of the results.

The study was of value in three ways:- 1) it provided a basis for the design of a more detailed and elaborate study to be carried out subsequently, 2) it provided an indication of the magnitude of the parameter error estimates likely to be encountered, and 3) it allowed the analytical methods it was proposed to use in the main study to be assessed. For example, the importance of selecting parameter values so that the effect

of the factor under investigation was not suppressed due to the effect of other more predominent factors was made apparent. It was found that the principal factors which determined the errors in the estimated parameters were the data error and the exponent ratio. The use of a weighting factor caused a reduction in the estimated parameter errors. Of lesser importance were the number of data points and the amplitude ratio.

In general, if more than ten data points are used, if the data error is less than 2%, and if the exponent ratio exceeds 4:1, then the parameter errors are likely to be less than 12%. If the exponent ratio is less than 2:1, and the data error is greater than 10%, the parameter errors are unlikely to be less than 25%. These parameter error estimates may be greater or less than these values depending on the various other minor factors. In practical terms, physiological parameters, computed by compounding the estimated parameters in various formulae, are unlikely to be useful if the individual parameters possess errors in excess of 25%.

2.1 Introduction to Preliminary Study

The purpose of the preliminary study was to prove the validity of certain error generation and analytical procedures and to obtain some insight into the relative importance of the various factors which were relevant to the problem. Small statistical samples (usually ten in each group) were used. Randomised error data were generated manually from tables of random normal deviates. Two different computer programmes were used one developed by D. C. Gilles (34) written in Algol and the other

by D. W. Marquardt (33) written in Fortran IV. Each of the programmes allowed the relative merits both of the programmes and of their special features to be assessed. A further purpose served by this preliminary study was to establish the definitions of terms which would be used to describe and discuss the results obtained in this and subsequent studies. Some indication of the practical significance of the errors in the estimated parameters in some typical applications were also sought in this study. It should be stressed that the purpose of the study was not to assess the limitations of exponential curve fitting procedures in general but to assess their potential within the limitations normally encountered in biological investigations, that is to say, in situations where the number of available data points is small and the data accuracy is relatively poor. The results of this study were used to design a large scale, more realistic statistical study, which is described in the subsequent chapters, and also to provide information on which to base the choice of curve fitting programme to be used in that study.

2.2 Method

2.2.1 General Considerations

In this investigation the function studied was $\sigma_1 \exp(-\lambda_1 t) + \sigma_2 \exp(-\lambda_2 t)$. The following variables were expected to influence the parameter estimates obtained by the computer:-

(1) The initial guess of the quantities σ_1, λ_1, σ_2, λ_2

(2) The ratio σ_1 / σ_2

(3) The ratio λ_1/λ_2

(4) The number and spacing of data points

(5) The error on the data.

The latter two parameters are of special interest in the biological or medical sciences since frequently the number of data points will be less than twenty, more usually about ten, and the data accuracy is frequently about ± 10%, although it may reach ± 2%. It is normally unlikely to be better than ± 2%.

Artificial data with a controlled random error were calculated for values of λ_1/λ_2 equal to 2, 3, and 4 (the absolute values for λ_1 were 0.2, 0.3 and 0.4, the value for λ_2 being held constant at 0.1) with normally distributed error values of 2%, 3%, 5% and 10% (see exact definition below). In the main part of this investigation the value of the ratio σ_1/σ_2 was held constant at unity (with absolute values for σ_1 and σ_2 of 0.5) and the number of data points was limited to eleven. It was found that the final results obtained with the Marquardt programme were independent of the initial guess within wide limits, and the possible influence of the initial guess on the final parameter estimate was not pursued further. The result obtained with the Gilles programme on the other hand was found not to be independent of the initial guess. In fact the latter programme was modified to include a subroutine which determined an initial estimate of the parameter which was then used to initiate the main iterative programme. For the purpose of this study this was not immediately relevent and the correct values of the parameters were used to initiate the fitting procedure. In this way one extra variable was eliminated from the study. The effect of

an incorrect initial guess served only to lengthen the time used to find a fit when using the Marquardt programme. Using the Gilles programme an incorrect solution may also result however. For each fixed combination of the variables, ten sets of data were examined, each with a different combination of random errors of the same magnitude.

The effects of increasing the number of data points, of varying the spacing of the data points, and also of varying the amplitude constants, were examined in a more limited investigation. Various methods of assessing the parameter errors were examined as compared with a statistical procedure used here in order to assess the relative merits of different methods of estimating the parameter errors. The importance of applying statistical weighting to the data points was also demonstrated since this possibility exists in the Gilles programme (see below) but not in the Marquardt programme.

2.2.2. Calculation of the Controlled random Error Data

If the values of all points in a given set of data lie within ± P% of their true values on 95% of the occasions on which they are measured, these data are defined as being subject to a "confidence limit" or "data error" of P%. The error is assumed to be normally distributed and, therefore, the value of any data point is within ± 2 standard deviations of the true value on 95% of the occasions on which it is measured. Therefore, if the error is P%, the standard deviation is equal to (exact value) x ½P/100. If this standard deviation is now multiplied by a random function, whose mean value is zero, and whose standard deviation is unity (a random normal

deviate or R.N.D.), then the error to be imposed on the exact value may be computed from the formula:

Error on exact value = R.N.D. x (exact value) x ½(P/100) . . . (1)

The value of the data point which is subject to a random error of P% is then formed by adding the error on the exact value, calculated as in equation (1) above, to the exact value of the data point.

Values of the random normal deviates were taken from mathematical tables (36) and the exact values of the function of interest at a series of times t, were calculated. The data values with superimposed random errors were then calculated. No error was superimposed on the point corresponding to t = 0 which was invariably taken to be equal to unity. This procedure was adapted since in practice data is frequently normalised to unity at zero time prior to analysis and a data point of this value at this time is assumed to be without error.

2.2.3 Curve Fitting Procedure

Two curve fitting programmes were used in this study. The first programme used was that developed by Gilles and Ben Hameid (37). This programme is written in Algol and was used on the KDF 9 computer in Glasgow University. It uses a least-squares minimizing procedure and weighting factors (relevant to the origin and accuracy of the data) may be applied. No parameter error estimates or graphical plot are provided by this programme. The second programme used was that developed by Marquardt (33) which is written in Fortran IV. This is a least-squares minimizing procedure for fitting any function to a set of data. With this programme

it is not possible to assign weighting factors to the data. Two different methods of estimating the parameter errors are provided by this programme. A graphical plot, obtained by an initial coding instruction, indicates the original data points, the computer calculated data points and a special sign indicates when these points coincide. An IBM 7090 Computer (Imperial College, London University) was used for running the programme.

2.3 Analysis of Results

Although the theory of confidence regions of non-linear parameters is not well developed, approximate parameter error estimates can be obtained and have been provided in the Marquardt programme, calculated by two different methods. The more commonly used method of estimating the confidence limit of the estimated parameter is based on the assumption of limited linearity within the confidence region. In the alternative method provided by the Marquardt programme, linearity is not assumed but it does assume limited coupling, i.e. low correlation, between the parameter estimates. This assumption does not often apply in exponential curve fitting. In the first method described above, the extent to which the assumption of a linear confidence region applies in any particular case will be very dependent on the values of the parameters, and may be expected to yield incorrect estimates on certain occasions.

In view of the above remarks, a simple statistical method estimating the parameter error was used to analyse the results obtained in this investigation. As mentioned above, ten sets of data were fitted for each combination of values of λ_1/λ_2, σ_1/σ_2 and data error. The standard

deviation and coefficient of variation for the estimates of each of the four parameters obtained from each set of data were then calculated. The assumptions and limitations of this method are discussed in Chapter 3. A value of double this coefficient of variation was then considered to be the estimated error on each parameter for the particular parameter values used. This procedure was adapted even though the implied assumption that the estimated parameter errors would be normally distributed was unlikely to be true. The method avoids the criticisms regarding linearity and coupling, mentioned above, although it can itself be criticised on other grounds. These criticisms are discussed subsequently (Chapter 3). A comparison has been made between the two methods calculated by the programme (the linear method being widely used in curve fitting procedures) and the statistical method devised to analyse the results.

2.4 Results

2.4.1 Effect of Data Accuracy and Exponent Ratio

Detailed results obtained with the two programmes, one with and without weighting factor are shown in tables 2.1, 2.2 and 2.3. In order to obtain a general impression of the trend of the results, the four individual parameter error estimates obtained for each group were averaged, and the results are presented in table 2.4. This procedure does however tend to hide some detailed trends, since for example, the errors estimated for λ_2 are always smaller than those of λ_1. It does, however, allow some general assessment of the whole exercise to be readily made. The average value of the four parameter error estimates has been called the "mean

parameter error" in table 2.4.

It can be seen from these tables, 2.1, 2.2 and 2.3, that the parameter errors may vary from about 3% (ignoring the single value of zero), for data with 2% accuracy and an exponent ratio of 4:1 and weighting factor applied, to over 100% with data of 10% accuracy, a 2:1 exponent ratio and no weighting factor. The estimates of the amplitude errors do not differ markedly despite the changes in exponent ratio although the slower exponent always appears to be estimated with greater accuracy than the faster (greater) exponent. In some cases, where the error on the data is large and the exponent ratio is small, the computer programme failed to converge to a final solution. Ultimately this caused overload of a particular storage locality, which stopped the iteration process. In such cases, either no result at all is obtained, or physically unacceptable parameters were presented as a solution (for example, a negative amplitude). If the effect of these particular values was such as to change the average value of more than one of the four parameters by more than two standard deviations then these values were omitted, since a single value of this kind may grossly affect the estimate of the final value of the individual parameter error. Although it is appreciated that this arbitrary exclusion of some of the data is liable to introduce a bias to the results, this bias is such as to suggest a smaller error than actually exists. If the resulting error is already so excessively large as to be of no practical value, this bias will not affect the conclusions, provided it is appreciated that the bias exists.

It is apparent from these results, that with data of accuracy ± 10%,

the parameter errors are seldom less than 20%, and may rise in the case of small exponent ratios to over 80%. It should be emphasized that these estimates were obtained using only eleven equally spaced data points.

2.4.2 Effect of Change in Amplitude Constants

Three amplitude ratios were compared at two data error values of 2% and 5% and exponent values of 0.3 and 0.1. No consistent trend emerged from this particular investigation although the amplitude ratio was varied from 2.3:1 to 1:2.3. A preliminary conclusion is that the amplitude ratio is substantially less important than both the exponent ratio and the data error in determining the value of the parameter errors. It is possible that the effect of the amplitude ratio may become more apparent, if the effect of the exponent ratio is reduced sufficiently, for example, in those functions with a high exponent ratio.

2.4.3 Effect of Increased Number of Data Points

To examine the effect of increasing the number of data points, the function $y = 0.5 \exp(-0.3t) + 0.5 \exp(-0.1t)$ was examined with data having errors of 2% and 10%, and the results obtained with 11 and 21 data points are presented in table 2.5 and are summarised in table 2.6. The effect of increasing the number of data points on the 10% error data has been to reduce the estimate of the mean parameter error by approximately 25% using the Marquardt programme and by approximately 33% using the Gilles programme. This improvement affects all the parameters. The rate of improvement in the parameter estimate diminished as the number of data

points increases. The practical problem of how many samples must be taken should be decided by consideration of the improvement in the overall accuracy of the physiological quantity which is liable to be achieved by taking extra samples above that which is minimally required also for other investigations.

2.4.4 Effect of Weighting Factor

It is evident by examination of table 2.4 that when comparison can be made between sets of data composed of equal numbers of observations, the effect of using a weighting factor is to reduce the parameter error, and that this effect is more marked as the mean parameter error decreases. This is most apparent if the results obtained with the Marquardt programme are compared with those obtained with the Gilles programme using a weighting factor. The weighting factor used is proportional to the inverse of the square of the observation and is used when the data have a constant relative error (Gilles, personal communication). From the comparison of the results obtained with the Gilles programme with and without the weighting factor applied it is evident that the effect of the weighting factor is to reduce the parameter error by approximately one-third. It will be seen below that the effect of incorporating a weighting factor may be equated with the effect of increasing the number of data points from 10 to 20 which in practice may not be feasible. The failure to incorporate a weighting factor correction is a significant disadvantage in any exponential curve fitting method.

2.4.5 Effect of Unequally spaced Data

It might be expected that extra data points taken when the function was changing more rapidly would improve the parameter estimates. To examine the quantitative aspects of this proposal, the function $y = 0.5 \exp(-0.3t) + 0.5 \exp(-0.1t)$ was again examined, first with values of $t = 0, 2, 4, 6, 8, 10, 12, 14, 16, 18$ and 20 and then with values of $t = 0, 0.5, 1, 2, 3, 4, 6, 9, 12, 16$ and 20. No general reduction in the parameter errors was apparent for this particular function when the results of the two sets of data were compared. When the function is changing more rapidly, an increased sampling frequency does not inevitably lead to an improvement in accuracy of the parameter estimates. Whether or not such an improvement is achieved will depend also on the absolute rate of change of the function.

2.4.6 Assessment of Methods for Estimating Parameter Errors

The results calculated by the statistical procedure used in this paper were compared with the estimates calculated by the Marquardt programme using the linear and non-linear methods mentioned previously. Amplitudes of 0.5 and exponents of 0.3 and 0.1 were used with eleven equally spaced data points. The results of this comparison are presented in table 2.7. It is apparent that for this particular function, the parameter errors estimated by assuming a linear confidence region are comparable with the purely statistical estimate for the data with the larger data error. The agreement is less good, proportionally, with the other set of data. The estimate based on non-linear regions appears to be badly

out. It may, however, be unwise to extrapolate the good result achieved here by the linear method to other functions without further evidence, and further more detailed work on this point is indicated. The poor result of the non-linear method may be associated with the high cross correlation between the parameters.

2.5 Discussion

2.5.1 Practical Significance of Results

The purpose of this preliminary and limited statistical investigation of exponential curve fitting was to establish and assess methods of analysing the results, and to obtain some insight into the relative importance of the factors which effect the parameter errors. The scope of the investigation was restricted so as to establish the limiting values of the parameters and data errors which would allow useful conclusions to be drawn. That is to say, the range of data errors was selected on the assumption that ± 10% could usually be attained in well controlled biological investigations, and ± 2% was unlikely to be achieved but was possible in certain circumstances. Similarly, the exponent ratio was made to vary from 4:1, which may be expected to be readily fitted even with large data errors, down to 2:1.

It was found that these expectations were largely justified and that it was possible to assess from the results some indication of the errors to be expected in the parameters derived from a particular set of data. The statistical procedure used for analysis of the results was compared with the two alternative procedures provided by the Marquardt programme

for estimating the parameter errors. There would seem to be a certain risk in ascribing too much reliability to estimates of the parameter errors based on the non-statistical procedures, sometimes provided by exponential curve fitting computer programmes.

To determine practical implications of the results presented above, data were generated from parameter values which are typical of those found in two types of investigation, i.e. bone metabolism studies and cerebral blood flow estimations. Sets of data with 11 and 21 points, and with errors increasing linearly from zero up to 10%, were analysed. An exponent ratio of 4:1 and an amplitude constant ratio of 5.7:1 were selected. The results (table 2.8) indicate that a mean parameter error of approximately 12% might be expected with eleven data points, decreasing to about 8% with 21 data points. However, the individual parameter errors are of greater importance in this situation, since σ_1 and λ_1 have much smaller errors than σ_2 and λ_2. In practice, for example in the case of cerebral blood flow estimated by a two exponential analysis of the clearance curve of radioactive gas from the head, this would mean that the error in estimating grey matter flow from 21-point data would be between 2.5 and 5%, and that the error in estimating flow in white matter would be between 13.5 and 18%. These errors have been calculated on the assumption that the parameter errors, estimated statistically, are independent. The effect of these parameter errors in the case of a bone metabolism study is less easy to estimate and depends on the manner in which the physiological quantities of interest are computed from the parameters derived by two exponential curve analysis of the blood activity curve.

In the assessment of bone metabolism, using the formulae of Glass and Nordin (37) and the same parameters as used in the previous example, the errors in the various constants of the proposed two compartment model may be calculated. The error in the compartment size V_1 would be 7% and the error in the bone formation rate K would be 27%. The error in bone exchange rates, K_{12} and K_{21} would be 49% and 17% respectively, and the error in the excretion rate would be 26%. These comparatively large errors may be considered fairly typical of the parameter errors associated with simple biological models, and derived from well controlled in vivo data.

2.5.2 Application of the Statistical Procedure for Estimating Parameter Errors

The statistical method of assessing parameter errors may also be applicable to a practical situation where a curve fit has been carried out on a single set of data. If the probable error of each data point can be estimated, and the parameters of the curve have been found by the computer, then sets of randomised error data, based on these parameters, can be generated using the method previously described. These sets of data can then be analysed. This operation can be repeated a sufficient number of times to obtain a statistically significant estimate of the parameter errors for this particular case. This method does however require a realistic assessment of the experimental errors in the original data.

CHAPTER 3

Design of the Experiment and Methods

Summary: The overall and detailed design of the theoretical investigation of the factors limiting the fitting of two exponentials and three exponentials to data is discussed based on the experience obtained in the preliminary study. An alternative basic approach to the same investigation is presented. The data for the study was generated automatically using a specially written computer programme. The programme output was written directly on to magnetic tape in a form compatible with the input requirements of the Berman curve fitting programme, which was selected for this study. A detailed description of this generation programme is presented. The actual Fortran instructions for the generation programme and a description of the mode of operation of the Berman programme are presented.

In analysing the results by the statistical method used previously there is an inherent assumption that the normally distributed errors superimposed on the data will produce a normally distributed range of parameter estimates. That this is not always the case is demonstrated by using a test for normality on a small group of sets of data. It is pointed out that, despite the measured non-normality, it appears to be possible to obtain meaningful results by the statistical analysis method. A further potential difficulty due to the occasional production of censored data by the curve fitting programme is shown to be relatively less important.

3.1 Design of the Curve Fitting Investigation

The design of the investigation was based on the experience obtained with the preliminary investigation described previously. The two practical examples discussed in section 2.5.1 of the previous chapter illustrate two possible ways in which the collection of data may be terminated, and raise a fundamental point concerning the design of the investigation. In the case of the measurement of cerebral blood flow, the limiting factor in determining the extent to which the data describing the function of interest may continue to be collected, is simply the sensitivity of the counting equipment, and is effectively independent of biological factors and physical characteristics of the tracer used. Also, the 'raw data', corrected only for background effectively describes the function to be studied. In the case of a bone metabolism investigation, using a short-lived isotope such as calcium-47, the 'raw data' does not define the function of interest, since the observed data must be corrected for decay of the isotope and also often must be expressed as a fraction of the initial data value or in terms of a standard, in order to obtain the required function for analysis. This may introduce a constraint at the beginning of the function, since, for example, the first point must go through 100% in a retention study. This type of study is terminated at an arbitrary time, which is effectively outside the control of the investigator, and is dependent on the metabolism of the subject under study, and time relationship of this metabolic turnover rate to the half life of the tracer being used. In practice, this presents the alternatives of either investigating the effect of the various parameters in defining the function, up

to values of t, such that the final value of the function is some fixed fraction of the initial value, or, investigating the effect of various parameters in defining the function up to a fixed value of t, which represents the time of duration of the biological study. Since it was not economically possible to study both of these cases in detail, it was arbitrarily decided to simulate the previous study of using a 'fixed time' investigation and investigate the effect of the limited definition of the function by the data in a small subsidiary study. It was also decided, in the interests of generality, and also for reasons associated with the programming of the data generation method described above, not to use a data point at zero time, so that the question of the accuracy to be assigned at the zero point did not arise in this investigation.

The preliminary investigation indicated that the range of parameters did, in fact, cover the region in which the parameter error estimates changed rapidly from small acceptable values to large values, which when compounded in order to calculate physiological variables, the latter were associated with excessive errors. The principal criticism of the preliminary study is associated with the small size of each batch on which a statistical estimate was being carried out. The maximum number in each batch was ten samples, but in fact, in many cases one or other of the curve fitting programmes failed to converge and the iteration ceased due to a variety of reasons mentioned in chapter 2. The rejection of unacceptable data frequently resulted in sample sizes much smaller than ten which in turn resulted in statistical estimates of doubtful value. Since 95% confidence limits were used to define the imposed errors and the estimated

parameter errors, it was decided to select a minimum of twenty samples in each batch, since Bessel's correction for the difference between the best estimate of the population variance obtained from the sample variance would not be in error by more than 5%, and need not therefore be applied in this instance.

3.1.1 Investigation of Two Exponentials

The same exponent and amplitude ratios (.2:.1, .3:.1, .4:.1 and .5:.5) were selected as in the preliminary investigation to examine the effect of the exponent ratio and data error on the parameter estimates. Only ten data points were normally used. When the effect of the amplitude ratio was investigated previously, an amplitude range from 2.3:1 to 1:2.3 was used with an exponent ratio of 0.3:0.1. With the small samples of 10 no significant effect was observed. In order to reduce the effect of the exponent ratio, a higher exponent ratio of 0.4:0.1 was used on this occasion and the range of amplitude ratios was increased to extend from 4:1 to 1:4. Extreme data error values of 2% and 10% were also used instead of values previously used.

To investigate the effect of increasing the number of data points, an exponent ratio (0.2:0.1) was used, in order to determine the extent to which the effects of the low exponent ratio on the parameter error estimates could be compensated for by increasing the number of data points. A greater range of numbers of data points was used (10, 20 and 60). To investigate the effect of a gradually increasing variable relative error as opposed to a fixed relative error, the effect of other factors was

minimised by using twenty data points and by using a high exponent ratio (0.4:0.1). When investigating the effect of variable spacing of the data, a very low amplitude ratio and high exponent ratio was selected in order to create conditions most likely to produce an observable effect.

To examine the effect of an incomplete observation of incomplete collection of data, a function was generated using 60 data points having high amplitude and exponent ratios. The last ten data points were removed and the function fitted once again. This process was repeated until the same function was fitted with only 20 points. When the investigation is carried out in this way, two variables are in fact being changed, 1) the number of data points, and 2) the period of observation. To assess the relative contributions of each of these factors to the parameter error estimates, in two cases 60 data points were used in the shorter observation periods and the parameter error estimates so obtained were compared. Under these conditions only the reduced period of observation is a relevant factor. In practice a reduced period of observation would normally be associated with a reduced number of data points. This investigation was not carried out in the previous study.

A further point of interest was to compare the parameter error estimates derived by the computer programme with those estimated by the statistical method described below. The results were examined in an attempt to determine whether limiting values of the parameter correlation coefficients could be used to indicate when the programme derived parameter error estimates may be used.

It is stressed that throughout this investigation, the correct

parameter values were used as the initial guess for the exponents. The detailed design of the investigation is presented in table 3.1.

3.1.2 Investigation of Three Exponentials

Whereas ten data points were taken as the standard number in carrying out the two exponential fitting investigation, this was considered inadequate for the three exponential investigation since the number of degrees of freedom, assuming independent parameters, would be limited to four. Accordingly, twenty data points were used as the standard number when investigating the effect of the exponent ratios and data errors on the parameter error estimates. The range of exponent ratios was extended to cover 3:1, 5:1 and 10:1, and this was the ratio between upper and middle and also the middle and lower exponents. In the case of the 10:1 exponential ratio, to allow for the effect of very high values of the exponent, whose effect might be reflected in only a small number of the data points, a different selection of exponents was also used with smaller absolute values, but maintaining the exponent ratio at 10:1.

The effect of the variation in the number of data points was investigated as before with 10, 20 and 60 data points using equal amplitude values and 3:1 exponent ratio. The effect of the amplitude ratio was investigated using an exponent ratio (5:1) and covering an amplitude ratio range from 5:1 to 1:5. In investigating the effect of variable spacing a 1:3 amplitude ratio and a 5:1 exponent ratio was chosen in order to assess the improvement if any, which could be expected in the early components of the function. The effect of incomplete data collection was investigated in a

manner similar to the two exponential investigation, by removing the last ten data points successively from 60 down to 20 data points, using 3:1 amplitude ratios and 5:1 exponent ratios. Once again an attempt was also made to separate the effect of reduced observation period and reduced number of data points in this part of the investigation. The detailed investigation plan is shown in table 3.2.

3.2 Data Generation Programme: Description of Operation

Test data for carrying out the statistical study was generated using specially written computer programme. The programme language is Fortran IV. The programme output could be obtained on punched cards or magnetic tape. Two versions of the programme were written to generate data for either two or three exponential functions. However, the particular programme described below could be readily generalised to allow the generation of data for the investigation of any function on which a controlled random error is to be superimposed.

The programme generates random numbers using the method of Pike and Hill (38). From these, random normal deviates are calculated (39) and are used to construct error terms which are added to the data points calculated for the function, using sets of variables, which are specified in the input data. The programme allows the functions to be described by either 10, 15, 20, 30 or 60 data points. Each data point may have the same relative error or individually selected error values. The spacing of the data points may be equally distributed or may be different and individually specified. The number of estimates used to perform a single statistical

test may also be specified. Several different sets of amplitudes and exponents may be used successively, along with several different sets of errors, and all data is then produced in one run of the programme.

The programme may be described by reference to the flow diagram presented in pages 176 and 177. Any odd number between 1 and 67108863 is first read in. This is required to initiate the random number generation routine. The amplitudes and exponents to be used to form the exponential functions are then read in. These are followed by six numbers which specify the way in which the generation programme is required to deal with the input data and which indicate the number of data points to be used, the number of statistical estimates in the sample, the number of sets of parameters, the number of error values. This data card also informs the programme whether to expect variable or fixed errors and equally or non-equally spaced data. The errors, variable or fixed and the spacing information, if variable is then read in. The errors are converted to fractional errors. This operation corresponds to stage (03) in the flow chart.

The programme now prints and writes the title of the tests on to tape or punches on to cards depending on which version of the programme is being used. This title consists of four numbers indicating the error, amplitudes, exponents and statistical estimate number. Any mandatory input entries required by the curve fitting programme to which the data is to be applied are now entered. In the case of this investigation, mandatory entries required by the Berman programme were generated at this point. A counter is now set to zero to be associated with the number of data points to be used.

The random number and random normal deviate are calculated as is also the value of the exponential function for one particular data point. The error terms are generated, as described in the preliminary investigation (Chapter 2), and added algebraically to the exponential function value at this data point. The value of the random deviate, the new value of the function due to the imposition of the error term and the independent variable (i.e. the value of 't') with which the function is associated is printed out and the latter two quantities are also written on to the magnetic tape in the correct format required by the curve fitting programme.

The programme then returns to point (04) and generates a new random number and repeats the cycle until the necessary number of data points on which an error has to be imposed have been generated. The mandatory output entries, required by the Berman programme, are now generated and the programme returns to (03) in order to repeat the cycle until the required number of estimates for the statistical test have been obtained. When this cycle is complete the programme returns to (02) in order to carry out the whole procedure again for new error values. Finally the programme returns to (01) if required by the input instructions, to read in a new set of parameter values.

The actual programme instructions are shown in Appendix 3.1. Although the mandatory entries and data formats have been written to conform with the requirements of the Berman SAAM-22 programme (Fortran II version) and the data has been written on to tape in card image form, the programme may be readily modified for use in association with any curve

fitting programme.

3.3 Selection and Description of the Curve Fitting Programme and some Special Features

Several alternative programmes were available for this study, but the Berman programme was selected for use because of certain local logistic advantages and also because of the versatile nature of the programme. It will accept data via magnetic tape, with only minor modifications to the lead-in cards, as well as by the more usual method of punched cards. This allowed the random error data to be generated and written on to magnetic tape directly. This tape could then be used as the input to the Berman programme, eliminating the necessity of intermediate handling of a vast quantity of data in the form of punched cards which would have had to be discarded as soon as it had been used. A second logistic advantage is that the Berman programme continues with a new set of data if a fault exists in the previous data. The Marquardt programme, for example, stops completely if only one set of data is faulty and the subsequent correct data are not examined.

One further reason for preferring the Berman programme is its use of weighting factors which were shown to improve the parameter estimates when the results of the Gilles and Marquardt programmes were compared in the preliminary investigation. The Marquardt programme does not use weighting factors. The Gilles programme does not yield error estimates, and one point of interest in this investigation was the possibility of obtaining information which might provide indications as to when the computer derived

error estimates may be quoted with reliability. A description of the Berman curve fitting programme is presented in Appendix 3.2.

3.4 Method of Analysing the Results

The printed output of the Berman programme includes estimates of the parameter errors based on the variance-covariance matrix for the unknown parameters. Such estimates are only valid when obtained in the neighbourhood of a least-squares fit and are not valid in the case of extensive non-linearities. The estimates are also invalid if there is strong interdependence between the variables. It was therefore decided to use the method developed in the preliminary investigation described in section 2.3 above. That is to say, the actual estimates of similar parameters were obtained on twenty occasions and the standard deviation of these parameter estimates were found. Twice the coefficient of variation was then taken to be an estimate of each parameter error.

There are several inherant assumptions in this method. It is assumed that the parameter estimates are normally distributed about the mean value. Although a truly random error was imposed on the original function it cannot be assumed that the parameter estimates are normally distributed around the true mean owing to the non-linear treatment of the data in the curve fitting procedure. Testing for normality has been an area of continuing statistical research mainly because many statistical procedures have been derived based on an assumption of normality. A knowledge that this underlying assumption is incorrect is a useful safeguard against too wide an application of what may appear to be useful analytical procedure. The

normality of the parameter estimates in several situations was tested using the criterion of Shapiro and Wilk (41). This test has been shown to be sensitive to a wide range of non-normality even with small samples (n = 20). The relative merits of various criteria for normality have been discussed by Shapiro and Wilk.

The results of the calculation on six sets of data are presented in table 3.3. The values of 'W' have been calculated according to the method of Shapiro and Wilk. In general small values of W are significant i.e. indicate non-normality. A graph relating the value of W (for samples of 20) to the probability of normal distribution is shown in figure 3.3. It will be observed from table 3.3 that sample 1 is effectively normal, sample 2 is non-normal to a 10% significance level, while sample 3 and 4 are non-normal to levels of 1% and 5% significance i.e. are highly probably non-normal. When the normality test was repeated on the logarithms of the data instead of on the raw data the results shown in table 1b were obtained. It is sometimes possible to 'normalise' skew data by taking the logarithm of each data element. It can be seen that the effect of taking the logarithms in four of the cases was to render the distribution less non-normal and in the case of sample 3 the improvement is quite marked. It can be seen however that taking the logarithm does not always improve the normality and whether an improvement is obtained is dependent on the form of the skewness, which is unpredictable.

The question remains as to whether a more meaningful indication of the spread of the parameter estimates would be obtained by calculating a "standard deviation" on the raw data neglecting the fact that it is non-normal

or alternatively by estimating a standard deviation on the logarithm of the data and deriving a 'confidence range' of the spread of the parameter estimates. To decide on this point the standard deviations were calculated on four sets of raw data, and on the logarithms of the same data. The coefficient of variation was calculated from the results obtained with the raw data. The actual values corresponding to plus and minus one standard deviation of the logarithm data were obtained and the difference between these two was expressed as a fraction of the actual calculated mean. The results are presented in table 3.4. It can be seen that in three cases the results are almost identical, although non-normal data is present. It would, therefore, seem that, despite the non-normality of the data, meaningful results can be obtained from a simple statistical analysis of the data.

The nature of the curve fitting procedure results in the production of censored data. Censored data is the term used to describe the situation where upper and lower limits have been set on the range of values which are acceptable and the number of estimates which lie above and below this range is known. This data should be distinguished from truncated data in which the number of data points above and below the given range is not known. This possibility of obtaining censored data arises because the Berman programme required a maximum value to be specified for each parameter and because the value of any of the parameters are not permitted to become negative, so that zero represents the lower limit of the value of each parameter. The upper limit is also required to be specified as input data to the curve fitting programme. If too high an upper limit is set, convergence of the iterative procedure may take an excessive amount of time. Several methods

have been described for estimating the true mean and variance of censored data and Tiku (42) has recently described a method of dealing with samples of normal data. This method is numerically easier to apply than iterative methods described previously (43, 44, 45) for normal samples. Plackett (46) has described a method which is not restricted to normal samples, but is numerically difficult to compute. It has been shown above that neither the samples composed of the raw data or logarithm of the data are predictably normally distributed. The simpler methods of estimating the true mean and variance of censored normal data cannot be applied. When the data is not censored the principal point at issue is whether or not the simple statistical method of estimating parameter errors described above, ignoring non-normality, is likely to be a closer approximation to the truth, than the computer derived estimates which assume linearity and non-correlation. In this situation an attempt to correct for slight censoring probably represents a second order correction and is probably barely justifiable. The effect of not allowing for the censoring of the data will be to indicate a smaller parameter error than actually exists. Highly censored data will only exist in those situations where excessively large errors are present and these results are therefore of least practical application. In the presence of the uncertainties previously mentioned and the practical implications of the results no attempt at correction for the presence of censored data was made.

In the absence of an obvious alternative procedure the method of calculating a "standard deviation" for all the data was used, even in those situations which did not fulfil the Shapiro-Wilk criterion of normality.

In all cases the calculated mean was used in estimating the coefficient of variation and not the true value of the parameter which was used as an initial guess to start the curve fitting procedure. Twice the value of the "coefficient of variation" was taken to be the parameter error.

APPENDIX 3.1

Random Error Data Generation Programme for use with the Berman GAAM-22 Programme

Fortran IV Statement

```
C       NP=6 MEANS 10 DATA POINTS
C       NP=4 MEANS 15 DATA POINTS
C       NP=3 MEANS 20 DATA POINTS
C       NP=2 MEANS 30 DATA POINTS
C       NP=1 MEANS 60 DATA POINTS
C       LQ=1 MEANS ALL THE POINTS HAVE THE SAME ERROR
C       LQ=2 MEANS DIFFERENT ERRORS FOR DIFFERENT POINTS
C       MN=1 MEANS POINTS EQUALLY SPACED
C       MN=2 MEANS POINTS UNEQUALLY SPACED
C       KT=NUMBER OF ESTIMATES FOR STATISTICAL TEST
C       KL=NUMBER OF SETS OF PARAMETER VALUES
C       IR=NUMBER OF DATA ERROR VALUES
        DIMENSION F(100),X(100),Y(100),A(100),Z(100),C(10),L(10),B(10)
        REWIND 22
        REAL L
        DO 50 I=1,100
        DO 50 N=1,10
        DO 50 J=1,10
        F(I)=0.0
        X(I)=0.0
        Y(I)=0.0
        A(I)=0.0
        Z(I)=0.0
        C(N)=0.0
        L(J)=0.0
   50   B(J)=0.0
        IP=125
        IL=0
        READ(5,26)K
        WRITE(6,26)K
    2   IL=IL+1
C       READ FIRST CARD OF NEXT CASE
C       READ IN PARAMETER VALUES
        READ(5,81)(B(J),L(J),J=1,3)
        WRITE(6,81)(B(J),L(J),J=1,3)
C       READ IN PROBLEM SPECIFICATION
        READ(5,31)NP,LQ,MN,KT,KL,IR
        WRITE(6,31)NP,LQ,MN,KT,KL,IR
        IN=60/NP
        GOTO(71,72),MN
C       READ IN VALUES OF TIME (X)
   72   READ(5,29)(X(I),I=1,IN)
   71   GOTO(61,15),LQ
   61   CONTINUE
C       READ IN VALUES OF ERROR
        READ(5,60)(C(N),N=1,IR)
        WRITE(6,60)(C(N),N=1,IR)
        NK=0
  100   NK=NK+1
```

```
      N=NK
      E=C(N)
      WT=.01*C(N)
      GOTO 200
C     READ IN VALUES OF ERROR (VARIABLE)
   15 READ(5,9)(A(I),I=1,IN)
  200 CONTINUE
      LE=0
   12 LE=LE+1
      GOTO(13,14),LQ
   13 WRITE(6,83)E,(B(J),L(J),J=1,3),LE
      WRITE(22,84)E,(B(J),L(J),J=1,3),LE
      GOTO16
   14 WRITE(6,85)(B(J),L(J),J=1,3),LE
      WRITE(22,86)(B(J),L(J),J=1,3),LE
   16 WRITE(22,87)
      WRITE(22,88)
      WRITE(22,89)
      WRITE(22,90)WT
C     IK=COUNTER FOR NUMBER OF DATA POINTS
      IK=0
    1 GOTO(42,41,40,39,38,37),NP
   37 IK=IK+6
      I=IK/6
      GOTO 17
   38 GOTO70
   39 IK=IK+4
      I=IK/4
      GOTO 17
   40 IK=IK+3
      I=IK/3
      GOTO 17
   41 IK=IK+2
      I=IK/2
      GOTO 17
   42 IK=IK+1
      I=IK
C     CALCULATION OF RANDOM NUMBERS
   17 IP=K*IP
      M=IP/67108864
      IP=IP-(M*67108864)
      R=FLOAT(IP)
      R1=R/67108864.0
      IP=K*IP
      M=IP/67108864
      IP=IP-(M167108864)
      R=FLOAT(IP)
      R2=R/67108864.0
C     CALCULATION OF NORMAL DEVIATE
```

```
      X1=SQRT(-2.0*ALOG(R1))
      T=6.2831853*R2
      X2=X1*SIN(T)
C     WE NOW HAVE NORMAL DEVIATE (X2)
      GOTO(43,44),MN
   43 SK=FLOAT(IK)
      X(I)=SK/3.0
   44 F(I)=B(1)*EXP(L(1)X(I))+B(2)*EXP(L(2)*X(I))+B(3)*EXP(L(3)*X(I))
      GOTO(46,47),LQ
   46 Z(I)=F(I)*E*X2/200.0
C     WE NOW HAVE CORRECTION TERM Z(I)
      Y(I)=F(I)+Z(I)
      WRITE(6,5)X2,Y(I),X(I)
      GOTO 45
   47 Z(I)=F(I)*A(I)*X2/200.0
      Y(I)=F(I)+Z(I)
C     NOW WE HAVE ARTIFICIAL DATA
      WRITE(6,66)X2,Y(I),X(I),A(I)
   45 WRITE(22,91)X(I),Y(I)
      IF(IK-60)1,6,6
    6 WRITE(22,92)
      WRITE(22,93)
      WRITE(22,94)
      WRITE(22,194)
      WRITE(22,92)
      WRITE(22,92)
      L(1)=-L(1)
      L(2)=-L(2)
      L(3)=-L(3)
      WRITE(22,95)L(1)
      WRITE(22,96)L(2)
      WRITE(22,196)L(3)
      L(1)=-L(1)
      L(2)=-L(2)
      L(3)=-L(3)
      WRITE(22,92)
      WRITE(22,97)
      WRITE(22,98)
      WRITE(22,198)
      WRITE(22,92)
      WRITE(22,92)
      WRITE(22,92)
      WRITE(22,92)
C     LE=COUNTER FOR NUMBER OF ESTIMATES FOR STATISTICAL TEST
      IF(LE-KT)12,7,7
    7 GOTO(28,4),LQ
   28 IF(NK-IR)100,4,4
C     IL=COUNTER FOR NUMBER OF BATCHES OF DATA PUT IN
    4 IF(IL-KL)2,70,70
    5 FORMAT(2F20.4,F10.1)
```

```
 9  FORMAT(5F10.1)
26  FORMAT(1I10)
29  FORMAT(5F10.2)
31  FORMAT(10I5)
60  FORMAT(10F5.1)
66  FORMAT(2F20.4,2F10.1)
81  FORMAT(6F10.4)
83  FORMAT(1H ,3H1E=,F4.1,2H *,6F6.2,2H *,I2)
84  FORMAT(3H1E=,F4.1,2H *,6F6.2,2H *,I2,31X)
85  FORMAT(1H ,8H1E=VAR.*,6F6.2,2H 1,I2)
86  FORMAT(8H1E=VAR.*,6F6.2,2H *I2,32X)
87  FORMAT(30H  000.000          4         5,50X)
88  FORMAT(70H                                    .01        .98
   1        .98    ,10X)
89  FORMAT(51H            OPTIONS                              2 ,29X)
90  FORMAT(42H 103.                                        ,F13.7,2H 2,23
   1X)
91  FORMAT(10H   3.      ,2F15.7,40X)
92  FORMAT(5H   26,75X)
93  FORMAT(25H    1              1.      ,55X)
94  FORMAT(25H    2              1.0     ,55X)
194 FORMAT(25H    4              1.0     ,55X)
95  FORMAT(12H    0   1   ,F13.2,29H                    3.0       ,26X)
96  FORMAT(12H    0   2   ,F13.2,29H                    3.0       ,26X)
196 FORMAT(12H    0   4   ,F13.2,29H                    3.0       ,26X)
97  FORMAT(51H    3   1                                   1.0    ,29X)
98  FORMAT(51H    3   2                                   1.0    ,29X)
198 FORMAT(51H    3   4                                   1.0    ,29X)
70  WRITE(22,101)
101 FORMAT(1H9,79X)
    STOP
    END
```

APPENDIX 3.2

General Description of the Curve Fitting Programme

The description of the Berman programme which follows is based mainly on the paper of Berman, Shahn and Weiss (18). The description of the programme is limited to its use for exponential curve fitting although the programme has been written as a general purpose programme designed to fit data to physical or mathematical models. This is achieved by adjusting the parameters of the model until a "best" fit is obtained. The programme is written in Fortran II and has been compiled for use on an IBM 7090 computer having a 32K word storage capacity.

The computer programme is applicable to a mathematical model which can be described by a discrete number of parameters, x_i, and for which a number of response functions

$$F_j(t) = F_j(x_1, x_2, \ldots\ldots x_m, t), \qquad (j = 1, 2, 3, \ldots\ldots n) \quad (1)$$

to a set of initial conditions can be specified.

The experimentally measured quantities, $Q_k(t)$, for such a system are usually a linear combination of the functions F_i:

$$Q_k(t) = \sum_{j=1}^{m} \sigma_{kj} F_j(t) \qquad (k = 1, 2, \ldots\ldots 1) \quad (2)$$

where the σ_{kj} are time independent coefficients and are either known or unknown. For the special case where the measured quantity is proportional to the function F_k, equation (2) reduces to

$$Q_k(t) = \sigma_{kk} F_k(t)$$

Given a particular mathematical model, initial conditions and initial

estimates of the parameters, x_i, the programme estimates the values of $F_i(t)$ analytically by means of a relevant subroutine. Values of $F_i(t)$ corresponding to each experimental datum are obtained. Since the data correspond to measurements at specific times t_k, equation (2) becomes

$$Q_k = \sum_{j=1}^{h} \sigma_j F_{jk} \qquad (k = 1, 2, 3, 4, \ldots\ldots\ldots m) \qquad (3)$$

where m is the number of experimental observations and h is the total number of the original σ_{ij}.

Solving for the unknown σ_j in terms of Q_k and the known σ_j, by rearrangement,

$$\sum_{j=1}^{r} \sigma_j F_{jk} = Q_k - \sum_{i=r+1}^{h} \sigma_i' F_{ik} \qquad (k = 1, 2, \ldots\ldots\ldots m) \qquad (4)$$

where σ_i' represents the unknown σ_j and r is the number of unknown σ_j. Equations (4) are known as equations of condition. Using matrix notation, the set of normal equations generated from the equations of (4) is

$$(F^T w F)(\sigma) = (F^T w Q) \qquad (5)$$

where σ is a column matrix of the σ_j

F is an r x m matrix of the F_{jk}

F^T is the transpose of F

Q is a column matrix of the elements $(Q_k - \sum_{i=r+1}^{h} \sigma_i F_{ik})$

and w is a diagonal matrix in which the element w_{kk} represents the relative statistical weight of the k^{th} observation.

The least squares solution for σ follows from (5)

$$\sigma = (F^T w F)^{-1} (F^T w Q)$$

The standard error in the k^{th} variable is derived by taking the square root of the k^{th} diagonal elements of the matrix formed by dividing the number of degrees of freedom into the product of the matrix $(F^T w F)^{-1}$ and the weighted sums of squares of the fitted data. The matrix formed as described in the previous sentence is the variance-covariance matrix. The correlation coefficient between the i^{th} and j^{th} variable is given by the square root of the ratio of the i, j^{th} element to the product of the i^{th} and j^{th} diagonal elements. A decoupling is obtained indirectly between the variables x_j and σ_{ij}, by the method of fitting used, which partitions a single interdependent variable space into two smaller independent spaces. To accelerate convergence, the variables are assigned upper and lower limits. If the variable reaches a limit the variable is fixed at the limit and treated as a constant for the remainder of the iteration.

CHAPTER 4

Results of the Theoretical Investigation of the Factors Affecting Curve Fitting of a Two Exponential Function

Summary: Seven different factors which affect the parameter errors obtained by curve analysis were investigated. With ten data points, an exponent ratio of 3:1, and an amplitude ratio of unity, parameter errors of less than 20% are obtained when the data error is ± 2%. However when the exponent ratio reduces to 2:1, the parameter errors exceed ± 30%. When amplitude ratio is greater than 1:1, the parameter errors are less than ± 15%, but when the amplitude ratio falls to 1:4, the errors may exceed ± 50%. Increasing the number of data points to 60 reduces the parameter errors by a factor of 3. If the accuracy of the data is ± 10%, the reduction obtained by increasing the number of data points is less significant. With 60 points of 2% accuracy, the average parameter error with a 2:1 exponent ratio and unity amplitude ratio, is ± 20%. By varying the sampling frequency, a further small reduction in the errors associated with one pair of parameter errors may be achieved.

If the data error is not constant but is allowed to gradually increase, then the 'equivalent' data error, for the purpose of estimating the likely parameter errors may be estimated by a sample graphical method. Failure to collect data over a time which adequately defines the function will result in poor second parameter estimates being obtained. This suggests that extrapolation of a double exponential curve is potentially hazardous. If

data is collected until the effect of the earlier exponential term has become negligible, parameter errors of ± 5% with 60 5% data points and 4:1 amplitude and exponent ratios can be achieved.

The validity of the computer derived parameter errors were found to be more closely related to the absolute value of the error than to the cross correlation coefficients between the parameters.

4.1 Results

4.1.1 Exponent Ratio and Data Error

The effect on the parameter estimates of variation of the exponent ratio and of the data error can be discussed by reference to table 4.1 and figure 4.1. The detailed information used to obtain table 4.1 and figure 4.1 is presented in table 4.1.1. As a general conclusion, changing the data error from 2% to 10% and also reducing the exponent ratio from 4:1 to 2:1 results in an approximately seven fold increase in the errors of all the parameters. In all cases the parameter errors associated with the more slowly varying component are smaller except in the case of the 2:1 exponent ratio data, when the amplitude errors are not consistently smaller. When the ratio is 4:1 increasing the data accuracy from 2% to 10% increases the parameter errors by a factor of four. A similar increase is observed in the case of the 3:1 exponent ratio. When the exponent ratio is 2:1, however, the effect of the data accuracy is negligible and no improvement is achieved even with data which is accurate to 2%, if only ten data points are available. With ten data points, if the amplitudes are equal, an exponent ratio of 3:1 between the exponents probably represents the lower limit of exponent ratio which is likely to yield useful parameter estimates. The

parameter errors will be approximately ± 15% at best, even when the data error is 2% with this exponent ratio. This finding confirms the observation of Riggs (145) that "when a multiple exponential function is used to fit biological data, the rate constants of successive terms almost always differ from each other by a factor of three!" Although Rigg has used the phrase "multiple exponential functions", the example which he uses to illustrate this remark is a two exponential function, with equal amplitude coefficients. The parameter errors will be approximately ± 30% with 5% data and ± 60% with 10% data. The lowest average parameter error, with an exponent ratio of 4:1 and with 2% data is about ± 12%, when the amplitudes are equal. The error on the second set of parameters is however only ± 8% on average. It should be stressed that these remarks only apply to functions described by ten data points.

4.1.2 Amplitude Ratio

If the amplitude ratio is increased to a value of 4:1 when the exponent ratio is 4:1, with 2% data, the average parameter error falls to about ± 7%. With these parameter values the error of the more rapidly changing parameters has dropped from about ± 18% with equal amplitudes to about ± 6% while the error on the second parameters is about ± 9% (see figure 4.2, table 4.2 and table 4.2.1). This represents little or no change in the value of the parameter errors for the parameters associated with the slower exponential terms, however, a substantial fall occurs in the errors associated with the other pair of parameters. As the amplitudes ratio decreases and becomes 1:4, there is little change in the second pair

of parameter errors but a substantial increase in the parameter errors of the first pair of parameters is apparent. This reaches ± 55% when the amplitude ratio is 1:4 with 2% data and ± 115% when the data has 10% accuracy. With 10% data the effect of the amplitude ratio is reduced but even at best the parameter error estimates are about ± 15 to 20%. When the amplitude ratio is 1:4, the effect on the function of the parameters associated with the first exponential term becomes negligible so rapidly, that when only ten data points are used, only one or two data points reflect the effect of the small amplitude component term on the overall function. This accounts for the large parameter errors associated with this term when the amplitude ratio is low (i.e. less than unity). These results indicate, that when the exponent ratio is greater than 4:1, improved parameter estimates will be obtained as the amplitude ratio increases above unity, but as the amplitude ratio is reduced below unity the parameter error estimates increase rapidly. In other words, an exponent ratio of greater than 3:1 does not guarantee that small parameter errors will be achieved unless the ratio of the amplitude terms is greater than unity, when only ten data points are available.

4.1.3 Number of Data Points

It has been shown that when the exponent ratio was 2:1, improvement in data accuracy did not result in any decrease in the parameter errors if only ten data points were used. When the number of data points is increased from ten to sixty some reduction in parameter errors is achieved with 10% data but only by a factor of about two. (Figure 4.3, table 4.3

and table 4.3.1). In fact, even with 2% data, only a factor of three reduction in parameter errors is achieved, by going from ten to sixty points. An interpolation may be carried out from figure 4.2 in order to estimate the parameter errors which would be obtained with 5% data for a function whose amplitude and exponent ratios were 4:1. This may be compared with the results presented in table 4.6, when 60 data points are used. It can be seen that by increasing the number of data points from ten to sixty the parameter errors are reduced from an average of about ± 14% to about ± 3½%. A comparable reduction in parameter errors is achieved with the 2% error data, when the number of data points is increased from ten to sixty, in the presence of low exponent and amplitude ratios. Although an improvement can be obtained for 2:1 exponent ratio data if the number of points is increased from ten to sixty, a substantial reduction in data error would also be necessary, if an improvement of practical significance is to be expected. In such a situation, the absolute values of the exponent parameter errors is nearly ± 10% with 2% data and 60 data points. If, however, small parameter errors are already achieved due to high exponent and amplitude ratios, then a useful reduction in parameter errors is likely to be achieved by increasing the number of data points up to sixty, even if the data accuracy is only 5%. Since it is unlikely in practical situations that sixty samples would be available the information contained in this section is probably only relevant to those techniques where continuous analogue or digital recording of rapidly changing functions is being carried out e.g. cerebral blood flow clearance of radioactive gases. But even in this situation, the inclusion of a large number of sample

points with low accuracy will be unhelpful. The accuracy associated with each data point should be known. If it is too low, a single point of higher accuracy will be of more significance.

4.1.4 Variable Sampling Frequency

To investigate whether the adverse effect of a poor amplitude ratio can be overcome by some preferred sampling method, the spacing of the data points was varied so that the number of points in the earlier part of the curve was substantially increased. Instead of evenly spaced points from t = 2 to t = 20, values of t were chosen at t = 0.5, 1.0, 1.5, 2.0, 3.0, 6.0, 9.0, 12.0, 16.0 and 20.0. The results obtained are shown in table 4.4 and figure 4.4. No significant change in the parameter error estimate occurs in σ_2 or λ_2. The errors in σ_1 and λ_1 are reduced by spacing the data in this way, but only in the case of the 2% data. The reduction is only of the order of a factor of two however. This implies that if the data error is in excess of ± 5%, varying the sampling frequency by taking more early data points is unlikely to improve the parameter estimates although some slight improvement is achieved with ± 2% data. It appears, as in the results of the previous section, that if the parameter errors are not excessive, then the use of variable sampling frequency or increasing the number of data points can improve the accuracy of the parameter estimates. However, if the parameter errors are large, and the other primary factors such as exponent ratio and the data error are also unfavourable, then sampling frequency variations are unlikely to reduce the parameter errors to levels where the parameter estimates will be of practical value.

4.1.5 The Effect of a Gradually Increasing Data Error

In practice the function to be investigated is not always specified by data with a fixed relative error and the effect of a variable and gradually increasing data error was investigated. The error on the test data was allowed to increase linearly from 1% to 10%. Twenty data points were used. It is also of interest to determine whether or not a good estimate of the magnitude of the parameter errors can be obtained from examination of the fixed error information already obtained, even in a situation where the error is variable. The relevant data is presented in table 4.5 and 4.5.1. If a direct linear interpolation is carried out, assuming an average error of 5.5% the parameter error estimates are approximately 5% (absolute) too low. If the interpolation is carried out between the logarithm of the data error and the parameter error it can be seen that very close approximation can be obtained to the actual parameter error as estimated with the variable error data. This interpolation can be carried out simply using semi-logarithmic graph paper. This empirical procedure may be useful in practice if suitable fixed data error information is available. The interpolation procedure, which was used to obtain the results, was obtained from figure 4.5.

4.1.6 Incomplete Collection of Data

A situation which occurs frequently in practical investigations is one where an arbitrary decision must be made when to cease collecting data. Prolonged observation may be expected to improve the parameter estimates, however, this may increase the potential risk or discomfort to the patient

undergoing investigation, and may also introduce the possibility of allowing a change in measurement conditions to occur. If a constant relative error is required, then it may be assumed that it is not possible to change the sampling regime during the investigation. In this situation, two factors may be expected to increase the estimates of the parameter errors, 1) if the function is sampled over a limited period and 2) if the number of data points is smaller than is desirable. Both of these effects combine to increase the parameter error estimates as shown in table 4.6 and figure 4.6. The first six results presented in table 4.6 were obtained by using the same data on each occasion but removing the last ten data points, successively. This particular investigation was carried out using punched card data, not magnetic tape data, since the cards allowed this manipulation to be carried out very rapidly. It will be observed, however, that the slower amplitude and exponent parameter errors increase very markedly compared with the parameter error estimates associated with the more rapidly varying exponent. Although the latter parameter error estimates remain acceptable, when just less than half the data points are collected, the other two parameter errors begin to increase as soon as the number of data points decreases. The parameter error estimates eventually become so large that the parameter estimates are probably useless in practice. The extent to which this effect is due to the smaller number of points being used rather than to a limited definition of the function by the data can be assessed by reference to the last three results in table 4.6.

In obtaining these results, the number of data points was kept constant, but the extent to which the function was defined by the data was

allowed to decrease. The 0-13.3 results should be compared with the "40" results above, and the 0-6.67 results should be compared with the "20" results, since in both cases these correspond to the t values reached when only 40 and 20 data points were used in the earlier investigation. It can be seen that the effect of obtaining a smaller number of data points (40 instead of 60) is unimportant when the function is well defined by the data. However, if this is not the case, substantial parameter errors are inveitable although these can be reduced (in this case by 50%) by collecting a large number of data points, However, the errors in the second pair of parameters, which dominate the "tail end" of the function, are still excessively large, and probably useless in practice. This would suggest that additional reduction of the parameter errors obtained by using more data points is not worthwhile when experimental conditions do not allow data to be collected which will adequately define the function under study.

4.1.7 Computer Error Estimates and Cross-Correlation of Parameters

Besides producing estimates of the parameters and the parameter errors, the Berman programme also prints out estimates of the correlation coefficients between the parameters. The possibility that the magnitude of the correlation coefficient may be used as a guide as to when the computer derived parameter errors may be reliably used, was investigated. The relationship between the correlation coefficients and the difference between the statistical and computer derived parameter errors was examined. The results are presented in table 4.7. It has been pointed out previously

(Appendix 3.2) that the amplitude and exponent parameters are decoupled in the curve fitting procedure, and information concerning the correlation between these sets of parameters is therefore not available. The results presented in this section, therefore, refer only to the use of this particular curve fitting programme. Those results which were associated with parameter error estimates which included one set of censored data have been marked by an asterisk. In no case did any set of data contain more than two results, out of the twenty groups of values, which made up each set, where the parameter estimates reached limiting values i.e. were censored.

The difference between the computer and statistical estimates are presented in table 4.8 and the results are plotted in figure 4.7 and figure 4.8. In the graphs the points corresponding to censored data were omitted. Even when the correlation coefficient is below 0.5, there was one result where the difference between the statistical and computer result was 135% (figure 4.7). If all the results below a correlation coefficient of 0.75 are considered, then out of the 43 data points, 8 (18.5%) of these, indicate a discrepancy between the two methods of estimating the parameter errors, which is in excess of ± 20%.

However, when the computer derived error estimate is less then 10%, the average value of the difference between it and the corresponding estimate calculated statistically, in 28 samples, is only 1.6%. If the computer derived error estimate is less than 30%, then average value for the difference between this estimate and a statistically derived estimate is unlikely to be greater than 7.5%. It would appear that, in practice,

the absolute value of the computer derived error estimate is probably a more reliable guide to its validity, then the associated correlation coefficient, even though a low value of the latter quantity is usually associated with a low value of the computed error estimate. Parameters, for which the computed error estimates are above 30%, are unlikely to be of practical value, and the error estimate itself is of doubtful validity.

CHAPTER 5

Results of the Theoretical Investigation of the Factors Affecting Curve Fitting of a Three Exponential Function

Summary: Despite the use of larger exponent and amplitude ratios and 20 data points, very large parameter errors were obtained in almost every case, due mainly to the incomplete definition of the function by the data. When the 'time' of observation was increased, much smaller errors were obtained. With 60 equally spaced data points of 2% error, a 5:1 exponent ratio and 3:1 amplitude ratio, no parameter error exceeded ± 20%, with t = 30. With t = 20, although the first two pairs of parameters have errors of less than ± 20%, the errors in the last pair of parameters are over ± 100%. When the data error is increased to 8% and only 20 points are collected, using the same parameters and t = 30, the error in the second pair of parameters may exceed ± 20%, and in the third pair of parameters ± 70%. With the amplitude ratio of 1:1, and 20 data points of 2% error, a 5:1 exponent ratio and a value of t = 20, errors of not more than 52% are obtained. Increasing the exponent ratio to 10:1 yields 'fast' parameter errors less than 20%, but the final parameter errors exceed ± 100%. The effect of increasing or decreasing the amplitude ratio causes the parameter errors to vary between 3% to 200%. Increasing the number of data points from 20 to 60 reduces the errors by at least a factor of 2. Variable sampling frequency is relatively less important, unless the effect of the other factors has been substantially reduced. Prolonged collection of the

data is particularly important in fitting three exponentials. Extrapolation of an ill defined three exponential function may produce parameter errors in excess of ± 75%. In an exercise carried out on a function, which closely resembles published curves, errors exceeding ± 70% were found on one pair of parameters when 20 variable error data points (2 - 12%) were used.

5.1 Three Exponential Results

5.1.1 Exponent Ratio and Data Error

While keeping the amplitudes equal, the exponent ratio was increased from 3:1 between individual exponents to 10:1, keeping the absolute magnitude of the final exponent fixed at 0.02. To examine the effect of the the presence of the relativity large exponent, a second set of exponents, also with a 10^0:1 ratio but with lower absolute exponent values was also analysed. The most immediately obvious feature of the results obtained (tables 5.1, 5.1.1, 5.1.2 and figure 5.1) is the comparatively large parameter errors obtained, compared with those observed during the two exponential study. Even with exponent ratios as large as 10:1 and despite the fact that twenty data points were used, the errors are large. The smallest parameter error achieved with a 10:1 exponent ratio and 2% data was ± 13%, the smallest exponent error was ± 24%. When the large first exponent data was used, the error on the largest exponent was 112% with 2% data even with an exponent ratio of 10:1. It is also apparent that improving the data accuracy from 10% to 2% reduces the parameter error estimates somewhat but the effect is not very marked. It is clear that the relatively small number

of twenty data points, in association with an amplitude ratio of unity, still results in large parameter errors, even with 10:1 exponent ratios and 2% data. When only two exponentials are present, when the exponent ratio was increased by a relatively small amount, the parameter error estimates decreased rapidly. This did not occur in the case of three exponentials.

The sensitivity of the parameter errors to the absolute values of the exponent as well as to the exponent ratio, was demonstrated by comparing two sets of data with different absolute exponent values, while maintaining the exponent ratio constant. The very large error associated with the exponent value of 2.0 is reduced when the largest exponent value in the function is reduced. These changes are also reflected in the amplitude error estimates. However, the parameter error estimates associated with all the other parameters became considerably worse. It may be inferred from this result that the extent to which the function is defined by the data is at least as important as the exponent ratios in determining the parameter errors. This confirms the results previously presented concerning two exponentials in 4.1.6 above.

5.1.2 Amplitude Ratio

Amplitude ratios varying from 5:1 to 1:5 were used in association with an exponent ratio of 5:1, and the results are presented in tables 5.2, 5.2.1, 5.2.2 and figure 5.2. Once again the most striking feature of these results is the magnitudes of the estimated parameter errors. As the amplitude ratio decreases from 5:1 to 1:5, the parameter errors increase as an inverse function of the absolute parameter value, although there are some

exceptions to this. The parameter errors of the exponents do not exhibit any particular trend other then a general tendency to follow the errors associated with the amplitudes. When the data error increases to 10%, not unexpectedly, the parameter errors show a large increase, and in the case of one exponent the estimated error is about 316%. It must be remembered that this will certainly be an underestimate of the error. It would appear that for all practical purposes, with twenty data points, that unless the amplitude ratio exceeds 3:1, then the estimate of the smallest amplitude has no significance, even with data which is accurate to 2%. This implies that a two exponential fit to the data would probably describe the data adequately. Although one would expect that with a high amplitude ratio, if the function is examined for a sufficient length of time, the parameter estimates will decrease, this will not be of any value if the ratio is less than unity. The combination of only twenty data points and 10% error data would appear to rule out the possibility of meaningful parameter estimates being obtained, with three exponentials.

5.1.3 Number of Data Points

The results obtained when the number of data points was increased from 10 to 20 and then to 60 are shown in tables 5.3, 5.3.1, 5.3.2 and figure 5.3. Once again the parameter errors remain comparatively large even with 60 points and 2% data with an amplitude ratio of unity and a 3:1 exponent ratio. When the amplitude ratio is increased to 3:1 and the exponent ratio to 5:1 with 60 data points of 2% error, the result shown in the last line of table 5.4 was obtained. By increasing the amplitude ratio

and exponent ratio, comparatively good estimates are obtained of the four larger parameters, while the errors associated with the smallest parameters are still rather large. Reliable estimates of these latter parameters can only be obtained by prolonged observation of the function and also obtaining more data points. This may not be possible in a particular biological study. The reduced effectiveness of increasing the number of data points compared with the two exponential case described previously is a significant finding. Unless the exponent and amplitude ratios are adequate, even with 2% data, acquisition of a large number of data points appears to be relatively ineffective in reducing the parameter estimates. A sufficient number data points must also be obtained to completely define the function (see below 5.1.5).

5.1.4 Sampling Frequency

To investigate the effect of variable sampling frequency, a 5:1 exponent ratio and a 1:3 amplitude ratio was selected and values of t = 0.2, 0.5, 0.7, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0 and 20.0 were used. The results are presented in tables 5.4, 5.4.1 and 5.4.2 and figure 5.4. It is interesting to note that in the case of the 2% data that the effect of the variable spacing is to reduce the parameter errors in all the parameters. It might have been expected that the first parameter errors would be reduced and the final parameter errors increased. The result obtained is probably due to the strong cross-correlation which exists between the parameters. As mentioned previously, it is not possible to assess completely the extent of this

cross-correlation with the Berman programme. This interesting effect of the variable sampling frequency provides an important means of improving the possibility of obtaining useful results from the data, when fitting a three exponential function.

5.1.5 Incomplete Collection of Data

The results obtained on the incomplete collection of data using three exponentials are shown in tables 5.5, 5.5.1 and 5.5.2 and figure 5.5. As before, this investigation was carried out with data on punched cards and not on magnetic tape. The results were obtained by successively removing the last ten data cards from each set of data. The number of data points and the degree of definition of the function by the data are being reduced simultaneously by this process. The results follow the general pattern observed in the case of two exponentials. Despite reducing the observation period, from $t = 20$ to $t = 6.67$ the parameter error estimates associated with the first pair of parameters remain small and acceptable. The errors associated with the other two pairs of parameters increase rapidly. The parameter errors associated with the second exponential term are smaller than those associated with the third term. Once again the absolute magnitude of the errors is disconcertingly large. The separate effects of the number of data points and the extent to which the function is defined by the data, may be assessed from the last two results presented in table 5.5. The 0-13.32 results should be compared with the 40 point data result and the 0-6.67 result with the 20 point result. As observed in the case of two exponentials, the extent to which the function is defined by the data

is apparently much more important than the actual number of data points. Some reduction in parameter error may be obtained by increasing the number of data points, but in general, the parameter errors are not substantially reduced unless these also improve the definition of the function by the data. It may be inferred from these results that any attempt to extrapolate a three exponential function based on data which does not adequately define the function is an extremely hazardous procedure.

The importance of continuing the data collection, when this is possible, until the function under investigation has been as well defined as it is practically possible, is well demonstrated in the two results presented in tables 5.6, 5.6.1, 5.6.2 and figure 5.6. In the first case, parameters were used which allowed the function to be well defined by 60 equally spaced data points. The data error was 2%, and "t" was given values up to 30. The result may be compared with the one shown below it in table 5.6 which is taken from table 5.5. It can be seen that extending the period of observation to t = 30 has reduced the parameter errors substantially to such an extent that even the errors in the final pair of parameters may be considered acceptable. This effect was, however, slightly enhanced by the larger exponent values used. The possibility of getting comparable parameter error estimates by collecting a much smaller number of data points for a comparable time was then examined. 20 data points were selected at the intervals indicated in table 5.6, and to improve the analogy with a practical investigation, the data error was allowed to increase gradually from 2.1% to 11.6%, which we would expect from section 4.1.5 (Chapter 4) to be equivalent to the effect of an average error of

about 8.5%. We are therefore comparing 60 point 2% equally spaced data with 20 point, 8.5% unequally spaced data. Previous experience with functions poorly defined by the data would have led one to expect the parameter error estimates with the 20 point data to be substantial. The results obtained are shown in table 5.6. Although the estimated errors in the 20 point data are large, the results are uniformly better than those obtained with the 20 point 2% data also shown in table 5.6 (taken from table 5.2). That is to say, the much higher data error has been more than compensated for by a prolonged observation period, and the variable sampling procedure. Since from table 5.2 it is apparent that a substantial reduction in parameter errors accompanies a data error reduction from 10% to 2%, the inference may be made that a slight improvement in data accuracy, say limiting the maximum error to 7.5% instead of 11.6%, in association with a number of extra data points (say 10), would result in acceptable parameter error estimates for a three exponential function which adequately defines the data. Any reduction in exponent ratio below 5:1 or of amplitude ratio below 3:1, even with adequate definition of the function by the data, will require a substantial reduction in data error in order to obtain acceptable parameter errors. As can be seen even with the parameter values used, together with 2% data and sixty data points, one parameter error is still as high as 18%. The use of additional data points of poor accuracy is relatively useless. This latter point, which is a common practice will receive further comment in Chapter 6.

CHAPTER 6

Discussion and Conclusions of the Theoretical Study

Summary: Some relevant statistical facts concerning the theoretical investigation are presented. Between 200 and 1200 bits of numerical information were required to obtain each point on the graphs presented in the previous chapters.

A recommendation concerning the design of further studies of this type is made with particular reference to a weakness apparent in the design of the present study. This point concerns the importance of ensuring, that the extent to which the data define the function is held constant, independent of the parameter values.

The effect of some of the factors which were not studied in detail, such as the initial parameter estimates, are discussed. The results of the two and three exponential studies are summarized briefly, and the relevant importance of the various factors are assessed. The extent to which the data defines the function is as important as the data error and exponent ratio if information on all the parameters is required. If the data are inadequate in this respect, only some of the parameters will have acceptable errors. The problem of indicating to an investigator the optimal sampling frequency, number of samples, and the optimum duration of an investigation, has been shown to require certain basic information, if meaningful recommendations are to be made. If the data accuracy and the approximate form of the result are known, then the way in which this

information can be used to provide a meaningful answer, using the data available in the previous chapters, is illustrated, by means of a simple numerical example.

6.1 Some General Points

Before commenting in detail on the results presented in Chapter 4 and Chapter 5, some general comments concerning the theoretical investigation are relevant. Nearly every point presented in figures 4.1 - 5.6 is derived from twenty individual curve fitting procedures. Since each fitting was carried out using at least ten data points, and sometimes 20 or 60 data points, each single point on the graphs is derived from between 200 and 1200 bits of numerical data. Each bit of data is the product of a calculated random normal deviate and an exact number. The data generation, curve fitting and statistical analysis of the results were carried out using various computers (I.B.M. 7090 for the data generation, I.B.M. 7090 and an English Electric KDF9 for the curve fitting, and an Elliott 4120 for the statistical analysis). The acquisition of each point, therefore, represents a not inconsiderable amount of computer time. A total of 440 sets of parameter estimates were obtained in the three exponential investigation.

The magnitude of the parameter errors resulting from fitting exponential functions to data depends on many interelated factors, most of which have been investigated in the preceeding chapters. These factors are:- 1) the exponent ratio, 2) the amplitude ratio, 3) the data accuracy, 4) the sampling frequency, 5) the number of data points, 6) the extent to

which the function is defined by the data, 7) the form of the weighting factor, 8) the degree of correlation between the variables, 9) the initial guess and 10) the curve fitting method used. A complete analysis of the relative importance of these ten factors, by a multivariant analysis, would be a statistical computer task of some magnitude. Although technically feasible, with no analytical tasks of major complexity involved, the cost of such an investigation would be formidable, if not prohibitive. The total "7090" time taken for the preliminary study and the two subsequent investigations was approximately 20 hours, inclusive of the computer on-line output data processing time. The printed output even from this limited investigation was immense. This output could possibly have been reduced by modifying the Berman Programme, since much of the output was not required in this particular investigation. This was not attempted owing to the complexity of the programme. The results, which were obtained in Chapter 4 and Chapter 5, probably represent the minimum which is statistically acceptable, i.e. twenty investigations/sample, and a limited investigation of the effect and interaction of most of the variables.

6.2 The Design of the Investigation

Designing an investigation, to examine the effect of 10 interelated variables, in such a way as to obtain an indication of the relative importance of these variables, requires some prior knowledge, and perhaps intuition, if useful results are to be obtained with a minimum number of samples. In general the design was reasonably successful but one particular point was rather unsatisfactory. In Chapter 3 two alternative approaches

to the problem of the period for which the function should be observed were proposed.

1. Examining the function until such a time as value of the final data point is an arbitrary fraction of the value of the function at zero time.
2. Examining the function for a fixed time.

A third alternative is now proposed:-

3. Examining the function until such a time, that the ratio of the value of the final data point, to the value of the function at that time minus the contribution of the final pair of parameters to this value is, 10:1.

This third proposal is one attempt to ensure that the data always define the function to the same extent, independent of the particular values of the parameters. Thus, when investigating the effect of the exponent ratio on the parameter errors, the final value of 't', or the time, which will be used for each set of parameters, will be different, but the results will reflect more directly the effect of the exponent ratio and will not be affected by the extent to which the data defines the function, as in the present investigation. The same technique should be applied when testing for the effect of the amplitude ratio. For those investigations where the exponents and amplitudes are held constant, the time corresponding to the final data point should again comply with requirement 3, above, even though it is no longer a primary variable in such investigations.

In a practical situation the point in time at which collection of the data ceases may be variable and the value of the function at that time

relative to the initial value is also variable. In an optimum situation, however, it is probably desirable that data should not cease to be collected at least for a time which conforms approximately with the requirements of condition (3) above. In the design of any future study which might be based on the results obtained in this investigation, condition (3) is the recommended criterion in establishing the final 'time' (t) values, when investigating the effects of the exponent and amplitude ratios and the other factors on the parameter errors.

6.3 The Choice of Programme and Initial Parameter Estimates

Except in the preliminary investigation the effect of the different types of programme and the initial parameter estimate was not investigated. There is reason to suspect, however, that between those available programmes which allowed the use of different weighting factors (the Gilles and the Berman programmes) a superior fit may be obtained by one programme (see Chapter 7 below). No attempt has been made to analyse the possible reason for these differences which may be a function of the mathematical methodology. That is to say, the iterative procedure itself, the methods used to accelerate convergence, and the method of assessment of the goodness of fit, are all factors which may lead to the preference of a particular programme in a particular situation.

The initial estimates of the exponents, which the computer programme requires to initiate the iterative procedure, become more critical, if those factors which produce large parameter errors are predominant. The Berman programme contains constraints, such that, unless good initial

guesses are made, the iteration will not proceed, and an appropriate diagnostic message is printed out. This facility does not exist in the Gilles programme. The possibility of incorrect initial guesses affecting the result, to the extent of producing parameter estimates substantially different from those which would have been obtained if the nearly correct parameter values had been used as the initial guess, is especially relevant when the parameter errors themselves are large. If the initial guess can affect the result in this way, the parameter values are unlikely to be practically significant. Convergence to the final parameter values will, however, be more rapid if the nearly correct values are used for the initial guesses. With inaccurate data, if a large error is associated with a parameter, a wide range of parameter values may result in an equally good least-squares fit, and the programme will usually indicate the initial guess to be the final parameter value. One further characteristic of the Berman programme is, that if during the course of the iterative procedure, one of the parameter estimates reaches the predetermined maximum acceptable value, the value of that parameter is held constant at the maximum value, in subsequent iterations.

In this investigation the correct parameter values were used as the initial guesses, and the calculated errors, therefore, represent the minimum errors likely to be achieved in a practical curve fitting procedure. That is to say, larger parameter errors may have resulted if incorrect initial guesses had been used. In practice, the economic necessity of imposing a limit on the maximum number of iterations to be carried out, may have prevented convergence to the true final result if bad estimates had

been used for initial guesses. Even in this investigation, where the correct value was used as the initial guess, the programme occasionally did not converge to a final value, within the limit of five iterations which had been arbitrarily specified for each fitting procedure in this investigation. This probably does not affect the parameter errors estimated by the computer, but may affect the absolute value of the parameters. The effect on the statistical estimate of the parameter errors, which used these absolute values, is not easy to assess, but is probably small, since in most cases, the final values were achieved within five iterations.

6.4 The Two Exponential Study

If, as an arbitrary decision, it is assumed that parameter errors in excess of ± 25% are not of practical value, some specific conclusions may be drawn from the results of Chapter 4. It should be stressed that in all the previous investigations, when discussing the random error on the data, and also assessing the errors on the parameters, two standard deviations have been used, and not one which is more commonly employed. The correct definitions of the terms have been given in Chapters 2 and 3. From table 4.1 ten point equally spaced data, whose error is in excess of 2% and whose exponent ratio is less than 3:1 with an amplitude ratio of unity, does not yield parameter estimates of practical value. If the exponent ratio is increased to 4:1, an amplitude of 1:2 will just fail to produce acceptable parameter errors with equally spaced data of 2% error. However, with variable sampling frequency of 10, 2% data points, acceptable parameter errors may be achieved with a 1:3 amplitude ratio and a 4:1

exponent ratio (table 4.4).

With 10% data, an amplitude ratio of 4:1, an exponent ratio of 4:1, and variable sampling, acceptable parameter errors will be obtained with ten data points. For practical purposes, with only ten data points and with an average data error of about 5% a combination of 3:1 of both exponent and amplitude ratio represents the minimum values of parameter ratios which will yield results of practical significance. If the error is increased, or either ratio is reduced, parameter errors will be greater than ± 25%. Even if one of the ratios is increased, this may not ensure that the parameter errors are below ± 25% (see table 4.2). As the number of points is increased to 20, or even 60, it is still unlikely that ratios of less than 2.5:1 of both amplitude and exponent ratio can yield satisfactory results with data which is accurate to only ± 5%. Table 4.3 indicates the poor parameter accuracy which is achieved with 60 data points, if the exponent and amplitude ratios are too low, even where the data accuracy is good (± 2%).

In a practical situation, where the double exponential fit is imposed on the data, with ten data points whose accuracy is 5%, unless both amplitude and exponent ratio exceed 3:1, then an equally good fit, for practical purposes may be achieved by fitting the data with a single exponential function. Alternatively, if a better mathematical fit is achieved with a double exponential function, then not all the parameter estimates are likely to be useful. Such a result would indicate that more than one exponential term is present, but improved data accuracy, or an increased number of data points will be necessary if practical use is to be made of

the individual estimated parameter values. If the parameter errors indicated by the computer are less than 25%, the 'statistical' parameter error is likely to be within ± 7.5% of this figure.

6.5 The Three Exponential Study

Not one of the combination of variables which were investigated yielded a result in which all the parameter errors were below ± 20%, unless the period of observation of the data was extended beyond the time value, t, normally used during the investigation. This included a case where 60 data points were used in association with an exponent ratio of 5:1 and an amplitude ratio of 3:1 (table 5.3). It is apparent that in this investigation of three exponentials, the function was not followed for values of t which were large enough to allow accurate assessment of the final pair of variables. Although the complete definition of the function by the data is very important, it is apparent from the results presented in table 5.6, when only 20 points with errors varying from 2 - 11.5% were used, that despite adequate definition of the function by the data, substantial parameter errors were obtained. The degree of definition of the function by the data was not investigated in the preliminary study (Chapter 2), and was investigated only to a limited extent in Chapter 4. A preliminary study, before the investigation of three exponentials would have resulted in more useful results, since in particular, the 't' value in the main investigation would have been extended to 30 in all cases. This would have resulted in much smaller parameter errors for the exponent and amplitude ratios which were used. The order in which the various

factors were studied was the order in which they were presented in the text of Chapter 5. When the results of section 5.1.5 became available, it was not possible to re-examine the previous three exponential investigations, with a higher value of 't'. Because of the reduced 'period of observation' which applied during the three exponential investigation, the results must be interpreted with caution. This is best illustrated by comparing investigations (1) and (2) in table 5.6, when by simply extending the period of observation from t = 20 to t = 30, errors in the final parameters are reduced from 66% and 117% to 18% and 12% respectively. Reductions in the second pair of parameter errors also occured, probably due to cross-correlation effects.

One significant conclusion from this study is that unless the function is well defined by the data, and unless more than twenty data points are used, it is unlikely that useful information will be obtained on any but the first pair of parameters. Even this may not be obtained in the presence of an adverse amplitude ratio. In practice, it is seldom known that a particular function contains three, and only three, exponential components, and a solution in terms of three exponentials is usually imposed on the data from independent considerations. It is vital, therefore, to collect the data for as long as is practically possible and for as long as the data is meaningful, that is to say, for as long as the conditions of the investigation remain constant. The only practical means of reducing the time of observation is by a retrospective examination of the results. If in the case of the first few sets of data it is possible to remove a number of data points corresponding to the final period of the investigation and

no significant change in parameter and parameter error values is observed, then the observation period may be reduced. On examining 60 point 2% data in terms of three exponentials, if large parameter errors are obtained, it may be assumed either that there are only two exponentials in fact present, or that the period of observation has been insufficient. If it is not possible to extend the period of observation, then for practical purposes, it will be necessary to consider the data in terms of two exponentials only.

It is extremely unlikely that more than 60 data points of accuracy better than 2% would be obtained in the course of a biological investigation. It has been seen that, even with a 5:1 exponent ratio and a 3:1 amplitude ratio, it is barely possible to obtain useful results. It is, therefore, extremely unlikely that the analysis of such data can be usefully analysed in terms of a number of exponentials greater than three, except in very special situations. Such a situation may conceivably be one, in which independent information is available, from other measurements, which provides detailed dependence relationships between the parameters, or provides known limiting values for parameters. Only then, might it be possible to fit more than three exponential terms to biological data.

6.6 Relative Importance of the Factors Affecting the Parameter Errors

The importance of the exponent ratio and data accuracy in determining the parameter errors in a curve fitting procedure have been appreciated qualitatively for some time. However, the comparable importance of the

requirement that the data points should be collected over a time which is adequate to define the function completely is not so widely appreciated. Expressed in other terms, extrapolation of multi-exponential curve should always be avoided. Riggs (145) has provided one example of the hazard of such extrapolations. Several further points associated with this particular topic emerged in the investigation. In the case of two exponentials, for example, although the errors in the parameter estimates associated with the latter part of the curve begin to increase as soon as the final data are removed from the curve fitting calculation, the errors associated with the other pair of parameters remain small, and the parameter estimates are potentially useful. This latter point makes the 'adequate definition of the data' factor perhaps slightly less important than the exponent ratio and data accuracy factors, since too small a ratio or inadequate accuracy preclude the acquisition of any useful parameter information at all.

Although a high amplitude ratio reduces the data accuracy and exponent ratio necessary to achieve acceptable parameter errors, a reduced amplitude ratio, produces the opposite effect although the latter may be compensated for to some extent by a larger number of data points. If the data error is not excessive, increasing the number of data points reduces the parameter errors, but this does not occur with inaccurate data. Varying the sampling frequency is relatively less important than the other factors.

Since the exponent and amplitude ratios are intrinsic properties of the function under study and are not under the control of the investigator, the order of importance of the main experimental factors is therefore as

follows: 1) Data accuracy, 2) Adequate definition of the function by the data, 3) Number of data points, 4) Sampling frequency. The relative importance of the secondary experimental factors such as 1) the initial parameter guesses, 2) the type of weighting factors, 3) the choice of curve fitting procedure, have not been studied in detail. The same order of importance applies to the fitting of three exponentials with the added complication that the interdependence of the factors is greater. The definition of the function by the data is a more critical factor and the acquisition of 20 data points is probably the minimum requirement. The sampling frequency is much more important if three exponentials are present than in the case of two exponentials.

6.7 Application of the Results to a Practical Investigation

The results described in the previous chapters can be of use in several different ways. When an investigation is proposed, certain basic information is required. The first point concerns the data accuracy likely to be attained. This can usually be estimated by calculation, and, where possible, by reference to previous work. For example, if a curve based on the clearance of radioactivity from the blood is to provide the basic data, then such factors as pipetting errors, radioactivity counting errors, errors in biochemical procedures etc. must be taken into account in estimating the final data error. The possibility of reducing the data error by taking duplicate or triplicate samples, should not be overlooked.

Having estimated the likely error to be found on the data, further information is required. Assuming that it is proposed to analyse the data

in terms of two exponentials, for how long can the data be collected, and how many samples will it be possible to obtain? The data should be collected for a time which is sufficient to define the function adequately. Avoiding, for the moment, the precise explanation of the term "define the function adequately", this implies that some prior knowledge of the general form of the result is a vital prerequisite in answering this question. This indicates that further preliminary information should be obtained. A representative example of the type of curve likely to be obtained in practice should be available. A simple double exponential analysis of such a curve by, say, a 'tail stripping' method, will provide approximate values for the exponent and amplitude ratios. This preliminary analysis will also indicate the time for which the faster exponential component is likely to affect the latter part of the curve.

It is now possible to explain the term "define the function adequately" used above. When the influence of the initial exponential term on the curve has effectively disappeared, which one can arbitrarily take to mean that the numerical value of the first exponential term is less than one tenth of the numerical value of the second exponential term, then at least five more data points should be obtained in that part of the curve. This part of the curve will now be effectively mono-exponential. Five points will allow just three 'degrees of freedom', which is probably the minimum necessary to allow the iterative procedure some flexibility. More data points are always desirable, but this number represents the minimum required over the 'end' of the curve.

The total minimum number, of data points may now be estimated by

reference to the tables presented in the previous chapters. For example, if the exponent ratio is, say, 3:1 with unity amplitude ratio, then reference to table 4.1 will indicate that at worst, if only ten samples are taken, approximately 35% parameter errors may be expected with 5% data errors. If, however, the amplitude ratio is about 4:1, then one may expect by reference to table 4.2 the average parameter error to fall to about 20%. If this parameter error is still considered excessive, then more data points will be necessary. It should be emphasised however, that in acquiring the basic information the data points were equally distributed and no attempt was made to ensure an adequate number of points in the 'tail' of the curve. Reference to table 4.3 would suggest that the improvement associated with an increase in the number of data points from 10 to 20 would reduce this error to about ± 15%. As indicated in table 4.4, some general improvement may be achieved by variable sampling frequency and this is likely to reduce the error to about ± 10%. The estimated parameter error is probably of the order of ± 10%, if twenty 5% data points are obtained, and this is confirmed to some extent by examination of table 4.6. If an inadequate observation time is available, then the large errors in the parameters associated with the latter part of the curve, may increase the errors in the other parameters also, due to correlation between the parameters.

An assessment of the data errors, and examination of a typical curve, would suggest that if twenty samples can be obtained, parameter errors of approximately ± 10% will be probable, in this particular example. If it is not possible to improve the accuracy of the data, then the only way in which the parameter errors can be reduced further is by increasing the number of

data points. Whether or not the parameter accuracy obtained is considered adequate depends on the calculations which will be carried out subsequently using these parameters. For example, the parameter values may be used individually in some formula. Alternatively they may be used to obtain average values for each parameter in a large number of patients. These average values may then be combined in a formula.

The initial decision to interpret the results in terms of a given number of exponentials is usually quite arbitrary. A further analysis which should form part of a preliminary study is to assess whether the data is consistent with one, two or three exponentials. This point has been raised in Chapter 1 and a description of one application of this procedure is described in Chapter 7. It should be pointed out, however, that testing for significance between various exponential fitting procedures is not a simple mathematical procedure, although standard 'F-ratio' tests have been used in the absence of alternative proposals. Another hazard with the 'preliminary study' procedure is the possibility, certainly present if the preliminary curve is obtained from a pathological group, that this particular study may not be typical of the whole group. This criticism may only be answered by widening the scope of the preliminary study. A detailed preliminary investigation may also indicate the extent to which the proposed experimental regime may be relaxed without unduly effecting the accuracy of the results. It may also provide information about the optimum analytical procedure which should be used.

If a short-lived isotope is used in a clearance study, or the rate of clearance is rapid, the duration of an investigation is often outside the

control of the investigator. The collection of data is automatically brought to an end when the radioactive samples can no longer be assayed. Alternatively, difficulties associated with the length of time for which the physiological conditions, associated with the investigation, remain constant, may limit the duration of the test. This latter point is particularly important in studies with which delay affects may be associated e.g. in bone metabolism studies. The results obtained in Chapter 3 and 4 suggest that it would seem prudent to continue to collect data during an investigation as long as it is practically possible, provided that the experimental conditions remain constant. Investigation of preliminary data may indicate that the observation time can be reduced, but in the absence of quantitative information of this type data collection should continue for as long as possible.

6.8 Conclusion

The theoretical study described in the previous chapters presents a quantitative examination of most of the factors which effect the parameter errors in fitting two and three exponential terms to data derived from biological investigations. Such data are unlikely to be more accurate than ± 2% and are unlikely to contain more than 60 data points. In the absence of any previous comparable study, and in view of the large number of interdependent variables, it was necessary to design the investigation in such a way as to get the maximum amount of information with the minimum number of individual studies. Some of these factors, such as the effect of the initial parameter guesses, were not studied, but the importance of this omission was minimised in this investigation by using the correct parameter value as the

initial guess. This ensures that the parameter errors quoted are the minimum likely to be obtained. The difference between different types of curve fitting procedures was not examined, since this represents a complete field of study on its own. This statement also applies to the study of the effect of different weighting factors. The relationship between the parameter errors and the cross-correlation coefficients was investigated in order to examine the extent to which the computer derived errors may be useful in practice.

Those factors outside the control of the investigator, the exponent and amplitude ratios, were allowed to cover a range at one extreme of which acceptable parameter errors were obtained, while at the other extreme, the parameter errors indicated that the results were of no practical value. The interaction between the exponent and amplitude ratio and the various factors under the control of the investigator, such as the data accuracy, the number of data points, the sampling frequency and the period of observation, were examined. The study has indicated in quantitative terms the effects of these parameters and also provided some indication of their relative importance. The results allow the parameter errors, likely to be encountered in an investigation, to be estimated. They also provide information which enables an investigation to be planned in such a way as to obtain the most meaningful results. The commonly asked questions:-

How many samples are required?

How long should the investigation be continued? and

When should the samples be taken?

are shown to be highly interrelated questions, which can be answered only if

further information, in particular the data accuracy and the approximate form of the expected result, is available. The results provided in this investigation give some indication of how to answer these questions in a meaningful manner, if the appropriate information is available. In the event of a further work being carried out along the lines described in this investigation, recommendations have been made concerning changes in the experimental design to limit the major effect in each individual study to that of one variable only. This particularly applies to the method of ensuring that the data defines the function to an extent which is independent of the values of the function parameters.

Although the results of the main investigation have been obtained using only one of the numerous exponential curve fitting programmes which are in existance, this particular programme is generally available and has been widely used. It permits the use of any type of weighting factor and the results obtained with programmes of comparable sophistication are unlikely to be very different, although simpler fitting procedures may produce larger "minimum" parameter errors. The very fact that such programmes are so readily available, may increase the tendency, which many investigators understandably possess, towards fitting multiexponential functions to their data. This has often been done without devoting too much attention to the fundamental problems of the data accuracy and intrinsic form of the function being imposed on the data. The published literature contains numerous examples of investigations in which the experimental data is not sufficiently accurate to justify the complex analyses to which the data has been applied. Such examples have probably detracted excessively from the potential

usefulness of exponential curve fitting. With the information provided in the preceding chapters, it is hoped that a meaningful examination of the limitations which the data impose on the proposed analytical procedure, will be possible. This will enable the potential value of the results to be assessed before the experiment or the data analysis is actually carried out.

CHAPTER 7

The Interpretation of Radioactive Uric Acid Clearance Data

Summary: Uric acid metabolism has been studied by measuring the variation with time, of the concentration of uric acid in the urine in twenty patients, including four patients with normal uric acid metabolism. The results were examined using two different exponential curve fitting methods. In certain cases a mathematically significant improvement in fit to the same set of data was obtained, by describing it with a double rather than a single exponential function. However, different results were obtained by the two different methods. A possible reason for the different results may be the criteria used for assessing the goodness of fit. The presence of a second exponential would suggest the existence of uric acid in a second physiological form, or pool, as has been proposed previously by other workers. The parameters of a two compartment model are presented for each of the patients, where appropriate. These parameters include pool sizes, turnover rates, and the errors in these quantities. Using one curve fitting method, a strong correlation between the presence of two exponentials, and the clinical diagnoses of gout was observed. In five of the twenty cases, the mathematical analysis would suggest that the existence of a second pool is highly probable and its presence is linked with the suggested presence of microcrystals of urate in the blood of gout patients. In two cases of gout, the second pool was in excess of 20% of the total uric acid pool. The accuracy with which the total pool size and turnover rate may be estimated from serum measurements has also been investigated.

7.1 Introduction

Labelled uric acid can be used to estimate the pool size and turnover of uric acid in man. Benedict, Forsham and Stetten (46), Green, Bendich, Bodansky and Brown (47), Bishop, Garner and Talbott (48) and Seegmiller, Granzel, Laster and Liddle (49) have used nitrogen-15 labelled uric acid. Carbon-14 labelled uric acid has been used by Sorensen (50) for the same purpose. A curve is obtained by plotting the relative specific activity of uric acid in the urine against time and the pool size is usually estimated by extrapolating the curve to zero time. Bishop indicated that several of his curves, and those obtained by Benedict, were not straight when plotted on semi-logarithmic paper. This finding was confirmed by Sorensen. Bishop analysed the curves empirically into two exponential components in order to extrapolate accurately to zero time. Sorensen (51) proposed a two compartment system to account for this phenomenon and pointed out its association with a patient suffering from tophaceous gout. The observation by Bishop of curved rather than straight clearance curves was not confined to patients with tophaceous gout. These observations posed several problems, not the least of which is to determine the optimum method of analysing the data. If a mathematically significant improvement in fit is obtained by analysing the data in terms of a double rather than a single exponential mathematical function, a method of estimating pool size and turnover rate in this situation is required. The correlation between the presence of uric acid in more than one "pool" or physiological form and the clinical findings was used in this investigation to assess various analytical procedures. The problems of careful urine collection pose many difficulties and the possibility of

carrying out turnover studies from serum samples only has been investigated.

7.2 Methods

100 μc of C-14 labelled uric acid, of specific activity between 0.05 mc/mg and 0.12 mc/mg, were completely dissolved in 100 ml of isotonic saline by heating, but the solution was not allowed to boil. After cooling, 50 ml were injected into a sealed sterile bottle through a 0.22 μ millipore filter, and the solution was used immediately. The uric acid content of the dose solution was measured immediately after injection by a slightly modified version of the Praetorius method (52) and standards for urine counting were immediately prepared as described below.

The patients were kept on a low purine and low protein diet for one week. A blood sample, to be used as a background sample, was obtained. Immediately after the patient had emptied his bladder, 30 μc in 30 ml were slowly injected intravenously. Blood samples were taken at increasing intervals over an eleven-day period. The blood was centrifuged and the serum was removed, and deep frozen.

Six twelve-hour urine collections were made, followed by eight twenty-four hour collections. The urine volumes were measured. Care was taken to ensure that each patient emptied his bladder just prior to the end of each collection period. The importance of this point will be discussed below. The uric acid concentration of all the urine samples and of the majority of the serum samples was measured by the Praetorius method.

7.2.1 Assay of ^{14}C Uric Acid in Urine

Two standards were prepared by diluting 1.0 ml of the dose solution to 100 ml, then adding 5 and 15 ml of the 1/100 solution to 250 mg of inactive uric acid which had been dissolved in 10 ml of 0.73% (w/v) lithium carbonate, and diluted to 35 ml with distilled water. The tubes were placed in a boiling water bath and the uric acid reprecipitated by the dropwise addition of 2N acetic acid. After cooling to room temperature, the precipitated uric acid was collected in a sintered glass funnel and washed with distilled water until the eluate was neutral. The crystals were dried overnight at 80°C and stored in a desiccator until assayed. Any breakdown products of uric acid in the dose solution which were produced during preparations were eliminated by this procedure. Immediate preparation of standards ensured that no significant breakdown of the uric acid could occur in the dose solution since the time of the injection.

Urine samples of known volumes, increasing from 10 to 200 ml over the period of investigation, were removed from the twelve and twenty-four hour collections. To these samples 250 mg of inactive uric acid were added and the samples were then processed using the method described by Sorensen (50).

Approximately 25 mg of the pure uric acid extracted by this method were weighed in the counting vials, and after gently tapping the vials to break up any accumulations of uric acid crystals, 20 ml of scintillator gel were added by pipette (53). The vials were shaken vigorously to disperse the suspension evenly, cooled at + 4°C, and counted in an automatic liquid scintillation counter. Duplicates were made of at least four samples. Two 25 mg samples of each standard were prepared in the same way, and two

background samples of 20 ml of the gel were used. A total of 30,000 counts were obtained from the majority of samples, the lowest number of counts being 7,000 when the sample to background ratio was 7:1.

The percentage of the injected dose contained in each standard sample, and the weight of uric acid originating from urine, in each sample, was calculated. The percentage of the dose/mg of uric acid in each urine collection was then estimated and the results plotted against the midtime of the collection period.

7.2.2 Assay of C-14 in Serum

A standard solution was made by diluting 1.0 ml of the dose solution to 500 ml. Duplicate serum, standard and background samples were prepared by adding 1.0 ml of serum, standard solution and inactive serum, respectively to 10 ml of scintillator (54). The samples were shaken, and kept for three hours, to allow the protein precipitate to settle. They were then cooled for twenty minutes at + 4°C, and counted in an automatic liquid scintillation counter. 10,000 counts were obtained in samples taken up to two days, and the total counts decreased to a minimum of 1,000 for the last sample. Internal standards were added, and the samples were reshaken, allowed to stand for three hours, and cooled, before recounting.

The percentage of the dose/mg of uric acid in each serum sample was calculated and the results plotted against the time of sampling.

.2.3 Data Analysis

Two exponential curve fitting programmes with facilities for applying

different weighting factors were available for the data analysis. One of these was developed by Gilles and Ben Hameid (34). The second programme used was that devised by Berman (18) for simulation and analysis of biological models, which was used for the exponential curve fitting investigation described previously. An advantage of this programme over the Gilles programme is that error estimates of the curve parameters are also obtained.

There were three specific points of interest in the data analysis problem. The first of these concerns the choice of curve fitting programme. The data were therefore submitted to both programmes, using initially a weighting factor on each data point inversely proportional to the square of the observation. In each case the data were fitted both with a single and a double exponential function. The goodness of fit of each of the functions to the data was then examined to determine whether a statistically significant improvement in fit was obtained by fitting a double rather than a single exponential function to the data. The improvement of fit, if any, which was achieved, was assessed using an 'F-ratio' test. It is appreciated that the use of such a significance test with exponential functions is questionable, because it incorporates a 'degrees of freedom' term involving the number of data points and the number of fitted variables, which does not allow for correlation between the variables (9). In the absence of a simple alternative, however, this method was used to assess the Berman results. The 'SIGMA' value printed out by the Berman programme represents the summation of the square of the differences between the observed and the final estimated values, each term being multiplied by the relevant weighting factors, and divided by the number of data points less the number of variable exponents.

This permits the F-ratios to be calculated readily. The same test of an improvement of fit is available as part of the printed output of the Gilles programme. If the 'Probability of F' printed out by the programme is less than 0.05, then the improvement in fit is considered to be significant.

The second field of interest in this investigation concerns the weighting factor to be applied to the data points. The data were re-examined using both programmes, and substituting a weighting factor proportional to the reciprocal of the observation, since the primary sample procedure consisted in collecting samples over a fixed time, and therefore such a weighting factor may be more relevent to this situation than the one used previously (Gilles, personal communication 1967).

Thirdly, using the weighting factor proportional to the inverse of the observation, the data were re-examined leaving out the first data point. There were several reasons why this data point may be suspect. The initial sampling period was over the first twelve hours after the injection. If the patient's bladder was not completely empty at the time of the injection of labelled uric acid, the initial concentration may be too low. If the time for complete mixing in the first pool, assuming more than one does exist, is slow, then the measured urine concentration may be too high. Finally, the points on the clearance curve have been associated with the midtime of the sampling period. In the most rapidly changing curve examined, this plotting procedure would have introduced a 4% error in the time associated with the first data point.

The results were compared with the clinical findings on each patient, and the correlation between the existence of two components and the presence

of gout was examined.

The Berman programme was then used to analyse the data in terms of a one compartment model, and also in terms of a two compartment model in those cases where two components had been indicated by curve analysis using the Gillea programme, when a weighting factor inversely proportional to the observation was applied. Estimates of the parameters of the model and the errors in these parameters were obtained in this way. This was a model fitting as opposed to an exponential curve fitting procedure. The error in the data due to sampling assay and manipulative procedures has been estimated to be ± 5%. The estimate was calculated from the computer derived errors in the curve parameters in those cases which were unequivocally found to be monoexponential. Since the average value of the ratio of the exponents in those cases where two exponentials were found, was greater than 4.5:1, and the data accuracy was of the order of ± 5%, acceptable errors may be expected in the derived parameters with the number of data points used in these investigations.

7.3 Results

The results of the mathematical analysis are summarised in table 7.1. The urine uric acid concentrations and their corresponding times are presented in tables 7.2 - 7.5. One set of data is plotted in figure 7.1. These times are the mid-times of the collection periods. The urine data were first examined using a weighting factor inversely proportional to the square of the observation. The data obtained for the Berman programme are shown in table 7.6, and the results are summarised in column 3 or table 7.1.

These may be compared with the results obtained with the Gilles programme shown in column 4 of table 7.1. When the weighting factor is changed to one which is inversely proportional to the observation, the effect on the results obtained with the Berman programme is negligible, see column 1, table 7.1. However, when the Gilles results (table 7.7) are examined, it is apparent that there is a strong correlation between the derivation of two exponentials from the data, and the clinical diagnoses of gout. The data were re-examined, using the Gilles programme, with the earliest sample point (at 0.25 days) omitted, and the results compared with those obtained previously. These results are summarised in columns 5 and 6 of table 7.1. The marked influence which the early sample exerts on the fitting procedure is apparent. In those cases where no result is given in table 7.7, the programme failed to converge, when attempting to fit two exponentials to the data.

Although the correlations between the diagnosis of gout, and the detection of the two exponential functions in the data, does not hold absolutely throughout the series, the data from nine out of the thirteen untreated gout patients were better fitted by a double exponential function. Of the remaining two patients who yielded data which were fitted with two exponentials, the data from one of these, number 6, yielded one exponential only if the first sample point was not included in the analysis. This was a normal subject.

The total exchangeable pools and fractional turnover rates, calculated by the Berman programme, are presented in table 7.8. A two compartment model was used whenever an improved fit had been obtained with two

exponentials by the Gilles programme, with all data points, and a weighting factor inversely proportional to the observation. However, in several cases, this meant that the Berman programme was using data in which an improvement of fit with two components had not been indicated by exponential analysis using the Berman programme itself. In five of these cases, the parameter error estimates, not surprisingly, are considered excessive. For these five cases, the value of pool size and fractional turnover rate obtained from the single compartment model were therefore inserted in the table. The turnover rate (mg/day) was calculated from the fractional turnover rate and the pool size. When two compartment analysis was used, the size of the first pool only was used to estimate turnover rate.

The patients have been divided into five groups and the average pool sizes and turnover rates have been calculated from the values in table 7.8 and presented in table 7.9. (The association of high turnover rates with the presence of tophi is considered to be coincidental). The range in each group is also given, although because of the small number of patients in each group these must be interpreted with caution. The larger than normal pool sizes in the gout patients is apparent. The pool size of the gout patient on treatment with allopurinol is within the normal range but the turnover rate is well below normal.

On comparison with the values of Seegmiller (49), the pool size for normals is slightly higher (1186 mg compared with 1071 mg). The pool size in one patient with hyperuricaemia (No. 7) is higher than normal which also agrees with Seegmiller's findings on three patients. The average pool size in the gout group, who have normal turnover rates, (1499 compared with 1400

mg obtained by Seegmiller) and in the gout group with a high turnover rate, (i.e. greater than 800 mg/day) are comparable (2398 mg compared with 2450 mg). In this latter group the turnover rate was also comparable but slightly lower than Seegmiller's results (1008 mg/day compared with 1191 mg/day). The value obtained for the turnover rate in four normals is 740 mg/day which is close to an average value for three normals of 758 mg/day quoted by Benedict (46). Both these figures are higher than the average normal value of 622 mg/day measured by Seegmiller using ^{15}N labelled uric acid and the values of 671 mg/day measured by Sorensen (57). The reason for this difference is not apparent.

In the data from five patients, the Gilles programme indicated that two exponentials best fitted the data, the model parameter error estimates obtained by the Berman programme were not considered excessive, and the presence of two exponentials was independent of the presence or absence of the first data point. In these five patients, the presence of two pools is considered highly probable. The two compartments sizes, the transfer rates between the compartments, and the errors in these quantities, are presented in table 7.10. In the case of the three patients with tophaceous gout, the second pools represent 10%, 18% and 38% of the total uric acid pool. In the other two patients the second pool represents 10% and 22% of the total.

The possibility of obtaining reliable estimates of pool size and turnover rates from radioactivity measurements in the serum was investigated in thirteen patients and the data are presented in tables 7.11 - 7.15. The results are compared with those obtained from the calculations based on all the urine data in each patient, in table 7.16. The serum concentration

measured in samples taken between days 2 and 9 inclusive were used. These limits were taken to minimize any second pool effects, by eliminating the values taken before day 2, and to minimize the effect due to the appearance of breakdown products of uric acid in the serum by ignoring data taken after day 9. This criterion was evolved after inspection of the serum clearance curves. A monoexponential function was fitted to the data and the pool size and turnover rates were estimated.

There is a very high correlation (0.96) between the same quantities estimated by the different methods but the standard error of the estimates are 12.3% and 19.6% for pool size and turnover rate respectively. This would imply that although the serum method can probably be used to obtain useful estimates of the quantities in large scale population studies, the application of the serum results in a particular individual is likely to be less useful than a study performed by analysis of urine samples. The regression line of the urine and serum turnover values is shown in fig. 7.2.

7.4 Discussion

Obvious difficulties arise in interpreting the radioactive uric acid clearance data in terms of pool size and turnover rates when the clearance curve is not monoexponential. In general, attempting to treat such data as a monoexponential function will yield an overestimate of both pool size and turnover rate. In most cases no significant errors will be introduced but in cases associated with large pools and high turnover rates, the errors may be 20% or more. This is the difference in the estimate of both major pool size and turnover rate if the data from the patient 2, for example, are

analysed first as a monoexponential function and then as a double exponential function.

During the course of this investigation, several factors which are of importance in analysing this type of data emerged. The first point concerned the weighting factor to be associated with each data point prior to curve analysis. The weighting factor to be used depends on the sampling technique and measurement procedure. That is to say, there are two main considerations which determine the correct weighting factor which should be used. One is the primary sampling procedure and the other is the measurement techniques and manipulation. There were three stages in the sampling process in this investigation; firstly, the collection of urine over a fixed time period, secondly, taking a fraction from each collection sample, and thirdly, taking a fraction of the second samples for radioactive assay purposes. Fixed time sampling requires that a weighting factor inversely proportional to the observation should be applied. The data were analysed using this weighting factor. The data were also examined, for comparison, using a weighting factor inversely proportional to the square of the specific activity, and as shown, this yielded results which did not correlate as well with the clinical diagnosis. The effect of the secondary sampling procedure is probably such as to make the correct weighting factor which should be applied inversely proportional to the observation raised to a power whose value is between unity and 1.5. It should be stressed that the choice of the correct weighting factor is independent of the final method of assessing the results.

The method of obtaining urine samples by collection over fixed time

periods is an important consideration when the results are to be examined by exponential curve analysis. The concentration of radioactive uric acid in the urine collection represents the average concentration over the whole period since the last occasion on which the bladder was emptied. If the concentration is to be associated with a particular time value, it is important that the bladder should be emptied at the end of each sampling period. If this is not done then the time to which each concentration estimate refers may be subject to a comparatively large error especially during the early part of the investigation. One further more elementary point concerns assigning the time value to the mid-point of the collection period. This assumes a linear function, and it would be more correct to select a time corresponding to the average value of the function during this period. This error is small and corresponds to an error of only 4% in the time associated with the first data point, in the case of the most rapidly changing urine concentration curve observed.

The question still remains whether the initial curvature of the urine clearance data is due to a slow mixing effect or a second pool or possibly both of these. The effect of slow mixing would be most apparent on the first data point, for which, as mentioned previously, there are other grounds for suspecting its reliability. However, in none of the five cases in which it is suggested above that it is highly probable that a second compartment exists, was this result dependent on the inclusion of the first data point in the analysis. This would imply that even if mixing in the first compartment was not complete for twelve hours, the final result suggesting the presence of a second compartment, would have been unaffected. It seems

unlikely that the immediate uric acid pool would be associated with a physiological pool whose mixing time would greatly exceed twelve hours. The decision to include the first data point was based on the better correlation obtained when results were compared with the clinical findings.

The final outstanding problem posed by this investigation is associated with the choice of the curve fitting programme. The difference in the mathematical methods used in these two programmes, and also possibly, of more importance, the methods used to assess the goodness of fit is assumed to account for the difference in the results obtained. In Rossing's work on nitrogen washout curves (20) the criterion for optimum goodness of fit was minimizing the mean squared error ratio, which he says is "the logical criterion to use, if the assumption is made that the error is proportional to the size of the observation". This is in fact what was done in this study also. Danford (28) has discussed the sources of errors in various biological models, and also draws attention to the absence of suitable tests of significance when the real weights of the observations is unknown, and concludes that the choice of model, and therefore the method of testing for the significance of the goodness of fit "will be based on intuition coupled with understanding of the biological process under study". A study of the detailed mathematics of the various minimizing procedures, and methods of significance testing, and their relevance to particular types of problem, is outside the scope of this thesis, but would appear to be badly needed in the field of exponential curve fitting in biological investigations. The absence of parameter error estimates in the Gilles programme is a disadvantage. It must be stressed however that the theory of estimating confidence

regions of non-linear parameters is not well developed and any estimates obtained from the curve fitting procedures are only approximate. No mathematical criteria for selecting a particular curve fitting procedure for any specific problem can be given at this time.

7.5 Conclusion

The existence of a second pool of uric acid, or alternatively, the existence of uric acid in a physiologically different form, is highly probable in five patients of the twenty patients who were studied. In these patients no inconsistency exists between the number of exponentials present, the magnitude of the error estimates, or the effect of the presence or omission of the first data point. As has been pointed out by Boland (58), the popular hypothesis of the nature of gout at present "is that the genesis of the acute attack depends on the fresh deposition of microurate crystals in the joint, and that this is followed by the phagocytosis of the crystals during the ensuing inflammatory process". McCarthy and Hollander (59) demonstrated that the urate crystals could be found in almost all gouty effusions, while Seegmiller and his co-workers (60) found that when suspensions of microcrystalline sodium urate was injected into the joints of gouty patients, an inflammatory reaction could be produced which was similar to acute gouty arthritis. The exchange of uric acid between serum and tophi has been suggested by Seegmiller (57) and Sorensen (52), and it would seem reasonable to assume that the second pool demonstrated in this investigation represents either microtophi or the exchangeable surface of tophaceous deposits. The two compartments analysis procedure provides a method of obtaining a

quantitative estimate of the exchangeable uric acid associated with tophi or microtophi.

CHAPTER 8

Measurement of Cerebral Blood Flow by a Curve Fitting Procedure

Summary: The methods of investigating cerebral blood flow are reviewed. Inert ratioactive gases have been used to measure the total blood flow in the whole brain, the regional flow in certain parts of the brain, and the flow in grey and white tissue in particular regions of the brain. The low energy gamma radiation characteristics of Xenon-133, the most commonly used radioactive indicator for measuring cerebral blood flow, do not permit good localisation of the field in view of the collimator since the 80 keV gamma rays are readily scattered with little loss of energy, and this prevents the effective use of pulse height analysis to exclude the scattered radiation. The collimators normally used "view" a cone of tissue containing grey and white matter in roughly the proportions found in the whole brain. It is possible, however, by use of a suitable collimator to take advantage of the low energy radiation emitted by Xenon-133, in order to measure only the blood flow in the cortex of the brain, which is composed entirely of grey matter. As in the other similar methods, an internal carotid injection of the indicator is used, but the injection is prolonged over two minutes. The clearance data of the activity from the cortex are fitted empirically with a double exponential function from which the initial slope is calculated and the blood flow is estimated. The relevant theory of this method is presented. The response of the collimator has been studied in detail. The flow estimated have been corrected for the arterial pCO_2. The values obtained in

the four normal subjects agree very well with the results obtained by other workers using curve analysis for the estimation of grey matter blood flow. This would appear to confirm the validity of the method. In all the pathological cases, except one, a reduced cortical blood flow was observed. It is suggested that the method of "viewing" grey matter in the cortex only, by means of the special collimator, may make the detection of reduced flow in this region more readily detectable, in comparison with other methods, which rely on curve analysis to "separate" the grey and white contributions to the total count, and which measure all grey matter not only that in the cortex.

8.1 Methods of Measuring Cerebral Blood Flow; a Brief Review

Cerebral blood flow may be investigated in many different ways, some of these methods being quantitative, others semi-quantitative, i.e. suitable for estimating relative changes in blood flow, while others give purely qualitative indications of changes of flow. Several reviews of these methods have been presented in the literature (61, 62, 63, 64, 141). Cerebral blood flow may be expressed either in terms of flow (measured in ml/minute) or in terms of tissue perfusion (ml/minute/gm of tissue). Methods have been described which estimate perfusion in the whole brain ('total flow') and in individual of grey and white tissues. Measurements have also been made of perfusion in a particular tissue in a defined region of the brain ('regional cerebral blood flow'). In patients, these methods have been almost entirely associated with the use of radioactive inert gases. Although the estimate of regional blood flow is of particular interest in the context of this chapter, a brief survey of the methods used for investigating cerebral blood flow and

tissue perfusion, total and regional, is presented.

8.1.1 Miscellaneous Methods

Impedance plethysmography (rheoencephalography) (65, 66, 67, 68, 69) consists of detecting regional changes in electrical impedance of the brain due to variations in the cerebral blood volume, and thus cerebral blood flow. The method appears to have little or no particular advantages and indeed has been severely criticised by several authors (70, 71). Heat clearance techniques have been used in several different ways. In the method used by Betz (72), small heating coils are placed in two gold plates and heated alternatively. The temperature difference between the plates is measured by thermocouples. The clearance of heat by convection in the blood stream is a measure of the regional cerebral blood flow. Using the Krypton-85 clearance technique, (see section 8.1.5 below), this method has been calibrated (73). Gotoh and his co-workers have used a double thermistor method, in which two thermistor beads are placed in the jugular vein, the one nearer the brain being heated for a short period, and the jugular venous flow has been measured (74). A similar method of using heated thermistors has been applied to measure changes in local tissue blood flow by Cooper (75) and by Powers et al (76). The method appears to be useful for physiological studies in animals rather than humans. The clearance of hydrogen measured polarographically using platinum electrodes has been used to measure regional cerebral blood flow in cats (77). The theoretical basis of the method has been discussed by Aukland (78). Meyer and Hunter measured local cortical blood flow in humans by a polarographic method (79) and inferred the blood

flow from the measured oxygen tension.

The electromagnetic flow meter has been used to measure cerebral flow in animals (80, 81, 82) and in man (83, 84, 85). In the case of cerebral blood flow with its profusion of collateral pathways, direct flow measurements carried out with an electromagnetic flow meter do not permit conclusions to be drawn concerning the adequacy of perfusion of different regions of the brain. The technique can only be applied in association with neck dissection. Direct observation of the cerebral vessels in patients undergoing craniotomy (86) and arteriographic methods (87), have been used to estimate cerebral blood flow but these are essentially qualitative methods although Hilal has attempted to obtain quantitative information (88).

A disadvantage of methods asssociated with the insertion of a probe into the tissue, is the possibility of interaction between the probe, and the small region close to the probe of the tissue, which is being monitored. The possibility may always exist that this interaction effects the measured flow in an unpredictable manner. The methods described above which measure regional flow are limited in their clinical application since some of them can only be applied in association with surgical intervention which may not always be acceptable. Furthermore accurate quantitation is frequently difficult and indeed many of these methods have found more application in the laboratory rather than in clinical practice.

.1.2 Basic Theoretical Equations Used in the Measurement of Blood Flow

The dye dilution principle of Stewart (89) as applied by Hamilton and his colleagues (90) has been used to measure cerebral blood flow in man (91,

92, 93). Excellent papers dealing with the theoretical aspects of this technique, and the effect of violation of the assumptions have been presented by Meier and Zierler (94) and Zierler (95, 96). Their findings have been summarized by Lassen and Hoedt-Rasmussen (97). The estimation of flow is discussed in terms of three quantities, the time concentration curve, the mean transit time and the volume of the system. Since this theory is fundamental to the subject, the basic equations are presented here.

If q_o units of tracer are injected as a bolus at time zero and if observed outflow concentration be c(t), then the amount of tracer which leaves the system between times t and (t + dt) is c(t)dt.F, where F is the flow through the system in ml/unit of time. Since all the tracer eventually leaves the system

$$q_o = \int_o^\infty c(t).F.dt = F\int_o^\infty c(t).dt$$

$$\therefore \quad F = \frac{q_o}{\int_o^\infty c(t)dt} \qquad (1)$$

= injected quantity/area under the concentration-time curve

If the whole bolus arrives within the field of view of the detector before clearance starts, then the initial height of the clearance curve is a measure of the quantity of injected material. If V is the volume obtained when a tracer is introduced at a constant rate and allowed to distribute itself in the head, then it has been shown (93) that this is the volume to which a measurement of perfusion rate refers following a bolus injection. In the case of an external detector which measures a variation of activity and not concentration, equation (1) may be rewritten in the form,

$$F/V = q_o / \int_0^\infty q(t).dt$$

where q(t) is the activity present at time t. Since c = q/V, and if λ, the partition coefficient, is defined as V/W i.e. the volume of distribution of the indicator divided by the total weight of the tissue, we may write

$$F/_\lambda W = f/_\lambda = q_o / \int_0^\infty q(t).dt$$

where f is the perfusion rate (i.e. flow/gm of tissue). Then,

$$\frac{\text{perfusion rate}}{\text{partition coefficient}} = \frac{\text{initial height (H)}}{\text{area under the activity time curve (A)}} \qquad (2)$$

i.e. $$f = {}_\lambda.H/A \text{ ml/gm/unit time}$$

F.C(t) is the rate at which indicator is leaving the system at time t, and hence F.C(t)/q is the fraction per unit time of indicator which is leaving the system at time t. A frequency function of transit times, h(t), may be defined such that

$$h(t) = F.C(t)/q \qquad (3)$$

Since all the fluid entering the system at zero time must eventually leave then

$$\int_0^\infty h(t).dt = 1$$

Mean Transit Time

Consider a population of transit times in which t_o appears a_o times

t_1 appears a_1 times etc.

Then $\bar{t}$ (the mean transit time) x $(a_o + a_1 + a_2 + ...)$

$$= a_o t_o + a_1 t_1 + \ldots\ldots\ldots a_n t_n$$

$$\therefore \bar{t} = \frac{\sum_{i=0}^{n} a_i t_i}{\sum_{i=0}^{n} a_i} = \sum_{0}^{n} t_i (a_i/N)$$

where N = the total number of observations. Now a_i/N is the frequency with which t_i occurs, that is, the fraction of the total observations which includes t_i, in time between $t_i + (t_i + dt)$ and it is therefore equivalent to $h(t)dt$:

In terms of measured quantities $\bar{t} = \int_0^\infty t.h(t)dt$ (4)

and substituting from (2) $\bar{t} = \int_0^\infty \frac{t.Fc(t)dt}{q} = \frac{F\int_0^\infty t.c(t)dt}{\int_0^\infty c(t)dt}$

by substitution from (1).

i.e. Mean transit time, $\bar{t} = \frac{\int_0^\infty t.c(t)dt}{\int_0^\infty c(t)dt}$ (5)

Volume of the System

Let the volume of those particles which leave the system between times t and (t + dt) be called dV. The fraction of particles entering the system which require transit times in order to leave between t and (t + dt), is h(t).dt. Therefore the rate at which such particles enter or leave the system is F.h(t).dt, where F is the rate at which fluid enters the system. The volume of these particles is the time required for them to leave the system multiplied by the rate at which they leave. This may be expressed

mathematically in the form

$$dV = t.F.h(t)dt$$

$$V = \int_0^\infty t.F.h(t)dt$$

$$= F\int_0^\infty t.h(t)dt$$

Substituting from equation (4)

$$V = F.\bar{t} \qquad (6)$$

where $\bar{t}$ = mean transit time.

Equations (1), (2), (3), (4), (5) and (6) demonstrate the inter-relationship between perfusion, blood flow, mean transit time and blood volumes. Equations have also been presented by Zeirler, and by Lassen (97) which relate the equations referring to a bolus injection, to those which are relevant when a continuous injection or continuous inhalation method is used.

3.1.3 Measurement of Total Flow (methods not employing inert gases)

Using red cells labelled with Thorium-B, ^{51}Cr and ^{32}P, Nylin and his co-workers used the dilution curve method to assess cerebral blood flow (98, 99, 100, 101). By an intravenous injection method, the mean transit time in the brain was estimated and from this the cerebral blood flow was estimated. More recently a more direct method of estimating mean transit time has been proposed (102), based on the difference between the time between the peaks of the venous and carotid artery activity curves. A similar method has been used by Bell (103). Several workers have measured the transit time

in the brain and used this as an indication of total and also regional cerebral blood flow (104). The difficulties of carrying out an exact measurement of transit time, and of estimating the cerebral volume, which is required if absolute values of flow are to be calculated, remain however. Love and his colleagues have investigated different methods of calculating mean transit time (105) and Oldendorf estimated transit time by measuring the mode transit time using the points of inflection of the cerebral activity curve following an intravenous injection (106, 107). By using a specially shaped collimator he has attempted to obtain uniformity of response between the detectors to enable cerebral blood volume to be estimated (108). The problem of measuring cerebral blood volume severely restricts the possibilities of using cerebral blood flow methods based on transit time measurements.

Iodoantipyrine-^{131}I, an inert freely diffusible substance which readily crosses the blood brain barrier has been used by several wrokers to estimate cerebral blood flow. Jugular venous and femoral arterial sampling, associated with an external counter over the head, allows a Fick principle calculation of cerebral blood flow. This method has been used by Reinmuth et al (109, 110). Iodoantipyrine-^{131}I had previously been used by Saperstein (111) and Steiner (112) to estimate cerebral flow as a fraction of the cardiac output. A second isotopic label, ^{42}K (111) or ^{86}Rb (112), was used in order to estimate the non-cerebral cephalic fraction of the total flow to the head. No arterial or venous samples are required in this method but there are technical difficulties associated with the counting and calibration procedures.

8.1.4 Total Cerebral Flow using Inert Gases

The classical method of measuring cerebral blood flow using nitrous oxide devised by Kety and Schmidt (113) has been very widely applied in its modified form (114) to acquire much information concerning cerebral metabolism (115). The method consists of measuring the arterial-jugular venous nitrous oxide concentration difference, during inhalation of the gas in low concentration. The final venous saturation concentration enables the cerebral blood flow to be estimated from the formula

$$\text{Cerebral blood flow/gm of brain tissue} = \frac{C_{vt} \cdot \lambda}{\int_0^t (C_a - C_v)dt}$$

where C_{vt} = final venous saturation concentration at time t

C_a = arterial concentration

C_v = venous concentration

λ = brain-blood partition coefficient of nitrous oxide.

The accuracy of the method was improved by Lassen and Munck (116) who replaced nitrous oxide by the radioactive inert gas Krypton-85. Since the period required to achieve saturation is at least ten minutes, the method is not suitable for the study of rapid changes in cerebral blood flow. Lewis et al (117) attempted to overcome this disadvantage by the use of Krypton-79, and an external counter placed over the head, but the results obtained by this method are considerably higher than those obtained by the nitrous oxide method. This is attributed to the effect of extracerebral tissues and the attenuation of the gamma emissions by the brain tissue (118). To overcome several difficulties encountered with the Krypton-85 modification

of Lassen, McHenry (119), who also used Krypton-85, measured the desaturation phase instead of the saturation phase as in the original study. With the desaturation technique it is important to allow adequate time for saturation before beginning the measurement (120). The methods discussed in this section measure total cerebral blood flow per gram of tissue.

8.1.5 Regional Blood Flow using Inert Gases

Following the injection of Krypton-85 dissolved in saline into the internal carotid artery of a cat, Lassen and Munck estimated the cortical blood flow in a small region of the cat brain by monitoring the clearance of activity with a small end window geiger counter (121). This work was repeated on dogs and rabbits (122, 123) and a preliminary correlation between cortical blood flow and arterial carbon dioxide tension was established (124). This correlation was subsequently investigated in detail (125, 126). The method was also used on the exposed brain of a human (127). The arterial injection of krypton dissolved in saline was maintained for approximately two minutes following an initial bolus injection. The initial slope of the clearance curve was then estimated and the blood flow calculated from the product of the partition coefficient and the initial slope. The cortex-blood partition coefficient has been measured directly using a double isotope method (128), and by an in vitro procedure (142).

Estimations of regional blood flow through the intact skull have been carried out by Lassen and his co-workers, and others, using scintillation detectors, fitted with cylindrical collimators to achieve a degree of localisation. Krypton-85 (97, 129, 130, 131) and Xenon-133 (97, 132, 133)

have been used in this way. An internal carotid arterial injection of a bolus of radioactivity, without being followed by a continuous injection, was used. The regional blood flow may be calculated from the clearance curve by the height/area formula of Zierler (96). The clearance curve may also be analysed in terms of two components in order to assess the relative flow rates in grey and white tissue. The correlation between the so called outflow detection method of Kety and Schmidt and the residue detection method of Lassen and his co-workers has been discussed (97) and has shown to be essentially the same observation which can be represented by the same basic height/area formula. By using an internal carotid injection the contribution to the clearance curve of extracerebral tissue is effectively eliminated and the effect of recirculation of the radioactive tracer is kept to a minimum. The major criticism of the method is the necessity of performing an internal carotid arterial puncture. This restricts the scope of application of the method and limits its use in some clinical studies.

In an attempt to overcome this drawback, Mallett and Veall (139, 135) introduced their inhalation method. However in order to obtain an accurate assessment of the cerebral blood flow, allowance must be made for the effects of recirculation and the contribution to the measured count rate of extracerebral tissues. Veall and Mallett proposed an empirical correction factor, derived from the expired air curve, to allow for recirculation and analysed their data in terms of two exponentials. The estimated values for cortical flow obtained in this way were lower than those obtained by the injection method. Obrist and his co-workers (136) have also attempted to allow for both of these effects by a more elaborate procedure. Following

an inhalation period of two minutes the cerebral activity is monitored externally for at least forty-five minutes. A monoexponential clearance rate is assumed from thirty minutes onwards. The effect of this slowly varying component is subtracted from the early part of the curve. This remaining curve is assumed to be a composite two compartment curve which is also a function of the arterial concentration. The effect of arterial recirculation is allowed for by assuming that the exhaled air curve reflects the arterial concentration and an iterative procedure is used to find a best fit to the measured data. A further refinement in fit is achieved by allowing for the variable delay between the measured air curve and the head curve. The results obtained by this method are in good agreement with those obtained by the internal carotid injection method of other workers. However, the reliability of this method is still to be determined.

8.1.6 Measurement of Local Cortical Blood Flow through the Intact Skull

The internal carotid bolus injection method of Lassen would appear to be the most acceptable method available for measuring average regional blood flow in the brain. It has two major limitations. Owing to the unfavourable scattering characteristics of the 80 keV energy emitted by Xenon-133, even the relatively long (8 cm x 2.5 cm diameter) collimators used by Ingvar and his co-workers (130, 138) "view" a comparatively large segment of brain. Thus precise regional measurements with this type of open collimator is difficult. Secondly, although a two component analysis may permit the effect of the grey matter component of the clearance curve to be extracted, owing to the large volume of detection this cannot be readily associated

with any particular depth in the brain. The original prolonged injection-initial slope method of Lassen using Krypton-85, made use of the limited range of the Krypton-85 beta rays to limit the volume of cortical tissue viewed by the counter.

The initial slope method described below was used in an extension of this method to humans using Xenon-133 instead of Krypton-85 and measuring the clearance of radioactivity through the intact skull (137, 139). This work was carried out using a special depth focussing collimator (140) in order to confine the region of interest to the cortex, and to maintain the assumptions of the initial slope analysis method valid for practical purposes. The favourable response characteristics of the collimator which were achieved were due in part to the relatively high attenuation of the ^{133}Xe radiation by the brain tissue and to the natural curvature of the skull. The region viewed by this collimator was determined from the measured point source response. The detection characteristics resulted in an enhanced response to cortical tissue. The detailed study of the theoretical aspects of estimating blood flow by this method are presented followed by a detailed description of the method of obtaining the collimator response. The clinical results on eleven patients are presented. The initial slope in all cases was obtained by numerical differentiation of the function obtained by fitting a double exponential function to the isotope clearance data using the Berman programme.

8.2 Theory

8.2.1 List of Symbols

C_t = average concentration of inert gas;
C_{io} = initial concentration of inert gas in i^{th} tissue at onset of desaturation;
C_{ao} = concentration of inert gas in blood during continuous injection;
C_{go} = initial concentration in grey matter of inert gas;
C_{wo} = initial concentration in white matter of inert gas;
f_i = flow in i^{th} tissue in ml/g/min;
f_g = average flow in grey matter ml/g/min;
f_w = average flow in white matter ml/g/min;
f = average blood flow in region viewed by detector;
F = ratio of grey to white matter flow;
k_i = detector sensitivity factor for i^{th} tissue;
k_g = detector sensitivity factor for all grey matter;
k_w = detector sensitivity factor for all white matter;
K = ratio of grey to white matter detector response;
λ_i = partition coefficient between tissue and blood of i^{th} tissue;
λ_g = partition coefficient between grey matter and blood;
λ_w = partition coefficient between white matter and blood;
λ = average partition coefficient to tissue viewed by detector;
N_t = observed count-rate;
N_o = observed initial count-rate;
S_o = initial slope of clearance curve;
T = injection time;
w_i = weight of i^{th} tissue (g);
W = total weight of all tissues.

8.2.2 Derivation of Blood Flow Equation

The desaturation curve following the injection of an inert gas and assuming no arterial re-circulation can be shown (143) to be the summation

of n monoexponential functions, n being the number of different tissues and i signifying each individual tissue.

$$C_t = \sum_{i=1}^{n} (w_i/W)C_{io} \exp - (f_i/\lambda_i)t \qquad (1)$$

The observed count-rate can be expressed as,

$$N_t = \sum_{i=1}^{n} k_i(w_i/W)C_{io} \exp - (f_i/\lambda_i)t \qquad (2)$$

If the initial concentration in each tissue is the same, i.e. C_{io} is a constant and the geometrical response of each tissue is identical then the count rate relative to that at zero time is:-

$$N_t/N_o = \sum_{i=1}^{n} w_i/W \cdot \exp - (f_i/\lambda_i)t \qquad (3)$$

and the initial slope of the clearance curve is given by,

$$\left|\frac{dN_t/N_o}{dt}\right|_{t=0} = S_o = f/\lambda \qquad (4)$$

where $f = \sum_{i=1}^{n} w_i f_i/W$, the average blood flow in the tissues being examined. Let us now examine the effect of inclusion, in the field of view of the detector, of regions in which the initial tissue concentration is different from the tissue region of interest. Within the region of interest, it is assumed that initial concentration is identical, due to a prolonged injection technique. The other regions are represented as a single tissue. The region being monitored is principally grey matter and any grey matter outside the principal region of interest will require a negligible correction to equation (3) partly because of the reduced response of the detector to these regions, and partly because the difference in the initial concentrations

within these tissues, from that in the main region of interest, will be small. For the purposes of obtaining a correction factor for the effect of other tissues, it is assumed that the monitored region can be considered to be effectively composed of two tissues consisting of grey and white matter.

The initial concentration of tissue i, can be expressed (129) by the formula,

$$C_{io} = \lambda_i C_{ao}\left[1 - \exp(-f_i/\lambda_i)t\right]T \qquad (5)$$
$$\doteqdot C_{ao} f_i T$$

Therefore we may write,

$$C_{go}/C_{wo} = f_g/f_w$$

if $k_g w_g/W = Kk_w w_w/W$ and $f_g = Ff_w$, by definition,

$$k_g w_g C_{go}/W = KF.k_w w_w C_{wo}/W \qquad (6)$$

$$\therefore \quad N_t/N_o = KF/(KF + 1)\exp\left[-(f_g/\lambda_g)\right]t + 1/(KF + 1)\exp\left[-(f_w/\lambda_w)\right]t \qquad (7)$$

Therefore expressing the initial slope in fractional form, we obtain

$$\left|\frac{d(N_t/N_o)}{dt}\right|_{t=0} = S_o = \frac{KF}{(KF + 1)}.\frac{f_g}{\lambda_g} + \frac{1}{(KF + 1)}.\frac{f_w}{\lambda_w} \qquad (8)$$

By rearrangement,

$$f_g = (1 + 1/KF)\lambda_g S_o - (\lambda_g/\lambda_w)f_w/KF \qquad (9)$$

since $f_w = f_g/F$, equation (9) may be rearranged,

$$f_g = \frac{(1 + 1/KF)}{(1 + \lambda_g/\lambda_w . KF^2)} \cdot \lambda_g . S_o \qquad (10)$$

Assuming that $\lambda_g/\lambda_w = 0.66$ (80) equation (10) becomes,

$$f_g = \frac{1 + 1/KF}{1 + .66/KF^2} \cdot \lambda_g S_o \qquad (11)$$

The effect of a high value of K and F is such as to make equation (11) identical with equation (4). In the situation where no enhanced response is obtained by using a special collimator, the factor K would equal the relative tissue weights of grey and white matter. If the flow ratio is 4, a typical value, the magnitude of the correction term reduces from 1.19 (for a K factor of 1) to 1.07 with an enhanced collimator response (K = 2). This reduction in the absolute magnitude of the correction factor has a significantly reduced effect on the total error of the estimate of blood flow. For example, the effect of a 50% error in this correction factor would result in an additional error in estimating the cerebral blood flow of ± 7% which would be reduced when the enhanced response is achieved, using a suitable collimator, to ± 3.5%.

8.3 Physical Investigations

8.3.1 Apparatus

A scintillation detector and a special collimator, a ratemeter, incorporating a pulse height analyser (I.D.L. 1750), and a 11" potentiometric chart recorder (Honeywell), were used for measuring cerebral blood flow. The ratemeter and recorder were replaced by a scaler incorporating a pulse

height analyser (I.D.L. 1700), for the point source investigation of the collimator characteristics. An analyser gate width of 35 - 90 keV was used. The detector consisted of a 12.7 cm diameter by 0.63 cm thick sodium iodide crystal and a 12.7 cm diameter photomultiplier (Nuclear Enterprises Ltd.). The lead collimator shown diagrammatically in fig. 8.1 consisted of two segmented tapered circular annuli, cut in a 1.27 cm thick lead disc. Within the central cross-over region, a maximum area of the sodium iodide crystal is viewed. The detector response falls off rapidly outside this region. The mechanical dimensions of the collimator are shown in fig. 8.2 and fig. 8.3. The exposed area of the crystal is equivalent to an unobstructed area of a 2.16 cm diameter crystal. The background count rate of this detector and collimator is 8 counts/sec. The maximum count rate, following the continuous injection over two minutes of 1 mc of Xenon was about 200 counts/second.

The factors which affected the collimator design were 1) crystal size 2) anatomical and mechanical considerations 3) attenuation characteristics of lead at 81 keV, the principal energy emission of Xenon-133.

It is necessary to use a minimum thickness of lead consistent with adequate crystal shielding, since the depth response will be improved by keeping the angle between the conical apertures and the central axis as large as possible. Lead is not the optimum shielding material since the attenuation coefficient of tin exceeds that of lead at 81 keV, tungsten is better than lead by a factor of 7, and gold by a factor of 10. In order to improve the area localizing properties, a segmented geometry was adopted, the segments shielding approximately one third of the crystal exposed by the annuli.

8.3.2 Investigation of the Collimator Response

The response of the collimator to a point source in air was first measured. The point source consisted of a hollow perspex cylinder of 6.3 mm internal diameter and 3 mm internal depth. The wall thickness of the cylinder was 1.5 mm. The upper part of the cylinder base was extended and contained a recess into which a depth micrometer could be inserted. Very fine variations in the distance of the point source from the face of the collimator could thus be achieved. The point source was filled with water which had been equilibrated with Xenon-133 gas, and leakage was prevented by relying on a combination of a good mechanical 'push-fit' between each half of the cylinder and the use of an adhesive ("Durofix").

The collimator response to the point source in air is shown in fig. 8.4. The maximum response occurs 1.75 cm from the collimator face on the central axis. This is approximately the depth below the surface of the head of the lower edge of the cortex in the postero-frontal region. The width of the central axis response curve at 10% and 1% of the maximum response is 1.4 cm and 2.0 cm respectively. The maximum response at 2 cm and 3 cm off axis is 10% and 5%. Fig. 8.5 shows the response of the collimator to a point source of Xenon-133 within the upper part of an inverted, water filled, skull. The width of the central axis response curves at 10% and 1% of the maximum is 1.4 cm and 3.5 cm. However, due to the absorption of the Xenon-133 in tissue, the off axis response is reduced, yielding a maximum of 6.5% and 3.5% at 2 cm and 3 cm from the central axis. If the radioactivity were distributed uniformly throughout the cerebral cavity the total response of the detector may be estimated by volume integration of the point source response

curves shown in fig. 8.6. However, the brain contains numerous sulci, and cavities which do not contain grey or white tissue. The response of the detector system to activity in the brain must take account of this non-uniform distribution. In order to obtain a more realistic approximation to the actual response, a human brain which had been fixed in formalin was sectioned along planes parallel to that in which the collimator is placed during a cerebral blood flow measurement. These sections were photographed and the positives included a scale which allowed the magnification to be estimated. This was usually equal to unity within ± 2%. Concentric circles were ruled on each of the section photographs. The depth of each section below the brain surface was measured accurately.

The chord lengths subtended by each individual part of the brain in the section, excluding sulci, were measured along the circle whose diameters were 1 cm, 2 cm, 3 cm, etc. From the chord length, and the radius of the circle, the total length of the arc of the circle was calculated from the formula, arc length = $\frac{\pi r}{90} \times \sum \frac{\theta}{2}$, where $\frac{\theta}{2}$ is half the angle subtended by each chord at the centre of the circle. The area of brain tissue within each annulus 1 mm wide was then calculated. (Table 8.1a - 8.1i). From the measured response of the collimator to a point source in water and the measured area of each annulus, the response of the detector to each annulus of brain tissue at various depths was then calculated (Table 8.2a - 8.2i). It was assumed that the total thickness of the skin and bone over the surface of the brain would normally be 1 cm. This quantity was therefore added to the depth of brain when choosing the appropriate response value corresponding to each part of brain. These results were plotted on large scale squared

paper. Fig. 8.6 shows these annular responses plotted against the distance of each annulus from the central axis. By calculating the area under each annular response curve using a planimeter, the response of the detector to a plane of any given radius can be found. The planar responses at various depths for circles of radii equal to 1.0, 1.5, 2.0, 3.5 and 5.0 cm were determined by measuring the areas under the curves up to the specified radii. These results are presented in Table 8.3, and the results are plotted in fig. 8.7. In order to calculate the volume response of the collimator, the area under the curves of fig. 8.7 were estimated. However, since a thickness of 1 cm was allowed for skin and bone above the brain, the area corresponding to this thickness was excluded from the planar response curves when calculating the volume response. The results for the calculated volume response are presented in fig. 8.8. It can be seen that if the activity were uniformly distributed throughout the brain, 50% of the detected counts would be due to a cylinder of tissue of 1.5 cm radius and 2 cm deep.

In practice the activity is not uniformly distributed throughout the brain not only because of the presence of cavities and sulci, but also because of the non-uniform distribution of the grey and white matter within the brain. The grey matter predominates near the surface of the brain, and since collimator 'sees' mainly this region, the distribution of the grey matter in the brain is of importance, since it determines the factor K in equation 11 in the previous section. To investigate this, a tracing on paper was made of each section of a brain and the grey matter was outlined. The response characteristics were examined in the manner just described. The area of grey tissue in annuli of increasing radii were measured and also

the total area. The average response in each annulus was found from the point source response data (Table 8.2). The relative response of the grey matter in each plane section was then found by integrating the product of the area and response over the plane. (Table 8.4). From the planar response, by integrating over the depth of the brain, the volume response of the grey matter as a fraction of the total response was found. (Table 8.5). This fraction represents the factor K which is a measure of the enhanced response of the collimator to grey matter. In this case, the result of 2.14 indicates that the detector is twice as sensitive to activity in the grey matter than in the white matter.

The findings of the collimator investigation may be summarised as follows. If the activity was uniformly distributed throughout the brain, 50% of the count rate would be due to a cylinder of tissue of radius 1.5 cm and up to a depth of 2 cm below the surface of the brain. If the activity was distributed uniformly in both grey and white tissue, then the detector will have twice the sensitivity for the activity in the grey matter than in the white matter. Since in a normal subject the grey to white matter flow ratio is approximately 4:1, (equation 5, section 8.2.2) this would imply that the initial count rate in the detector due to activity in the grey matter will be approximately eight times that due to activity in the white matter, and as indicated, 50% of these counts will be arising in a small volume close to the surface of the brain.

8.3.3 Data Analysis

The clearance curves were examined and a reading taken at five second

intervals and the background was subtracted. 'Zero' time was usually taken to be within 10 seconds of the end of the injection. The data was analysed using the Berman programme by fitting a double exponential function to the data. The maximum count rate was usually about 200 c/s, using a ratemeter time constant of 1 second, which results in an initial probable error of approximately ± 3.5%. When the count rate had dropped to about half the maximum value, the time constant was usually increased to 3 seconds resulting in a probable error of about ± 3%. As the count rate decreased further the error increased. In the Berman analysis a constant relative error of ± 5% was assumed in order to set the weighting factor required by the Berman programme. The normalised initial slope was calculated substituting the parameters derived by the curve fitting procedure in the formula:-

$$\text{Normalised Initial Slope} = (\sigma_1\lambda_1 + \sigma_2\lambda_2)/(\sigma_1 + \sigma_2)$$

The error estimates derived by the Berman programme were not used to calculate errors in individual flow estimates, since they are not independent errors but also becasue it was felt that a more useful assessment of the results may be obtained from the spread of values observed in groups of similar subjects or patients. Nevertheless, the estimated parameter errors have been noted and are presented in Table 8.6.

.4 Clinical Investigations

.4.1 Method of Measuring Cortical Blood Flow

Measurements of cortical blood flow were made on anaesthetized patients undergoing cerebral angiography for suspected intracranial lesions. Anaesthesia was induced with thiopentone and maintained with 75% N_2O and oxygen.

D-tubocurarine (15 - 25 mg) was administered and additional doses of 10 mg were given as required. The patients were ventilated with intermittent positive pressure on a Barnet ventilator. An open circuit was used and the expired gases were led outside the investigation room through a thick-walled plastic tube. The collimator and detector described in section 8.3.1 were mounted on a special stand and lowered on to the patient's scalp with the centre of the collimator above the posterior part of the frontal cortex.

A catheter was inserted into the internal carotid artery via the common carotid artery. The position of the catheter was confirmed by radiography. About 10 - 15 ml of a saline solution of Xenon-133 was injected into the catheter, rapidly at first, and then more slowly over two and a half minutes. The arrival of activity in the cortex was monitored on the chart recorder and the clearance curve obtained on completion of the injection. A sample of carotid artery blood was taken after each flow estimation for the measurement of the arterial carbon dioxide tension.

8.4.2 Clinical Results

Cortical blood flow was measured in eleven patients, of whom four were diagnosed to be radiologically normal. The computer derived results are presented in Table 8.7. The pCO_2 correction factors were obtained from the relationship derived by Harper and Glass (125) in measurements on dogs. Over the straight portion of the curve the correction factor is 2.7%/mm Hg of pCO_2. This compares with the value of 2.1%/mm Hg used by Reivich (126) and the value of 2.5%/mm Hg derived by Kety and Schmidt (145). The cortical blood flow was corrected to a pCO_2 of 38 mm Hg. In five of the patients

duplicate determinations were carried out, in four of these after passive hyperventilation and in the fifth after hypoventilation. In three of these cases (marked with an asterisk in Table 8.7), the pCO_2 was outside the range (25 mm - 65 mm Hg pCO_2) within which it is considered that the pCO_2 correction factor may be applied with some degree of reliability, and these results were therefore excluded from the final assessment. In deriving the cortical blood flow, a partition coefficient of 0.84 (142) was assumed in all cases, since at the time when these measurements were carried out, the dependence of partition coefficient on haematocrit was not appreciated and the latter quantity was not measured. The normalised initial slope, which had been corrected for pCO_2, was also multiplied by a factor 1.07 as required by the theory (8.2.2) in order to calculate the flow. The clinical results are summarized and the diagnoses are presented in Table 8.8. In one case (F.R.) the result is included although the clearance curve was inadvertently lost, but the result is quoted in a preliminary publication by Harper, Glass, Steven and Granat (139). The result for this subject is very close to the mean value determined for the normal group and its inclusion does not substantially affect the mean value.

The average cortical flow in the normal group was found to be 0.820 ± .146 ml/min/gm, which agrees well with the value of 0.787 ± .148 ml/min/gm (pCO_2 of 38) quoted by Ingvar et al (138). The results in the pathological group are substantially lower than the normal mean in all cases except one. The mean flow in this group is 0.48 ± .156 ml/min/gm. This difference is statistically significant, 'p' is less than 0.01.

8.5 Discussion

This work was carried out to investigate the possibility of extending the 'initial slope' method of measuring cortical blood flow using Krypton-85, to humans, without the necessity of exposing the brain cortex. The latter condition required the use of a gamma emitting inert gas radioisotope. In order to maintain the theoretical assumptions on which the method depends, it was necessary to develop a collimator, which would effectively 'view' mainly the cortex. It was necessary however to be able to assess the effect of the contributions of other tissues. The low energy emission of Xenon-133 offered two advantages. The detecting crystal could be thin and this allowed the focus of the collimator to be close to the front face, while still achieving good detection efficiency. Secondly, the appreciable attenuation of the radiation in tissue favoured the preferential detection of cortical radioactivity. The major disadvantage of Xenon-133 is the difficulty in distinguishing between the scattered and unscattered components of the detected radiation, owing to the small change in energy which occurs with scattering at 81 keV. This implies that the 'field of view' of the collimator is much larger than might be expected from purely geometric considerations.

The problem, therefore, resolved itself into two distinct parts. The first part related to the construction and testing of a suitable collimator. In view of the highly non-uniform volume which was being investigated (one hemisphere of the brain), it was not possible to infer the detection characteristics of the collimator from a simple 'point source response'. The 'volume' response of the detector was, therefore, investigated, as described above. The rather large 'focal region' is due to the large scattered

component in the detected radiation. In order to achieve a finer 'focal region', it is necessary to use an alternative radioisotope to Xenon-133. One possibility is Krypton-85m, a four hour half life isotope which emits a 120 keV gamma ray. The partition coefficient of Krypton between blood and grey matter is about 10% higher than that of Xenon, which is an advantage.

The second part of the investigation concerns the analysis of the data obtained when the collimator was used to measure cerebral blood flow. Although the curves have been analysed into two exponential components, this analysis has been performed entirely with a view to estimating the initial slope. No attempt has been made to attach significance to the components, i.e. in effect a 'total curve' analysis has been performed. Any other suitable mathematical function may equally well have been fitted to the data. In other methods which use 'total curve' analysis (e.g. the height/area formula of Zierler) it is only possible to obtain a flow measurement which is a weighted average of blood flow in all tissues. To measure blood flow in grey and white matter normally requires curve analysis to be carried out on the clearance curve and the region of brain being assayed is a truncated cone of tissue through one hemisphere. In the method described above, essentially cortical grey matter blood flow is being estimated without recourse to exponential analysis. The good agreement between the normals measured by this method and by the curve analysis method would appear to confirm the validity of this procedure. However, the more confined regional properties of the initial slope method described here make this technique more sensitive than other procedures to changes in cortical blood flow.

The analysis procedure does, however, present certain difficulties. For example, if one is interested only in the initial slope of the clearance curve, how much of the curve is necessary in order to obtain a reliable estimate of the initial slope? If too much of the curve is included in the analysis, the effect of the latter portion of the clearance curve may unduly affect the estimate of the initial slope, since the assumption that the [redacted] relative concentration of tracer initially in the tissues of interest, is equal, is no longer valid. The extent to which the estimate of the initial slope is dependent on the selection of the 'time zero' point is also unclear. Many of these uncertainties become relatively unimportant, if a high counting rate is achieved by the detector so that the data accuracy is high. In this case, the very early part of the curve only may be fitted with two exponentials using say 10 data points each of at least 2% accuracy. If the clearance curve is followed for 50 seconds and a data point is taken every five seconds and averaged over that period, then approximately 2500 counts must be detected in each period, or a counting rate of 500 c/second must be achieved on the last point. Since 50 seconds is just over a normal half time of clearance from the cortex, this would imply that as initial count rate of 1000 c/s (error ± 3%) is required. From Table 4.2 (Chapter 4) one would expect about 5% parameter errors in this situation. Since the value of the initial slope is largely dependent on the larger parameters (which have a smaller error), the error in the initial slope estimate is probably less than 15%. This figure is arrived at by assuming the parameters are independent, using the normal 'propagation of errors' formula. However, when the maximum count rate is low, as it was

in this investigation (200 c/s), the estimate of the initial slope is less accurate. The lower accuracy of the data is usually associated with a higher correlation between parameter estimates, which increases the value of the computer derived parameter error estimates. With this count rate it is necessary to use data derived over a period longer than fifty seconds in order to perform a curve fitting procedure which is to be meaningful. A five second sampling period was continued up to five minutes. In five cases, where the clearance curves were predominantly monoexponential, the fractional errors in the exponents were 3.3%, 3.9%, 2.9% and 10% and 8.8%, which are effectively the errors in the initial slope. However, the estimated errors are greater in the double exponential curves. The statistical procedure for estimating the errors, described in Chapter 2, may well be usefully applied here. It has not been done for two reasons. The low count rate achieved with the depth focussing collimator barely justifies extra effort which would be necessary to carry out the detailed error assessments of individual flow estimations by a statistical procedure. Secondly, the small number of subjects examined by the method served simply to assess the potential value of the method rather than serve as a detailed application study. The five measurements carried out on the four normal subjects may be classed as a single homogeneous group, which has been compared with the remaining measurements carried out on a non-homogeneous group of patients with confirmed pathology. The difference between the means of the two groups is highly significant, but of more specific interest is the difference between the normal mean and each individual estimate. In this case the accuracy of each determination is of importance. The accuracy of the individual

measurements may be improved by using a larger injection quantity of activity or exposing more crystal area. It is possible that the use of a different isotope would substantially increase the sensitivity since with less scatter, a comparable resolution could probably be achieved with greater sensitivity. Other alternatives are a higher injected dose or a larger crystal. In view of the minute radiation hazard compared with that received from cerebral angiography, the use of the increased dose would appear acceptable when the two types of investigations are being carried out simultaneously.

Because of the rapid variation in response with depth it is of importance to keep the collimator in contact with the skin in order to get maximum sensitivity to cortical activity. The skin or bone will not receive significant amounts of activity unless substantial backflow occurs down the internal carotid artery. This was normally not expected to occur with the prolonged injection method used in this investigation. If a significant air gap is present between the collimator and skull, or if the skull and skin thickness were excessive, reduced sensitivity as well as a reduced rate of clearance might be expected. It is considered extremely unlikely that the observed differences between the normal and pathological cases could be accounted for in terms of a technical artefact of this type.

The variations in successive measurements under different conditions of pCO_2 may well be associated with insufficient time being allowed to obtain a steady condition before the second measurement was carried out. Since it was considered desirable to take the patient off anaesthesia as rapidly as possible, only five minutes were normally allowed in order to

achieve equilibration following hyperventilation. It is possible that the reduced values of cerebral blood flow, when corrected for pCO_2, observed in the subjects in which repeated measurements were carried out may be related to this phenomenon. When this work was carried out, the dependence of the partition coefficient on haematocrit was not appreciated and this factor was not measured. The error introduced by assuming a normal haematocrit in all cases is probably not significant, since within the haematocrit range of 35 - 45, the change in partition coefficient is only ± 0.035 in mean value 0.84, i.e. the error introduced is at most about 4%. None of the patients who were investigated exhibited any unusual symptoms, which might suggest an abnormal haematocrit.

The clinical results demonstrate a significant difference in cortical blood flow between the normal and abnormal group. Of particular interest is the very low cortical flow observed in the case of subject Hutcheson, in who marked spasm was observed on the carotid angiograph. In only one subject (Scott) did there appear to be a normal flow in the presence of proven pathology. It may be that the clear difference in the cortical flow between the normal and abnormal group, is associated with the sensitivity of the cortex to the presence of such abnormal conditions. Fieschi (131) has observed that where a reduction in flow has been measured in patients with ischaemia, the major change was found in grey matter flow (33%), while the reduction in white matter flow was only 23%. McHenry (145) found a 27% reduction in total blood flow compared with normals in a group of patients with middle cerebral or internal carotid occlusion. This compares with the 37% reduction in cortical flow found in the pathological series described

here. In some individual patients, the flow was less than 50% of the normal cortical flow. These figures correlate reasonably well with what would have been expected from Fieschi's observation.

8.6 Conclusion

Although other workers refer to 'regional' measurements, these measurements are usually 'regional' in only the crudest sense, with large overlapping regions being 'viewed' by the individual detectors owing to their poor volume response. Very few, if any, such responses have appeared in the published literature on the measurement of cerebral blood flow. The properties of the collimator described above are not ideal. The collimator represents an attempt to obtain a genuine regional flow measurement, where the region is sufficiently small to be anatomically significant and where it is also of limited depth and does not, as in other methods, see a 'cone' through one half of the brain. The acceptable response of the collimator has reduced the use of exponential curve analysis to an empirical curve fitting procedure. The good agreement between this and other methods, together with the very much improved localisation properties of the depth focusing collimator would suggest that a more sensitive version of the collimator may have useful application in clinical practice. A more 'regional' measurement of the type possible with this collimator may be even more sensitive to cortical blood flow changes in the presence of disease, since the latter tissue is being viewed to the relative exclusion of other tissues by the method described. Provided sufficient activity can be injected in order to improve the accuracy of the method, then the initial slope can be estimated after one or

two minutes observation, instead of the ten or more minutes required by other methods. An estimate of the result can be obtained rapidly from the clearance curve, and the exact initial slope may be estimated subsequently by empirically fitting a double exponential function to the data.

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THE ANALYSIS OF ISOTOPE CLEARANCE DATA IN BIOLOGICAL SYSTEMS

by

H. I. GLASS, B.A., A.INST.P.

Thesis submitted for Ph.D. (Glasgow) June 1968

The Analysis of Isotope Clearance Data in Biological Systems

Abstract: Clearance curves resulting from biological studies using radioactive isotopes are frequently described mathematically in terms of the summation of a number of exponential terms. This allows the curves to be interpreted by reference to the physical characteristics of a model of the biological system. Numerous exponential curve fitting methods are now available which make use of digital computers. Despite the very widespread application of exponential curve analysis, a systematic study of the relative importance of the factors which affect the parameter errors has not yet been described.

A quantitative statistical study of the problem is described in this thesis with particular reference to the special limitations encountered in biological investigations. These limitations are firstly, the limited number of samples and, secondly, the relatively poor accuracy normally associated with such studies. The accuracy would not normally be better than ± 2% nor would the number of samples exceed 60. Of the ten principal factors which affect the errors in the estimated parameters, two of these, the exponent and amplitude ratios, are intrinsic factors dependent on the system under study. The principal factors under the control of the investigator are the number of samples, the data accuracy, the sampling frequency, and the duration of sampling, which determines the extent to which the data define the function under study. Other factors of lesser importance were not investigated in the same detail as those mentioned above.

Artificial data, on which a controlled random error was superimposed, were generated by a computer programme and recorded on magnetic tape in a format suitable for exponential analysis by the Berman SAAM-22 computer programme. The parameter errors were estimated by a statistical analysis of twenty curve fitting operations carried out on twenty different sets of data with a constant controlled random error. Twice the coefficient of variation, expressed as a percentage, was taken to be the parameter error. A range of exponential and amplitude ratios was investigated for two exponential and three exponential functions with data errors from 2 - 10%.

The study has indicated, in quantitative terms, the effects of the various factors on the errors associated with the estimated parameters, and also the relative importance of these factors. The results indicate the conditions which must be fulfilled if reliable results are to be obtained by exponential analysis. The information is also of value in designing investigations which will subsequently involve exponential analysis of the data. In view of the parameter errors encountered in the study of two and three exponential functions, it appears unlikely that analysis of biological data in terms of a greater number of exponentials will be helpful unless further independent information is available concerning the biological system under study.

Two clinical applications of exponential curve fitting procedures are described. In a study of uric acid metabolism, two different computer programmes were used to examine the same data. A mathematical significance test was used to indicate which sets of data were better fitted by a double rather than a single exponential function. It was found that with one of

these programmes only, when using a particular weighting factor on the data, a strong correlation exists between the indication of a double exponential function in the data and the clinical diagnosis of gout. This is interpreted as evidence of the existence of uric acid in two different physiological forms in gouty patients.

In the second study, the detailed investigation of a depth focusing radioisotope collimator, and its use in the measurement of local cortical blood flow in the brain, is described. By using this collimator, clearance curves of radioactivity from a very small volume of brain tissue in the cortex were obtained. The curves were analysed empirically by means of a double exponential curve fitting procedure, in order to determine the initial slope. No biological significance is assigned to the individual exponential terms. Since the collimator is designed to accept radiation originating specifically in the cortex, the detector is particularly sensitive to changes of flow in this tissue. The results obtained for cortical tissue in normals agree with the values of grey matter flow determined by other workers on much larger regions of the brain containing both grey and white tissues.

THE ANALYSIS OF ISOTOPE CLEARANCE DATA IN BIOLOGICAL SYSTEMS

VOLUME 2

by

H. I. GLASS, B.A.

Thesis submitted for degree of Ph.D.

to the University of Glasgow, June, 1968.

VOLUME 2

I N D E X

Tables of Chapter 2

TABLE 2.1

Dependence of Parameter Errors on Exponent Ratio and Data Error

Results obtained with the Marquardt Programme (11 data points; amplitudes 0.5)

Exponents	Data Error	Number of Valid Observations	Parameter Error $\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\lambda_1$	$\Delta\lambda_2$
.4, .1	2%	10	10.58	10.62	11.54	6.64
.3, .1		8	11.16	12.28	11.12	6.56
.2, .1		9	115.16	106.16	62.18	54.80
.4, .1	3%	10	15.90	14.64	17.26	10.12
.3, .1		10	38.24	39.96	27.02	22.16
.2, .1		10	113.10	152.32	71.16	81.52
.4, .1	5%	10	38.08	38.82	43.88	24.80
.3, .1		10	65.24	74.48	57.46	44.98
.2, .1		7	74.92	156.44	60.38	91.42
.4, .1	10%	10	41.36	30.60	46.36	19.98
.3, .1		10	89.98	101.62	59.44	70.62
.2, .1		8	85.90	197.00	82.48	95.74

TABLE 2.2

Dependence of Parameter Errors on Exponent Ratio and Data Error

Results obtained with the Gilles Programme I (without weighting factor)

(11 data points; amplitudes 0.5)

Exponents	Data Error	Number of Valid Observations	Parameter Error $\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\lambda_1$	$\Delta\lambda_2$
.4, .1	2%	10	11.22	11.52	12.00	6.40
.3, .1		8	9.12	10.38	9.44	4.52
.2, .1		4	76.16	44.70	32.28	16.98
.4, .1	3%	9	13.18	16.10	18.40	11.18
.3, .1		9	33.88	31.94	24.30	17.60
.2, .1		4	50.40	17.86	41.20	3.82
.4, .1	5%	9	34.88	33.08	38.52	21.44
.3, .1		6	29.06	19.70	34.36	10.84
.2, .1		3	45.76	64.40	57.54	33.56
.4, .1	10%	7	29.00	24.98	35.06	17.48
.3, .1		3	29.60	36.30	22.26	17.36

TABLE 2.3

Dependence of Parameter Errors on Exponent Ratio and Data Error

Results obtained with Gilles Programme II (with weighting factor)

(11 data points; amplitudes 0.5)

Exponents	Data Error	Number of Valid Observations	Parameter Error $\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\lambda_1$	$\Delta\lambda_2$
.4, .1	2%	10	7.06	7.34	8.92	0.0
.3, .1		9	17.18	20.06	14.60	2.88
.2, .1		7	56.44	52.34	22.70	19.64
.4, .1	3%	10	11.84	11.98	13.50	14.62
.3, .1		9	24.24	26.94	17.84	4.06
.2, .1		3	86.90	53.16	36.34	19.70
.4, .1	5%	9	28.20	27.70	35.44	16.86
.3, .1		10	41.90	39.18	45.16	19.18
.2, .1		5	60.92	46.32	61.56	60.16
.4, .1	10%	10	21.78	17.17	27.08	21.38
.3, .1		6	59.92	72.56	48.88	41.92
.2, .1			68.10	73.52	25.44	39.40

TABLE 2.4

Dependence of Mean Parameter Errors on Exponent Ratio Data Accuracy (Summary)

		Marquardt Programme		Gilles Programme		Gilles Programme II (with weighting factor)	
Data Error	Exponent Ratio	Mean Parameter Error (%)	Number of Valid Observations	Mean Parameter Error (%)	Number of Valid Observations	Mean Parameter Error (%)	Number of Valid Observations
2%	4:1	9.84	10	10.28	10	6.34	10
2%	3:1	10.28	8	8.36	8	13.42	9
2%	2:1	84.56	9	42.54	4	36.06	7
3%	4:1	14.32	10	14.72	9	9.68	10
3%	3:1	32.04	10	26.92	9	23.28	9
3%	2:1	104.52	10	28.32	4	22.96	3
5%	4:1	36.38	10	31.98	9	21.66	9
5%	3:1	60.54	10	23.48	6	32.72	10
5%	2:1	95.80	7	50.32	3	52.34	5
10%	4:1	34.62	10	26.64	7	23.18	10
10%	3:1	80.42	10	25.88	3	61.40	6
10%	2:1	115.28	8	-	-	-	-

TABLE 2.5

Dependence of Parameter Error on Number of Data Points

(amplitudes 0.5; exponents 0.2, 0.1)

Data Error	Number of Data Points	Parameter Error (Marquardt)				Parameter Error (Gilles with Weighting Factor)			
		$\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\lambda_1$	$\Delta\lambda_2$	$\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\lambda_1$	$\Delta\lambda_2$
2%	21	17.34	14.66	9.40	2.82	6.68	8.30	6.34	4.02
2%	11	11.16	12.28	11.12	6.56	17.18	20.06	14.60	2.88
10%	21	64.92	61.38	79.50	35.82	32.74	46.04	40.98	24.62
10%	11	89.98	101.62	59.44	70.62	59.92	72.56	48.88	41.92

TABLE 2.6

Dependence of Parameter Error on the Data Points (Summary)

	Mean Parameter Error %			
	Data Error of 2%		Data Error of 10%	
	21 points	11 points	21 points	11 points
Marquardt Programme	11.06	10.28	60.40	80.42
Gilles Programme (with weighting factor)	6.34	13.42	36.10	61.40

TABLE 2.7

Comparison of Methods of Estimating Parameter Errors

Method of Estimating Error	Data Accuracy	Parameter Error $\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\lambda_1$	$\Delta\lambda_2$	Mean Parameter Error
Non linear	2%	4.06	7.86	3.06	5.44	5.10
Linear	2%	22.68	24.96	14.88	14.16	19.10
Statistical	2%	11.16	12.28	11.12	6.56	10.28
Non Linear	10%	16.20	36.66	11.90	32.74	24.38
Linear	10%	80.02	51.32	83.64	79.62	73.65
Statistical	10%	89.98	101.62	59.44	70.62	80.42

TABLE 2.8

Parameter Errors in a Simulated Practical Investigation

Number of Data Points	Parameter Error				Mean Parameter Error
	$\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\lambda_1$	$\Delta\lambda_2$	
11	4.70	25.04	5.50	15.44	12.67
21	2.20	17.92	4.98	10.10	8.80

Tables and Figures of Chapter 3

TABLE 3.1

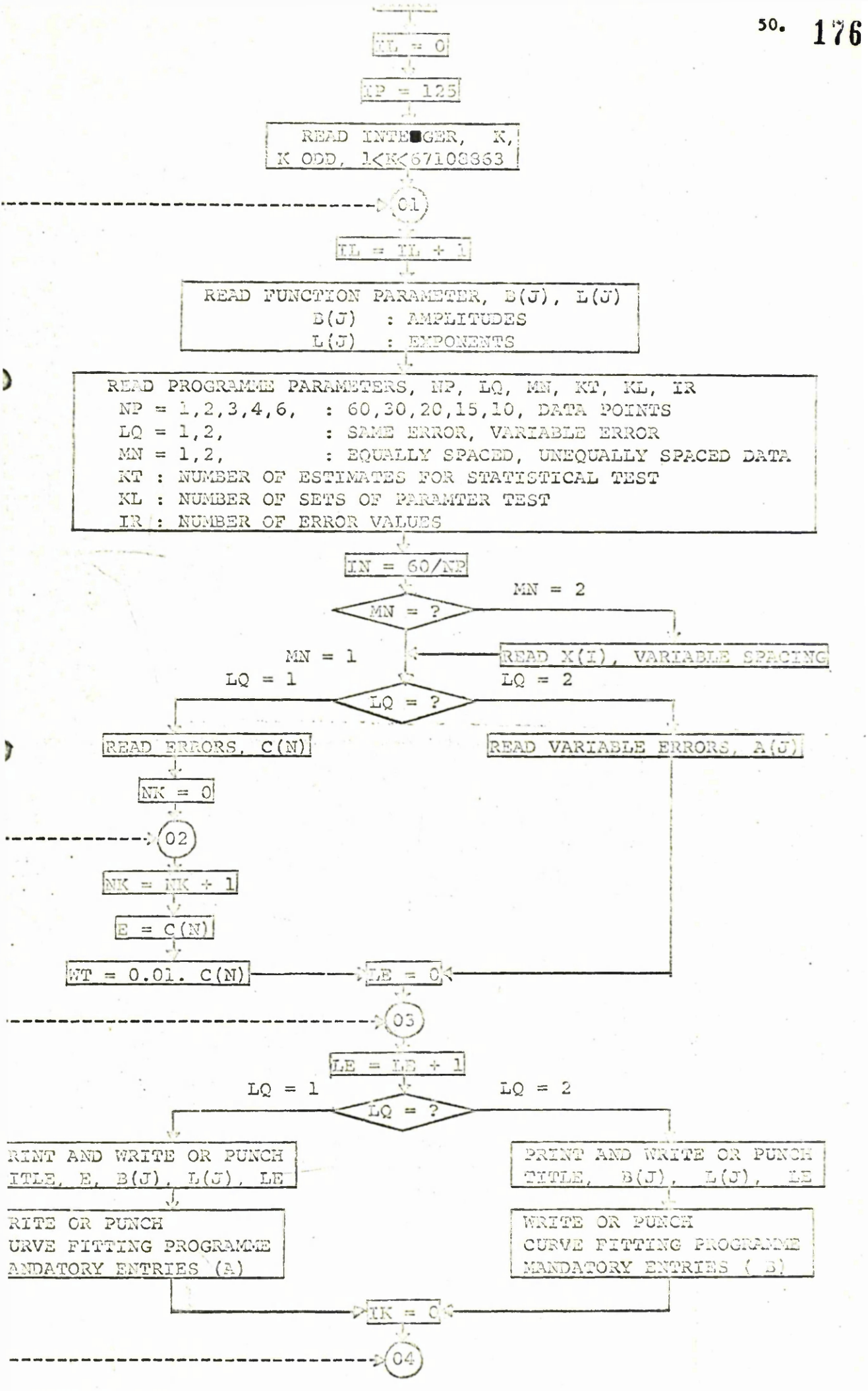
Design of the Experiment (Two Exponentials)

Investigation	Number of Data Points	Exponents	Amplitudes	Data Error
Exponent Ratio	10	.2, .1 .3, .1 .4, .1	.5, .5	2% 5% 10%
Amplitude Ratio	10	.4, .1	.8, .2 .66, .33 .5, .5 .33, .66 .2, .8	2% 10%
Number of Data Points	10 20 60	.2, .1	.5, .5	2% 10%
Variable Error	20	.4, .1	.5, .5	Variable 1-10% 2% 10%
Variable Spacing	10 (Variable)	.4, .1	.5, .5	2% 10%
	10 (Fixed)	.4, .1	.5, .5	2% 10%
Reduced Observation Period	60 50 40 30 20 60/40 60/20	.4, .1	.8, .2	5%

TABLE 3.2

Design of the Experiment (Three Exponentials)

Investigation	Number of Data Points	Exponents	Amplitudes	Data Error
Exponent Ratio	20	0.18, 0.06, 0.02 0.5, 0.1, 0.02 2.0, 0.2, 0.02	.33, .33, .33	2% 5% 10%
	10	0.18, 0.06, 0.02 2.0, 0.2, 0.02		2% 10%
Amplitude Ratio	20	0.50, 0.10, 0.02	.8063, .1613, .0323 .6923, .2308, .0769 .33, .33, .33 .0769, .2308, .6923 .0323, .1613, .8063	2% 10%
Number of Data Points	10 20 60	0.18, 0.06, 0.02	.33, .33, .33	2% 10%
Variable Data Spacing	20 (Variable)	0.50, 0.10, 0.05	.0769, .2308, .6923	2% 10%
	20 (Fixed)	0.50, 0.10, 0.05	.0769, .2308, .6923	2% 10%
Reduced Observation Period	60 50 40 30 20 60/40 60/20	0.50, 0.10, 0.02	.6923, .2308, .0769	2%


IL = 0
IP = 125
READ INTEGER, K,
K ODD, 1<K<67108863
01
IL = IL + 1
READ FUNCTION PARAMETER, B(J), L(J)
B(J) : AMPLITUDES
L(J) : EXPONENTS
READ PROGRAMME PARAMETERS, NP, LQ, MN, KT, KL, IR
NP = 1,2,3,4,6, : 60,30,20,15,10, DATA POINTS
LQ = 1,2, : SAME ERROR, VARIABLE ERROR
MN = 1,2, : EQUALLY SPACED, UNEQUALLY SPACED DATA
KT : NUMBER OF ESTIMATES FOR STATISTICAL TEST
KL : NUMBER OF SETS OF PARAMTER TEST
IR : NUMBER OF ERROR VALUES
IN = 60/NP
MN = ?
MN = 2
MN = 1
READ X(I), VARIABLE SPACING
LQ = 1
LQ = 2
LQ = ?
READ ERRORS, C(N)
READ VARIABLE ERRORS, A(J)
NK = 0
02
NK = NK + 1
E = C(N)
WT = 0.01. C(N)
LE = 0
03
LE = LE + 1
LQ = 1
LQ = 2
LQ = ?
RINT AND WRITE OR PUNCH
ITLE, E, B(J), L(J), LE
PRINT AND WRITE OR PUNCH
ITLE, B(J), L(J), LE
RITE OR PUNCH
URVE FITTING PROGRAMME
ANDATORY ENTRIES (A)
WRITE OR PUNCH
CURVE FITTING PROGRAMME
MANDATORY ENTRIES (B)
IK = 0
04

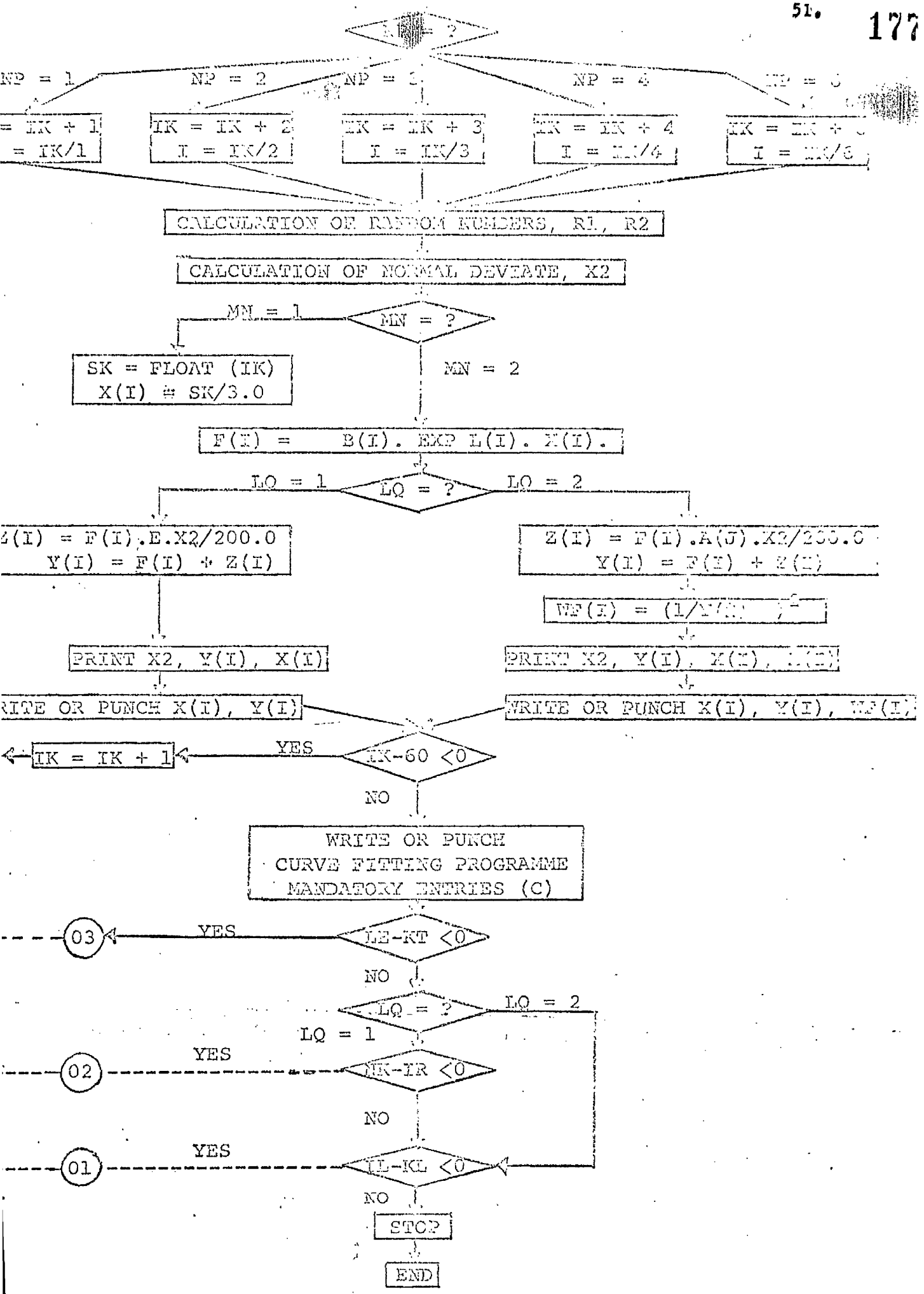

NP = ?
NP = 1
NP = 2
NP = 3
NP = 4
NP = 5
IK = IK + 1
I = IK/1
IK = IK + 2
I = IK/2
IK = IK + 3
I = IK/3
IK = IK + 4
I = IK/4
IK = IK + 5
I = IK/5
CALCULATION OF RANDOM NUMBERS, R1, R2
CALCULATION OF NORMAL DEVIATE, X2
MN = ?
MN = 1
MN = 2
SK = FLOAT (IK)
X(I) = SK/3.0
F(I) = B(I). EXP L(I). X(I).
LQ = ?
LQ = 1
LQ = 2
Z(I) = F(I).E.X2/200.0
Y(I) = F(I) + Z(I)
Z(I) = F(I).A(J).X2/200.0
Y(I) = F(I) + Z(I)
WF(I) = (1/Y(I))**2
PRINT X2, Y(I), X(I)
PRINT X2, Y(I), X(I), WF(I)
WRITE OR PUNCH X(I), Y(I)
WRITE OR PUNCH X(I), Y(I), WF(I)
IK-60 <0
YES
IK = IK + 1
NO
WRITE OR PUNCH
CURVE FITTING PROGRAMME
MANDATORY ENTRIES (C)
LE-KT <0
YES
03
NO
LQ = ?
LQ = 1
LQ = 2
IN-IR <0
YES
02
NO
IL-KL <0
YES
01
NO
STOP
END

TABLE 3.3

Normality Criterion Test on Six Samples of Data

(10 data points in each set, 20 sets of data in each sample)

Sample Number	Exponents	Amplitudes	Data Error	'W' Raw Data	'W' Log Data	Probability of Normality Raw Data	Probability of Normality Log Data
1	.4, .1	.8, .2	2%	.973	.976	78%	84%
2				.941	.948	24%	32%
3		.3, .6	10%	.871	.964	1.15%	59%
4				.907	.874	5.5%	1.3%
5		.5, .5		.954	.976	41%	84%
6	.3, .1		5%	.976	.928	84%	14%

FIGURE 3.1

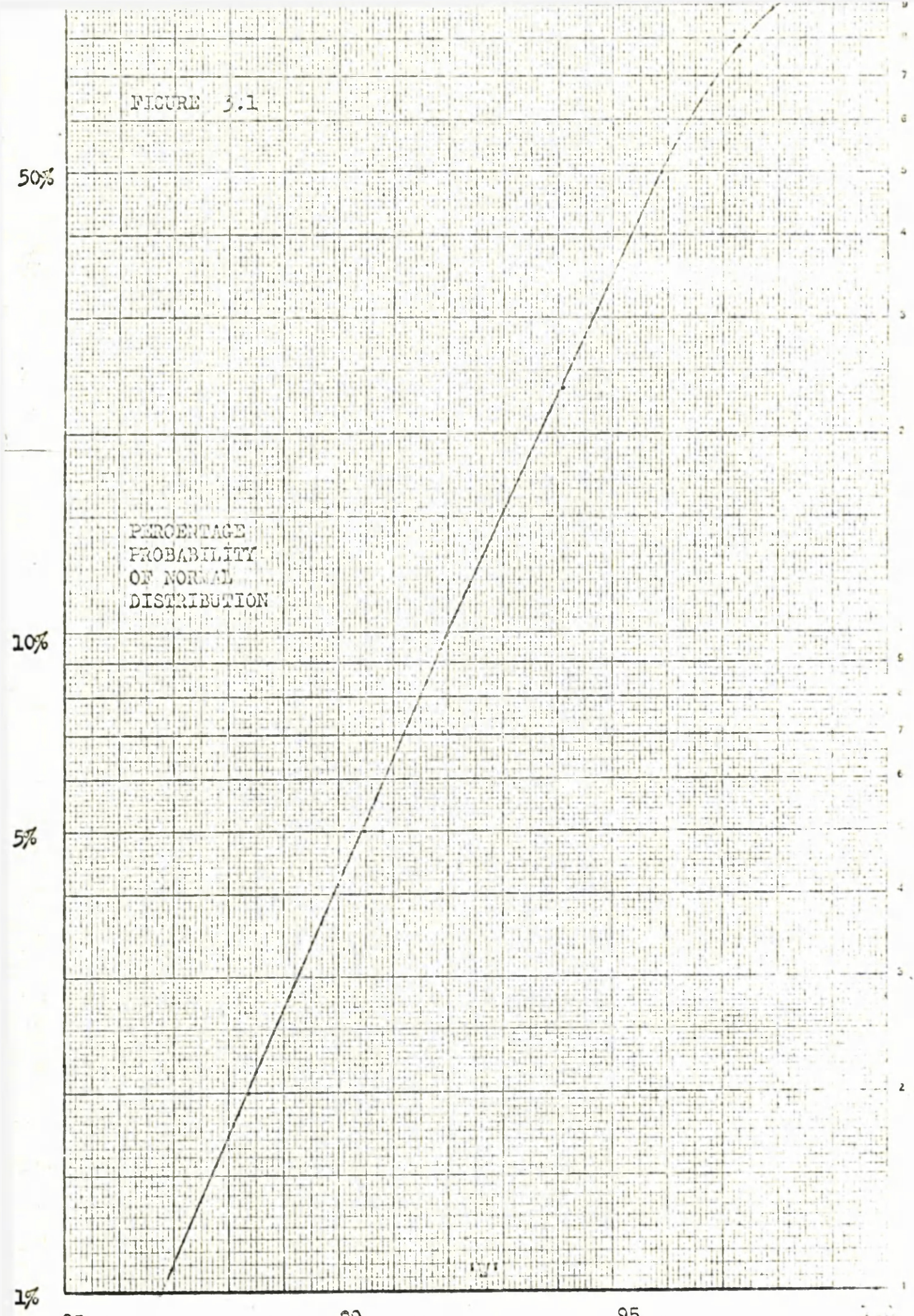

50%
10%
5%
1%
.85
.90
.95
PERCENTAGE PROBABILITY OF NORMAL DISTRIBUTION

TABLE 3.4

Comparison of Parameter Error Estimates

Probability of Normality		Computer Estimate on Raw Data	Statistical Estimate on Raw Data	'Confidence Limit' Estimate on Log Data
Raw Data	Log Data			
1.15%	59%	87.2	87.6	80.7
5.5%	1.3%	41.9	34.5	37.6
41%	84%	121.8	49.9	48.4
84%	14%	51.6	19.3	41.9

Tables and Figures of Chapter 4

TABLE 4.1

Dependence of Parameter Errors on Exponent Ratio

(amplitudes 0.5; 10 data points)

Exponents	Data Error	Parameter Error			
		$\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\lambda_1$	$\Delta\lambda_2$
.4, .1	2%	12.34	9.71	24.79	5.49
.3, .1		17.11	18.61	19.49	8.89
.2, .1		75.33	77.83	44.65	33.44
.4, .1	5%	26.60	28.90	46.46	15.38
.3, .1		31.29	39.29	45.82	17.65
.2, .1		67.31	97.67	56.22	38.14
.4, .1	10%	49.93	35.16	78.70	21.33
.3, .1		49.49	68.23	93.56	38.56
.2, .1		83.97	99.76	143.86	35.57

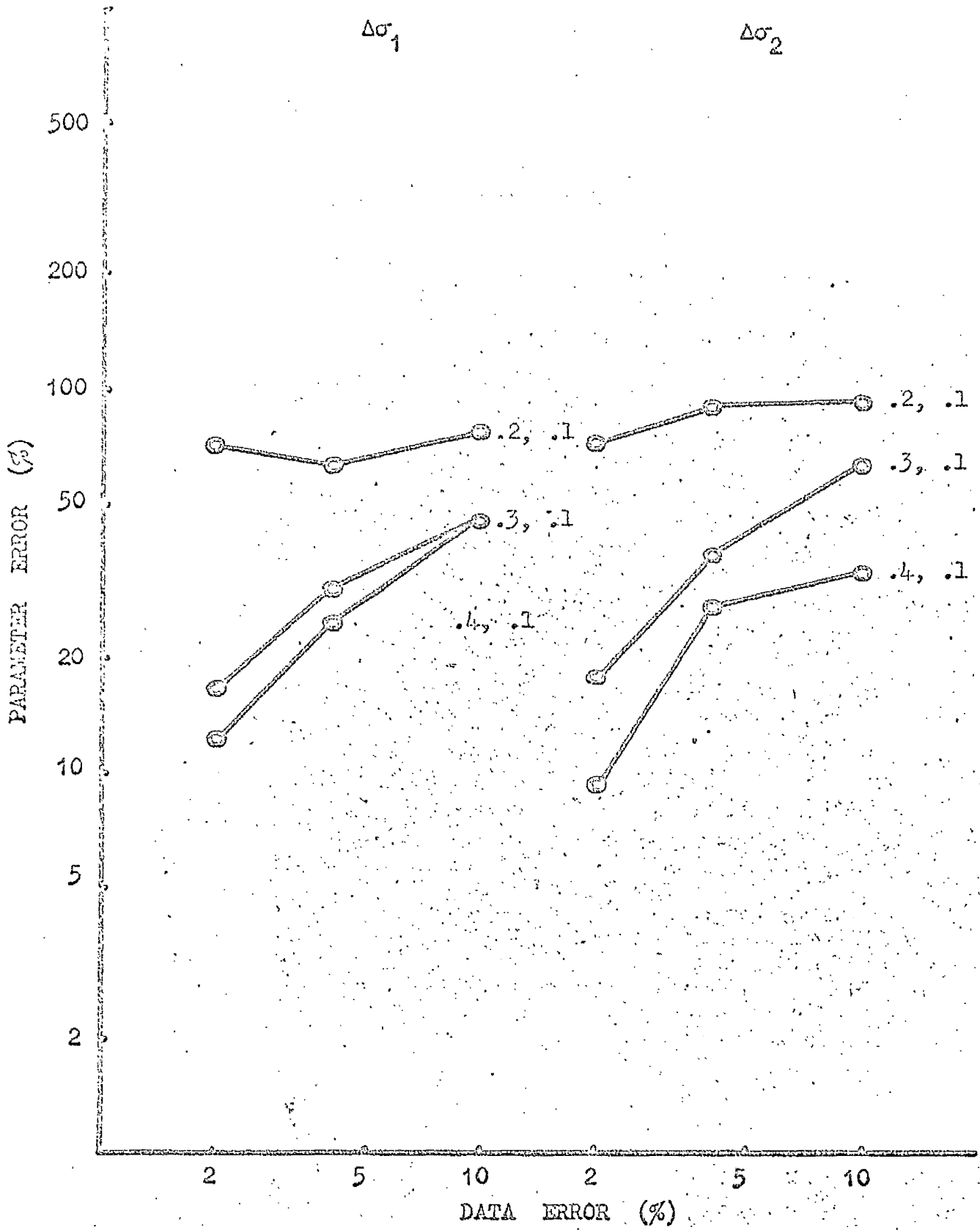


Fig. 4.1a Dependence of parameter errors on exponent ratio

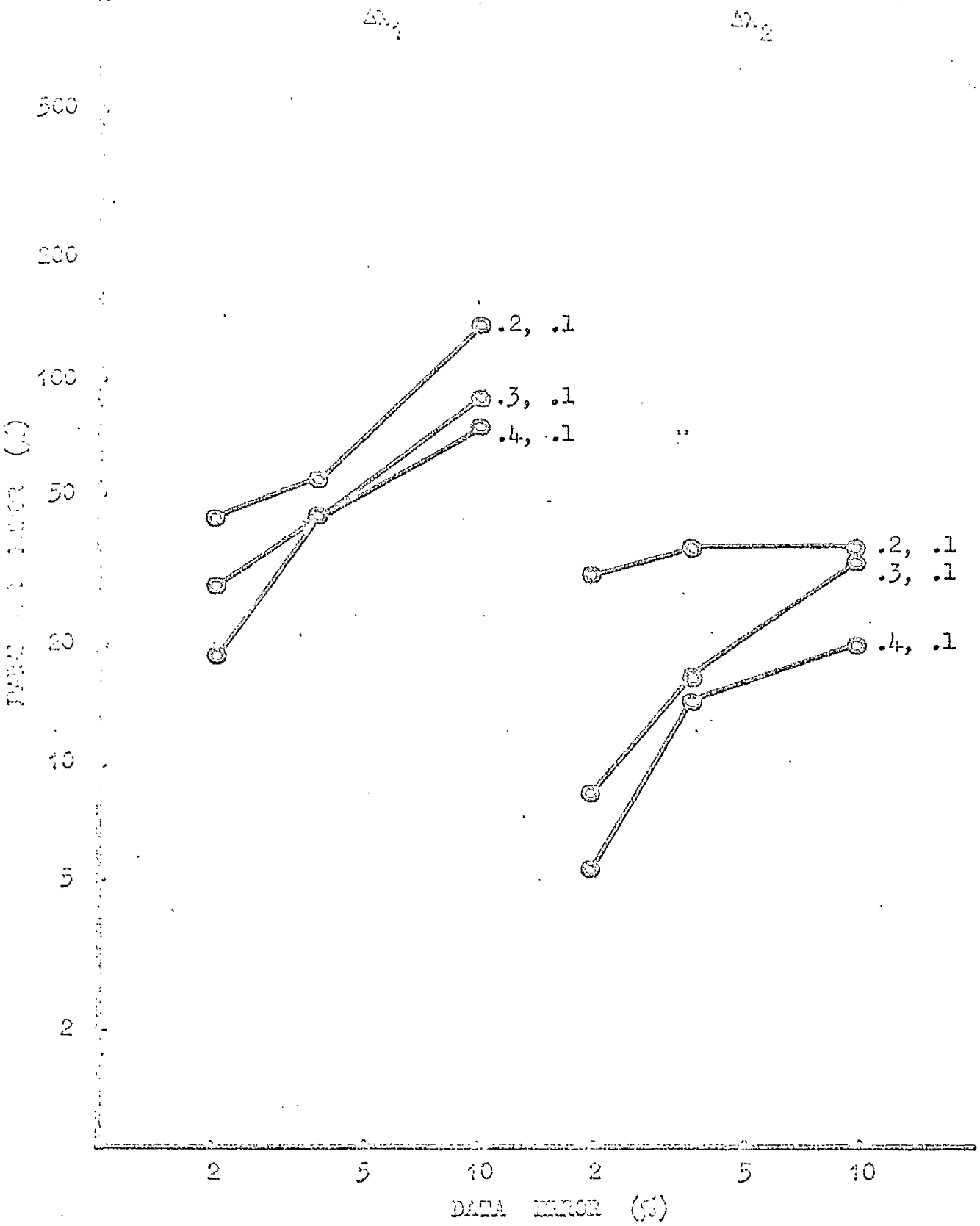


Fig. 4.1b Dependence of parameter errors on exponent ratio

TABLE 4.1.1

Dependence of Parameter Errors on Exponent Ratio (Detailed Results)

(amplitudes 0.5; 10 data points)

Error %	Exponents	σ_1			σ_2			λ_1			λ_2		
		Mean	S.D.	2 x Coef. of Var	Mean	S.D.	2 x Coef. of Var	Mean	S.D.	2 x Coef. of Var	Mean	S.D.	2 x Coef. of Var
2	.4, .1	0.5165	0.03187	12.34	0.4957	0.02407	9.71	0.4030	0.04996	24.79	0.09945	0.002733	5.49
5		0.5220	0.06945	26.60	0.4746	0.06858	28.90	0.3935	0.09147	46.46	0.09717	0.007478	15.38
10		0.6168	0.1540	49.93	0.4839	0.08508	35.16	0.4575	0.1800	78.70	0.09823	0.01048	21.33
2	.3, .1	0.5001	0.0428	17.11	0.5068	0.04715	18.61	0.3087	0.03006	19.49	0.1005	0.004468	8.89
5		0.5388	0.0643	31.29	0.4723	0.0928	39.29	0.3033	0.06949	45.82	0.0969	0.008562	17.65
10		0.6251	0.1547	49.49	0.4517	0.1541	68.23	0.3405	0.1593	93.56	0.09439	0.01820	38.56
2	.2, .1	0.4962	0.1869	75.33	0.5031	0.1958	77.83	0.2145	0.04789	44.65	0.09802	0.01634	33.44
5		0.5728	0.1928	67.31	0.4265	0.2083	97.67	0.1993	0.05603	56.22	0.09291	0.01772	38.14
10		0.5166	0.2169	83.97	0.5014	0.2501	99.76	0.2658	0.1912	143.86	0.09872	0.01753	35.51

TABLE 4.2

Dependence of Parameter Errors on Amplitude Ratio

(exponents 0.4, 0.1; 10 data points)

Amplitudes	Data Error	Parameter Error			
		$\Delta\sigma_1$	$\Delta\sigma_2$	ΔA_1	ΔA_2
.8, .2	2%	3.73	12.72	7.48	6.31
.66, .33		7.40	6.27	10.07	3.12
.5, .5		12.34	9.71	24.79	5.49
.33, .66		19.69	9.49	27.72	5.47
.2, .8		51.16	7.12	60.17	3.89
.8, .2	10%	20.89	33.65	25.70	15.89
.66, .33		32.55	63.37	62.99	166.67
.5, .5		49.93	35.16	78.70	21.33
.33, .66		87.62	34.53	93.12	88.95
.2, .8		116.21	25.37	113.47	15.19

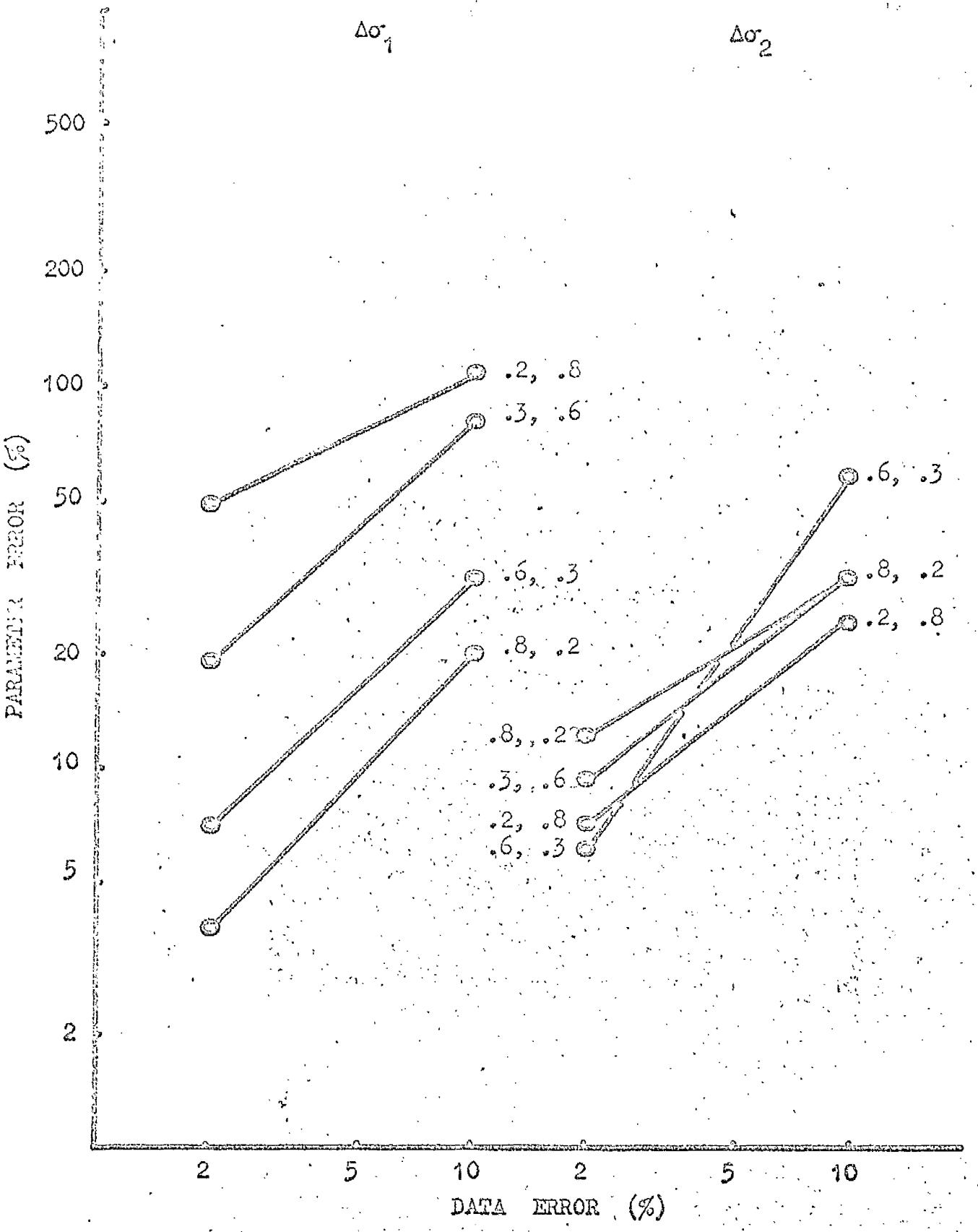


Fig. 4.2a Dependence of parameter errors on amplitude ratio

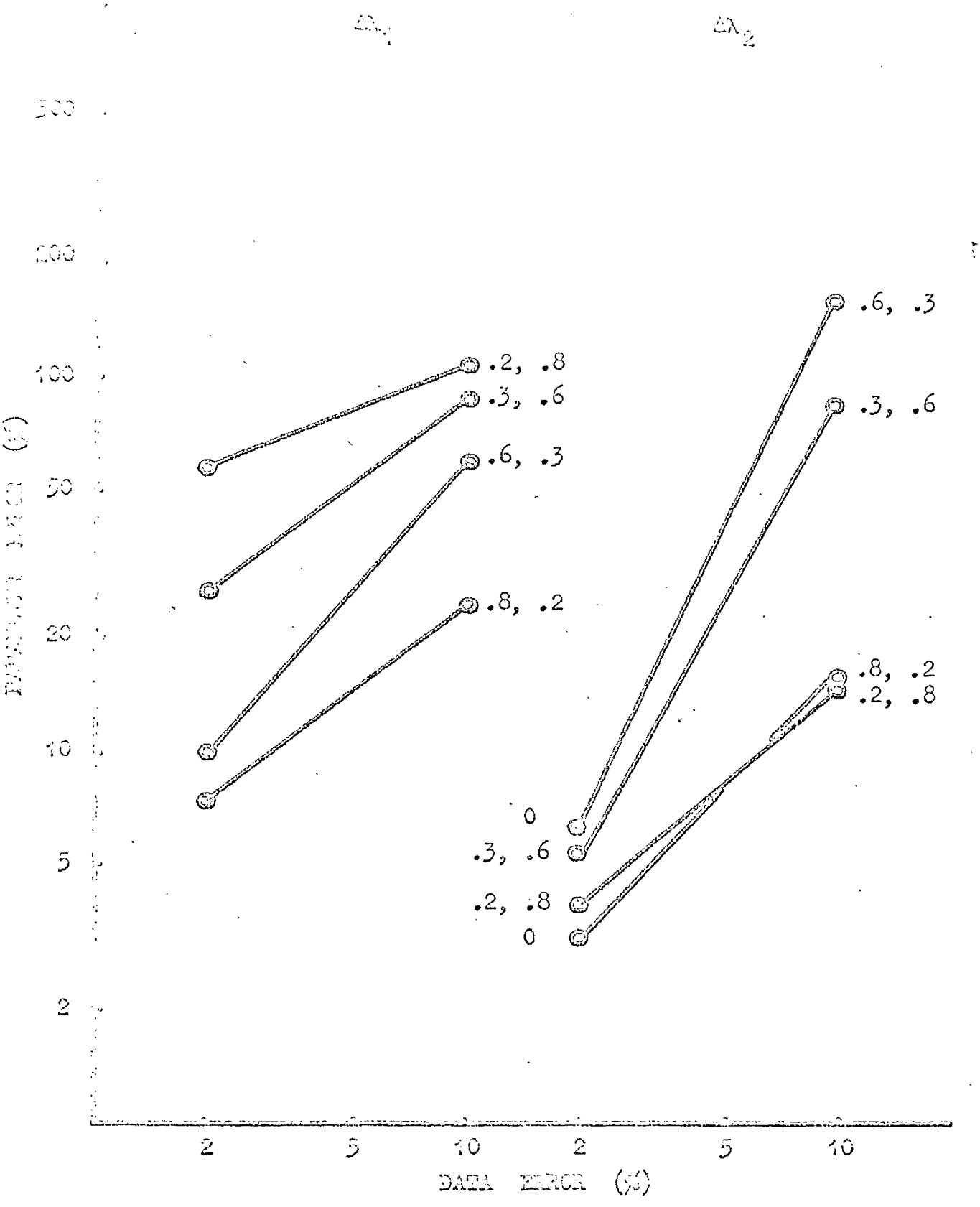


Fig. 4.2b Dependence of parameter errors on amplitude ratio

TABLE 4.2.1

Dependence of Parameter Errors on Amplitude Ratio (Detailed Results)

(exponents 0.4, 0.1; 10 data points)

Error %	Amplitude	σ_1			σ_2			λ_1			λ_2		
		Mean	S.D.	2 x Coef. of Var	Mean	S.D.	2 x Coef. of Var	Mean	S.D.	2 x Coef. of Var	Mean	S.D.	2 x Coef. of Var
2	.8, .2	0.8030	0.01499	3.73	0.1991	0.01227	12.72	0.4008	0.0150	7.48	0.09975	0.003151	6.31
10		0.8691	0.09081	20.89	0.2180	0.03668	33.65	0.4358	0.05603	25.70	0.10420	0.00969	15.89
2	.66, .33	0.6609	0.02447	7.40	0.3296	0.07283	6.27	0.4009	0.0202	10.07	0.09982	0.001561	3.12
10		0.7490	0.1219	32.55	0.3132	0.09925	63.37	0.4362	0.1374	62.99	0.1199	0.09992	166.67
2	.5, .5	0.5165	0.03187	12.34	0.4957	0.02407	9.71	0.4030	0.04996	24.79	0.09945	0.002733	5.49
5		0.5220	0.06945	26.60	0.4746	0.06858	28.90	0.3935	0.09147	46.46	0.09717	0.007478	15.38
10		0.6168	0.1540	49.93	0.4839	0.08508	35.16	0.4575	0.1800	78.70	0.09823	0.01048	21.33
2	.33, .66	0.3446	0.03393	19.69	0.6455	0.03067	9.49	0.3905	0.05412	27.72	0.9861	0.002699	5.47
10		0.4138	0.1813	87.62	0.6063	0.1047	34.53	0.4042	0.1882	93.12	0.1070	0.04759	88.55
2	.2, .8	0.2204	0.05638	51.16	0.8062	0.02872	7.12	0.4633	0.1394	60.17	0.1004	0.001953	3.89
10		0.3115	0.1810	116.21	0.7587	0.09626	25.37	0.4563	0.2589	113.47	0.09713	0.00738	15.19

TABLE 4.3

Dependence of Parameter Errors on Number of Data Points

(amplitudes 0.5; exponents 0.2, 0.1)

Data Error	No. of Data Points	Parameter Error $\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\alpha_1$	$\Delta\alpha_2$
2%	60	29.51	29.04	10.89	11.38
	20	27.17	29.85	12.44	9.39
	10	75.33	77.83	44.65	33.44
10%	60	54.99	50.47	23.09	15.34
	20	74.75	71.89	35.77	23.00
	10	83.97	99.76	143.86	35.51

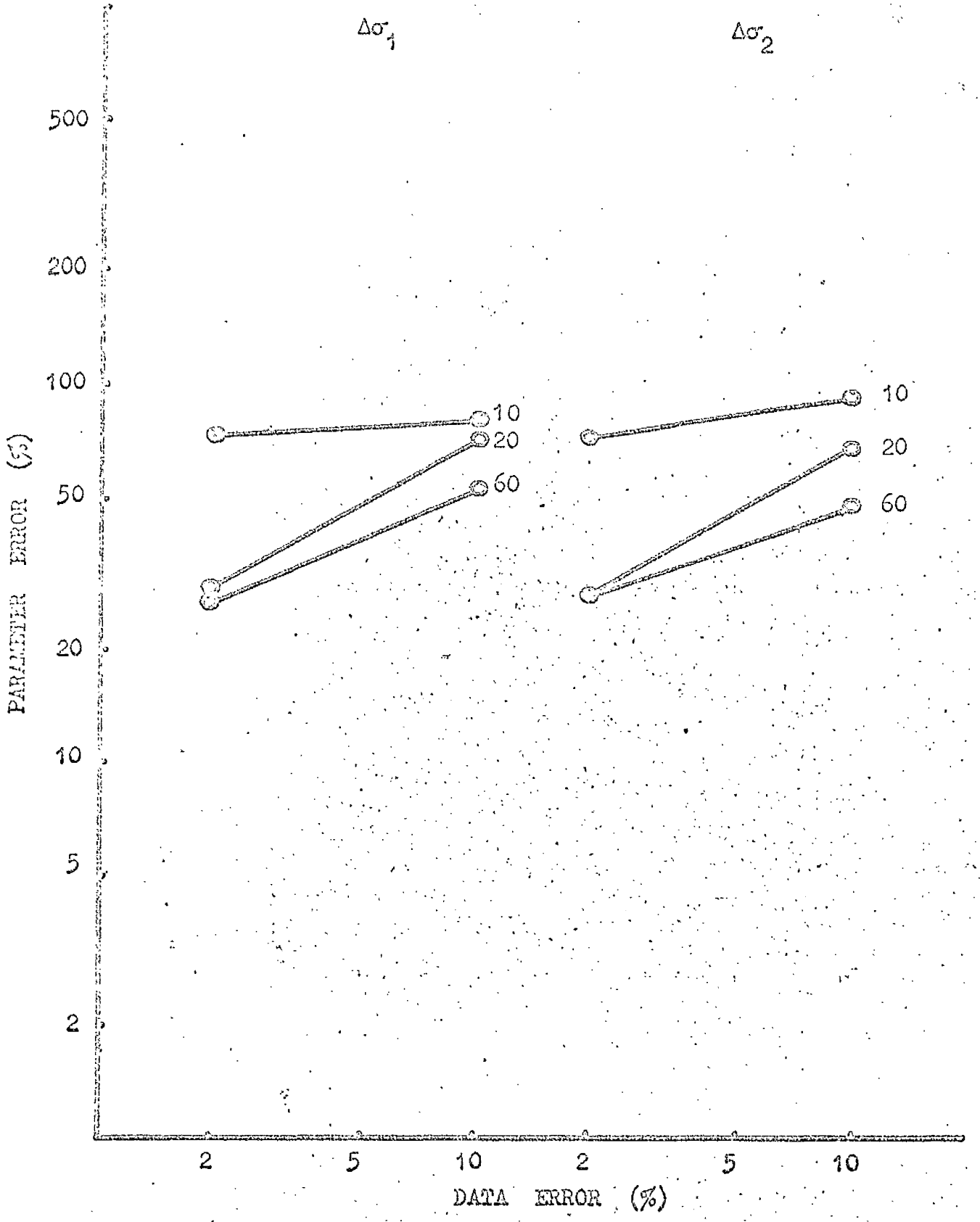


Fig. 4.3a Dependence of parameter errors on number of data points

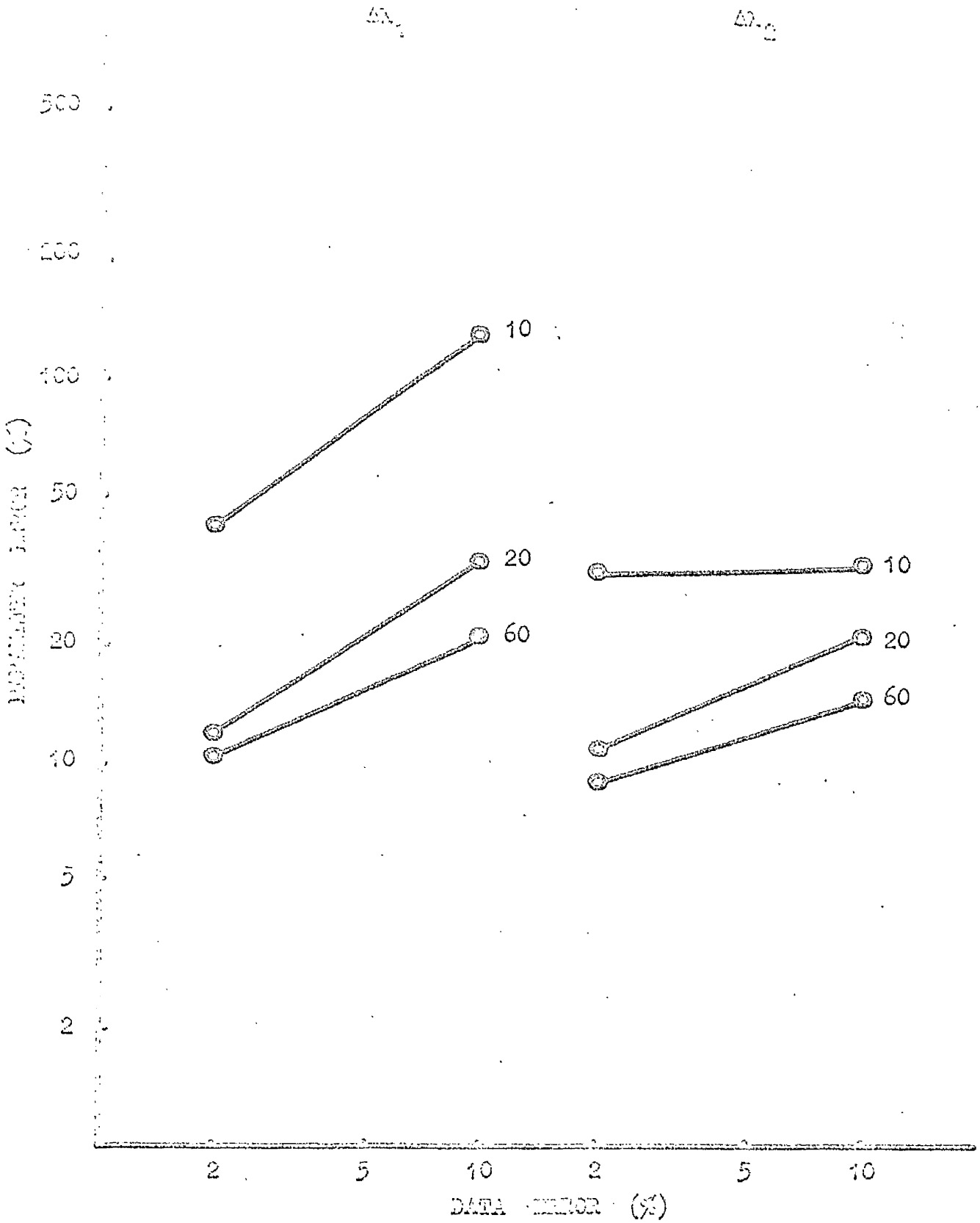


Fig. 4.3b Dependence of parameter errors on number of data points

TABLE 4.3.1

Dependence of Parameter Errors on Number of Data Points (Detailed Results)

(amplitudes 0.5; exponents 0.2, 0.1)

Error %	No. of Points	σ_1 Mean	σ_1 S.D.	σ_1 2 x Coef. of Var	σ_2 Mean	σ_2 S.D.	σ_2 2 x Coef. of Var	λ_1 Mean	λ_1 S.D.	λ_1 2 x Coef. of Var	λ_2 Mean	λ_2 S.D.	λ_2 2 x Coef. of Var
2	60	0.4891	0.07217	29.51	0.5109	0.07419	29.04	0.2029	0.01105	10.89	0.1004	0.005717	11.38
10		0.4753	0.1307	54.99	0.5040	0.1272	50.47	0.2002	0.02312	23.09	0.1005	0.007111	15.34
2	20	0.5180	0.07039	27.17	0.4818	0.07193	29.85	0.1980	0.01232	12.44	0.09872	0.004687	9.39
10		0.4808	0.1797	74.75	0.5026	0.1809	71.98	0.2045	0.03658	35.77	0.1011	0.01163	23.00
2	10	0.4962	0.1869	75.33	0.5031	0.1958	77.83	0.2145	0.04789	44.65	0.09802	0.01634	33.44
5		0.5728	0.1928	67.31	0.4265	0.2083	97.67	0.1993	0.05603	56.22	0.09291	0.01772	38.14
10		0.5166	0.2169	83.97	0.5014	0.2501	99.76	0.2658	0.1912	143.86	0.09872	0.01753	35.51

TABLE 4.4

Dependence of Parameter Errors on Sampling Frequency of the Data Points

(amplitudes 0.2, 0.8; exponents 0.4, 0.1; 10 data points)

Sampling Frequency	Data Error	Parameter Error $\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\lambda_1$	$\Delta\lambda_2$
Fixed	2%	51.16	7.12	60.17	3.89
Variable*		20.13	6.24	36.30	2.92
Fixed	10%	116.21	25.37	113.47	15.19
Variable*		93.42	33.90	107.06	13.97

* Data samples at t = 0.5, 1.0, 1.5, 2.0, 3.0, 6.0, 9.0, 12.0, 16.0 and 20.0

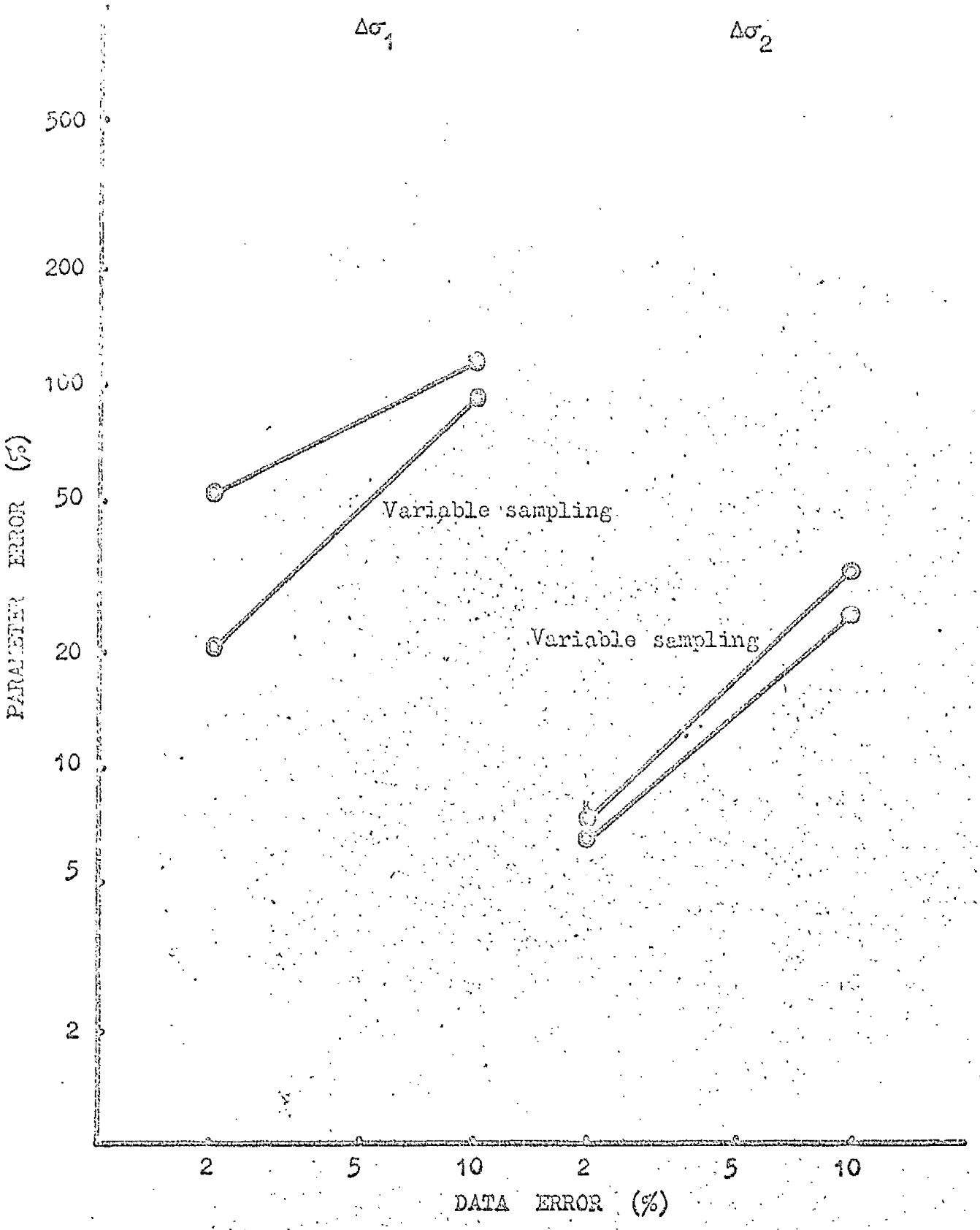


Fig. 4.4a Dependence of parameter errors on sampling frequency

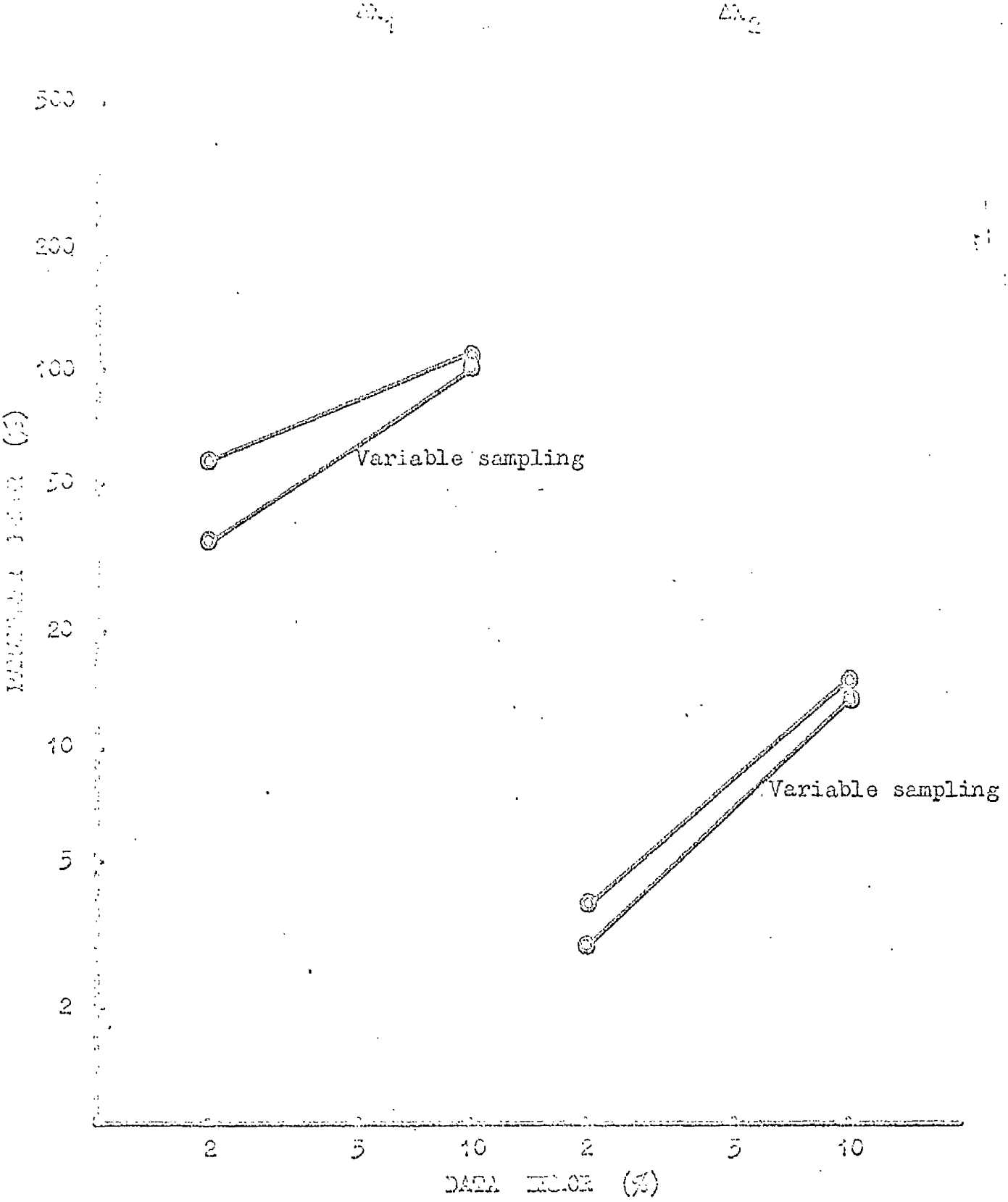


Fig. 4.4b Dependence of parameter errors on sampling frequency

TABLE 4.4.1

Dependence of Parameter Errors on Sampling Frequency (Detailed Results)

(amplitudes 0.2, 0.8; exponents 0.4, 0.1; 10 data points)

Error %	σ_1			σ_2			λ_1			λ_2		
	Mean	S.D.	2xCoef of Var	Mean	S.D.	2xCoef of Var	Mean	S.D.	2xCoef of Var	Mean	S.D.	2xCoef of Var
2	0.2204	0.05638	51.16	0.8062	0.02872	7.12	0.4633	0.1394	60.17	0.1004	0.001953	3.89
10	0.3115	0.1810	116.21	0.7587	0.09626	25.37	0.4563	0.2589	113.47	0.09713	0.00738	15.19
2	0.2000	0.02013	20.13	0.8016	0.02504	6.24	0.4191	0.07608	36.30	0.1002	0.001464	2.92
10	0.2175	0.1016	93.42	0.7486	0.1269	33.90	0.4431	0.2372	107.06	0.09785	0.006835	13.97

TABLE 4.5

Dependence of Parameter Errors on Variable Data Errors

(amplitudes 0.5; exponents 0.4, 0.1; 20 data points)

Data Error	Parameter Error $\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\lambda_1$	$\Delta\lambda_2$
2%	4.17	5.18	9.74	3.16
10%	30.71	32.41	39.84	17.80
1-10%	19.24	22.73	27.24	14.12
Estimation by linear interpolation	15.7	17.1	22.9	9.6
Estimation by semi-logarithmic interpolation	20.8	27.2	28.6	12.2

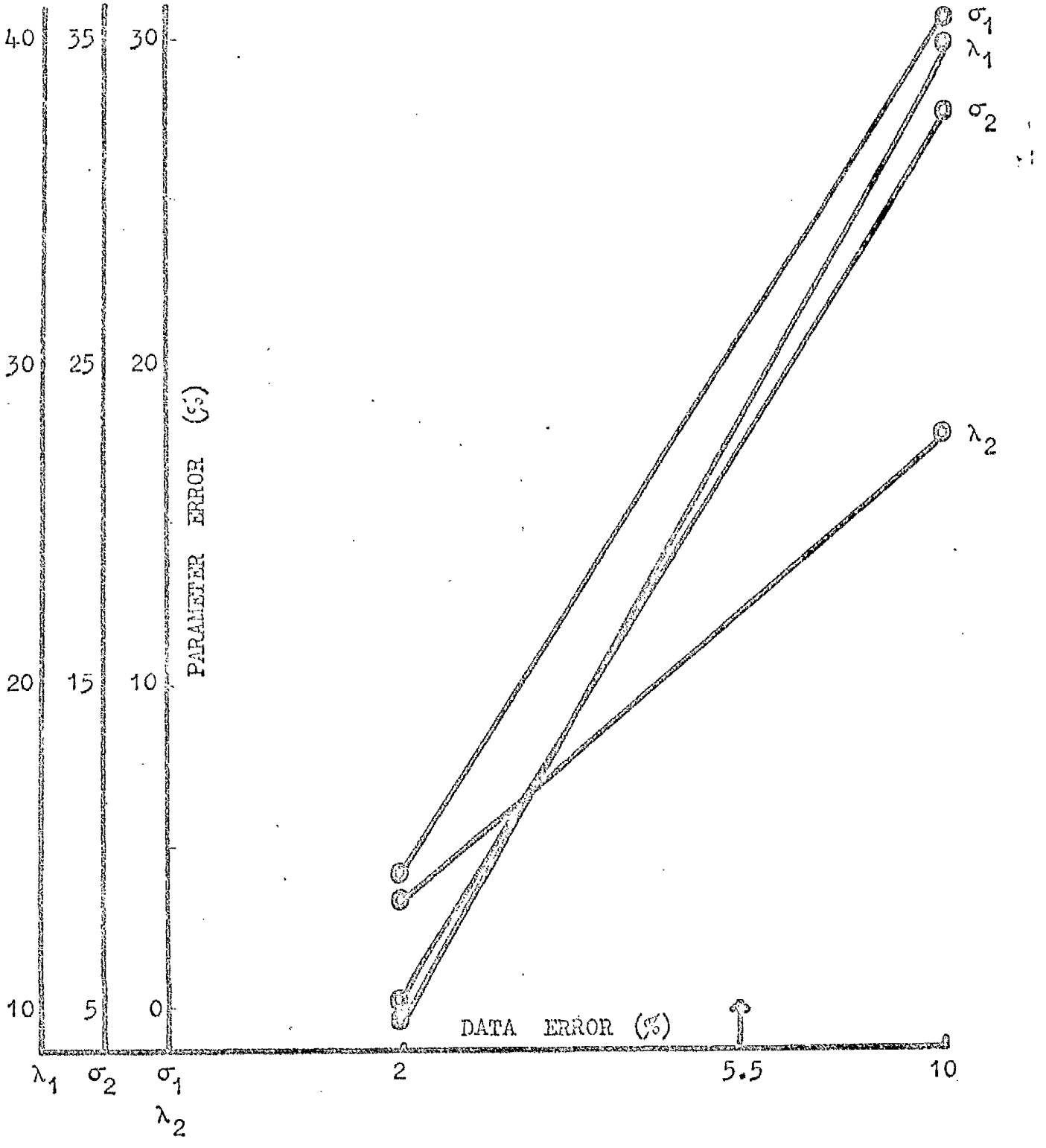


Fig. 4.5 Estimation of parameter errors from fixed error data results when data error varies by semilogarithmic interpolation

TABLE 4.5.1

Dependence of Parameter Errors on Variable Data Errors (Detailed Results)

(amplitudes 0.5; exponents 0.4, 0.1; 20 data points)

Error %	σ_1 Mean	σ_1 S.D.	σ_1 2xCoef of Var	σ_2 Mean	σ_2 S.D.	σ_2 2xCoef of Var	λ_1 Mean	λ_1 S.D.	λ_1 2xCoef of Var	λ_2 Mean	λ_2 S.D.	λ_2 2xCoef of Var
2	0.5035	0.0105	4.17	0.4962	0.01286	5.18	0.3968	0.01933	9.74	0.09942	0.001577	3.16
10	0.5185	0.07964	30.71	0.4780	0.07748	32.41	0.4301	0.08569	39.84	0.09943	0.008852	17.80
VAR	0.4075	0.03922	19.24	0.4910	0.05581	22.73	0.3980	0.05422	27.24	0.9893	0.006985	14.12

TABLE 4.6

Dependence of Parameter Errors on Incomplete Collection of Data

(amplitudes 0.8, 0.2; exponents 0.4, 0.1; 5% error)

Time	No. of Data Points	Parameter Error $\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\lambda_1$	$\Delta\lambda_2$
	60	4.23	4.59	2.62	2.93
	50	6.13	16.39	4.43	11.77
	40	5.68	25.95	8.09	18.55
	30	12.41	76.05	17.28	71.05
	20	42.29	121.55	30.16	140.93
0 - 20.0	60	4.23	4.59	2.62	2.93
0 - 13.32	60	5.91	24.87	8.50	16.91
0 - 6.67	60	14.22	71.58	11.92	78.61

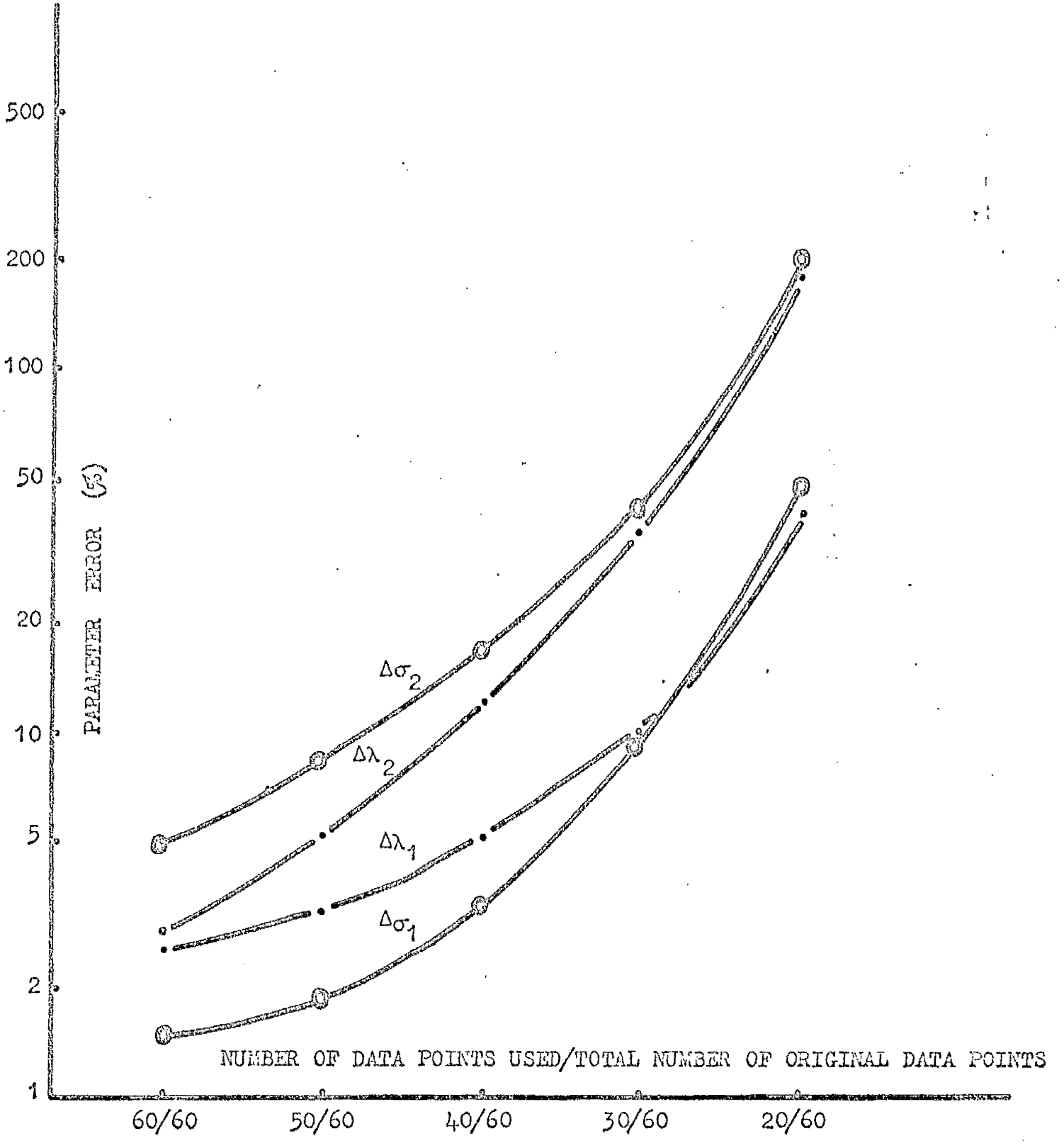


Fig. 4.6 Dependence of parameter errors on incomplete collection of data

TABLE 4.6.1

Dependence of Parameter Errors on Incomplete Collection of Data (Detailed Results)

No. of Points	σ_1			σ_2			λ_1			λ_2		
	Mean	S.D.	2xCoef of Var	Mean	S.D.	2xCoef of Var	Mean	S.D.	2xCoef of Var	Mean	S.D.	2xCoef of Var
60/60	0.8018	0.01699	4.23	0.1972	0.00453	4.59	0.3988	0.00523	2.62	0.09934	0.001458	2.93
50/60	0.8010	0.02458	6.13	0.1932	0.01584	16.39	0.3964	0.008794	4.43	0.09780	0.005759	11.77
40/60	0.8042	0.02284	5.68	0.1928	0.02502	25.95	0.3962	0.03206	8.09	0.09752	0.009046	18.55
30/60	0.8094	0.05036	12.41	0.1866	0.07096	76.05	0.3944	0.03409	17.28	0.08965	0.03185	71.05
20/60	0.7670	0.1622	42.29	0.3480	0.2115	121.55	0.4085	0.06161	30.16	0.09738	0.06862	140.93
t=0-13.32*	0.7787	0.02320	5.91	0.2153	0.02677	24.87	0.04111	0.01748	8.50	0.1045	0.00884	16.91
t=0- 6.67*	0.8162	0.05804	14.22	0.1847	0.06610	71.58	0.3919	0.02335	11.92	0.08462	0.03307	78.16

* see text for explanation

TABLE 4.7

Statistical and Computer Error Estimates and Correlation Coefficient

Correlation Coefficient	$\Delta\sigma_1$		$\Delta\sigma_2$		Correlation Coefficient	$\Delta\lambda_1$		$\Delta\lambda_2$	
	Stat.	Comp.	Stat.	Comp.		Stat.	Comp.	Stat.	Comp.
0.983	75.3	97.0	77.8	10.24	0.842	44.6	88.6	33.4	53.4
0.986	67.3	361.6	97.7	374.6	0.860	56.2	196.2	38.1*	143.2*
0.956	84.0	563.4	99.8	586.8	0.808	143.9*	441.4*	35.5	240.2
0.942	17.1	13.4	18.6	19.6	0.650	19.5	12.6	8.9	8.4
0.939	31.3	42.4	19.3	51.6	0.671	45.8	44.8	17.7	24.2
0.911	49.5	273.4	68.2	203.8	0.634	93.6	123.2	38.6	87.6
0.885	12.3	9.7	9.7	8.2	0.533	24.8	16.1	5.5	4.6
0.886	26.6	25.6	28.9	26.0	0.549	46.5	37.6	15.4	14.8
0.855	49.9	121.8	35.2	38.6	0.517	78.7*	109.8*	21.3	46.8
0.988	27.17	78.2	29.85	81.3	0.864	12.44	34.7	9.39	27.4
0.985	74.75	431.2	71.98	447.5	0.865	35.77	250.1	23.0	129.4
0.987	29.5	42.0	29.0	43.4	0.860	10.9	19.1	11.4	14.8
0.987	55.0	218.0	50.5	224.8	0.866	23.1	211.3	15.3	70.4
0.881	3.7	3.7	12.7	8.8	0.475	7.5	5.3	6.3	4.8
0.865	20.9*	24.0*	33.7	42.0	0.413	25.7	28.2	15.9	22.8
0.883	7.4	4.9	6.3	7.7	0.507	10.1	8.3	3.1	4.2
0.862	32.6	52.1	63.4	46.4	0.474	63.0	59.0	166.7	30.8
0.891	19.7	17.0	9.5	9.2	0.567	27.7	30.7	5.5	5.0
0.874	87.6	174.4	34.5	83.8	0.568	93.1	178.4	88.9	50.0
0.858	51.2	49.8	7.1	9.2	0.547	60.2	66.4	3.9	4.6
0.846	116.2	798.6	25.4	469.8	0.572	113.5*	578.4*	15.2	45.8
0.871	4.2	3.0	4.6	9.9	0.463	2.6	5.0	2.9	5.6
0.910	6.1	3.7	16.4	16.6	0.530	4.4	6.6	11.8	10.6
0.941	5.7	6.6	25.9	33.3	0.622	8.1	10.2	18.6	24.4
0.968	12.4	18.0	76.1	83.6	0.732	17.3	20.0	71.1	71.6
0.986	42.3	94.0	122.6	398.3	0.856	30.2	82.5	140.9*	375.8*
0.881	19.2	16.6	22.7	18.4	0.531	27.2	28.9	14.1	10.8
0.842	20.1	27.8	6.2	7.7	0.703	36.3	32.9	2.9	3.8
0.836	93.4	779.8	33.9	194.5	0.719	107.1*	285.6*	14.0	73.4
0.880	4.2	5.1	5.2	5.4	0.526	9.7	26.4	3.2	3.1
0.862	30.7	29.8	32.4	28.2	0.515	39.8	50.5	17.8	16.9

* Censored data

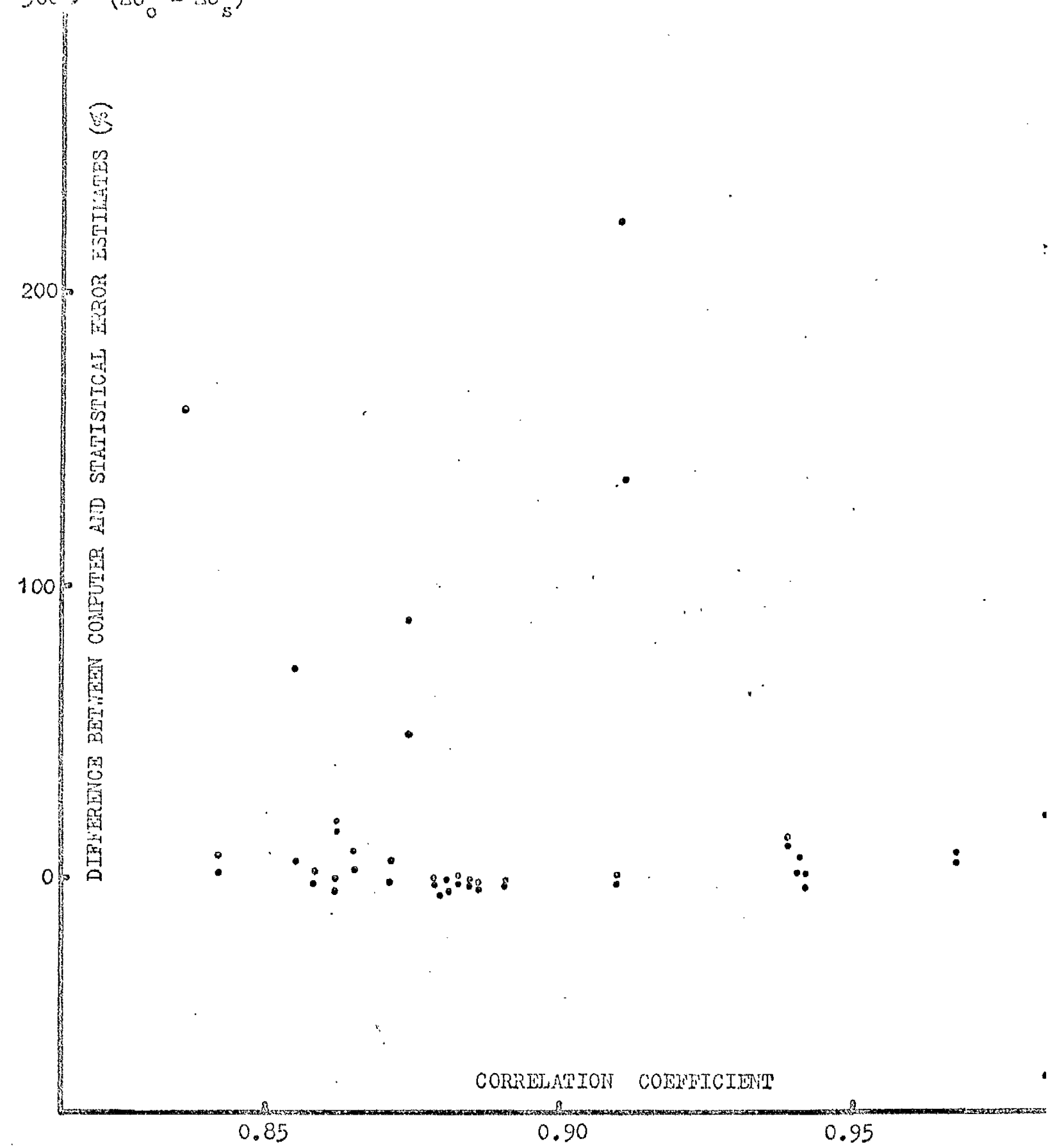


Fig. 4.7 The difference between computer and statistically derived error estim
plotted against the cross correlation coefficient. Seven points indi
in Table 4 have not been plotted here owing to their excessive values

TABLE 4.8

Dependence of Difference in Error Estimate and Correlation Coefficient

Correlation Coefficient	Difference in Error Estimates σ_1	σ_2	Correlation Coefficient	Difference in Error Estimates λ_1	λ_2
0.983	21.7	-67.6	0.842	44.0	20.0
0.986	294.3	276.9	0.860	140.0	105.1*
0.956	479.4	487.0	0.808	297.5*	204.7
0.942	-3.7	1.0	0.650	-6.9	-0.5
0.939	11.1	12.3	0.671	-1.0	6.5
0.911	223.9	135.6	0.634	29.6	49.0
0.885	-2.6	-1.5	0.533	-8.7	-0.9
0.886	-1.0	6.6	0.549	-8.9	-0.6
0.855	71.9	6.6	0.517	31.1*	25.5
0.988	51.0	51.4	0.864	22.3	18.0
0.985	356.4	375.5	0.865	214.3	106.4
0.987	12.5	14.4	0.860	8.2	3.4
0.987	163.0	174.3	0.866	188.2	55.1
0.881	0.0	-3.9	0.475	-2.2	-1.5
0.865	3.1*	8.3	0.413	2.5	6.9
0.883	-2.5	1.4	0.507	-1.8	1.1
0.862	19.5	-17.0	0.474	-4.0	-135.9
0.891	-2.7	-0.3	0.567	3.0	-0.5
0.874	86.8	49.3	0.568	85.3	-38.9
0.858	-1.4	2.1	0.547	6.2	0.7
0.846	682.4	444.4	0.572	464.9*	30.6
0.871	-1.2	5.3	0.463	2.9	2.8
0.910	-2.4	0.2	0.530	2.2	-1.2
0.941	0.9	7.4	0.622	2.1	5.8
0.968	5.6	7.5	0.732	2.7	0.5
0.986	51.7	175.7	0.856	52.3	254.9*
0.881	-2.6	-4.3	0.531	1.7	-3.3
0.842	7.7	1.5	0.703	-3.4	0.9
0.836	686.4	160.6	0.719	178.5*	59.4
0.880	0.9	0.2	0.526	16.7	-0.1
0.862	-0.9	-4.2	0.515	10.7	-0.9

* Censored data

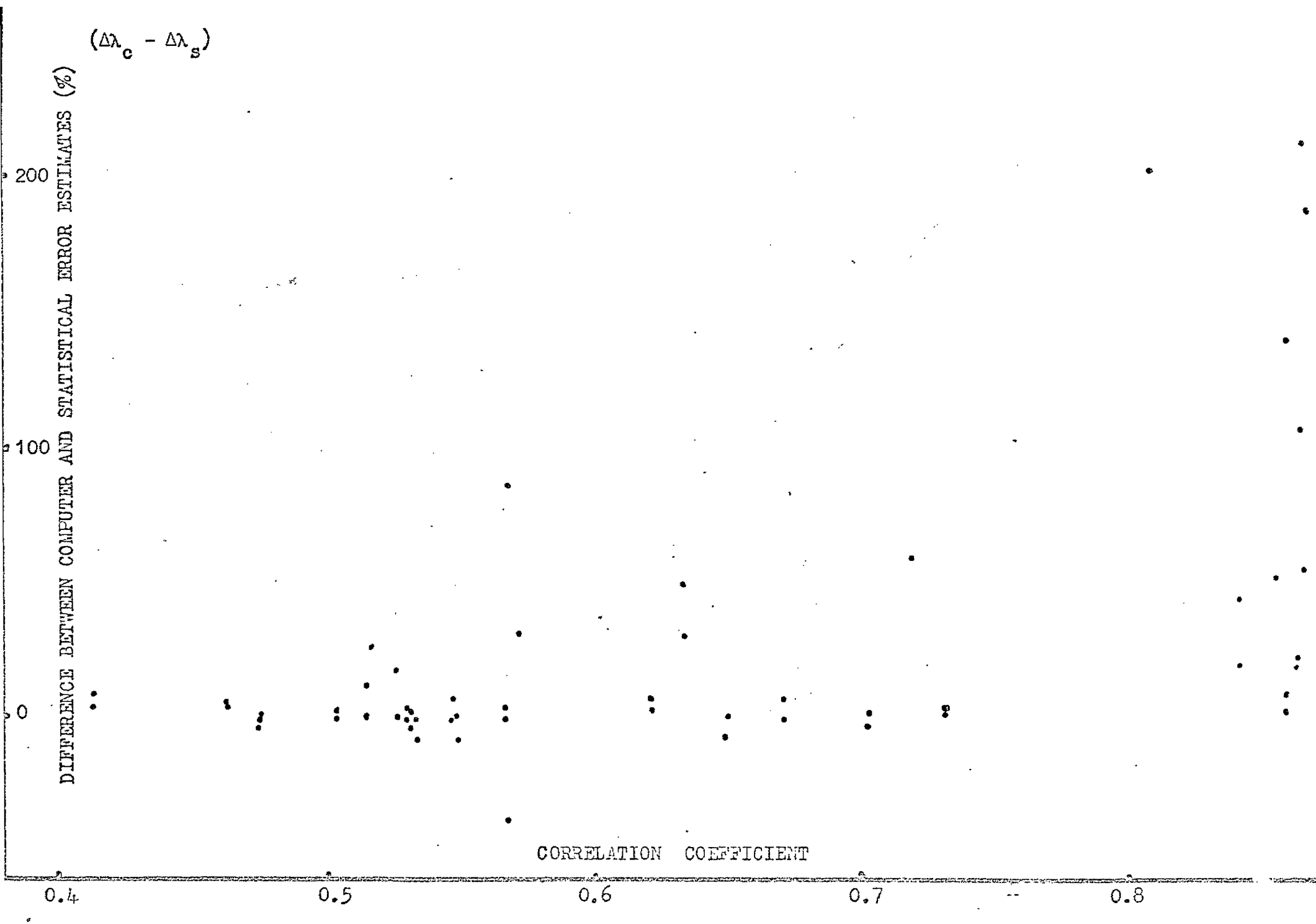


Fig. 4.8 The difference between the computer and statistically derived error estimates plotted against the cross correlation coefficient. Two points indicated in Table 4 have not been plotted here owing to their excessive values.

Tables and Figures of Chapter 5

TABLE 5.1

Dependence of Parameter Errors on Exponent Ratio

(amplitudes 0.33; 20 data points)

Exponents	Data Error	Parameter Error					
		$\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\sigma_3$	$\Delta\lambda_1$	$\Delta\lambda_2$	$\Delta\lambda_3$
.50, .05, .005	2%	22.78	89.13	74.74	33.11	82.08	195.86
2.00, .20, .02		112.41	13.01	15.52	63.73	24.64	32.35
.18, .06, .02		85.84	130.41	74.61	67.87	64.99	103.77
.50, .10, .02		29.07	38.64	43.81	25.01	51.78	48.61
.50, .05, .005	5%	52.50	89.42	124.86	69.89	119.87	219.14
2.00, .20, .02		145.50	23.13	29.19	73.60	42.78	70.64
.18, .06, .02		115.20	102.58	59.95	91.57	56.62	124.08
.50, .10, .02		48.71	83.44	62.78	54.77	53.26	100.04
.50, .05, .005	10%	145.03	69.20	84.29	180.26	109.93	276.20
2.00, .20, .02		249.11	56.95	34.04	189.38	92.35	93.70
.18, .06, .02		91.23	161.32	79.84	79.67	75.36	207.51
.50, .10, .02		82.84	71.30	63.16	77.68	59.45	175.14

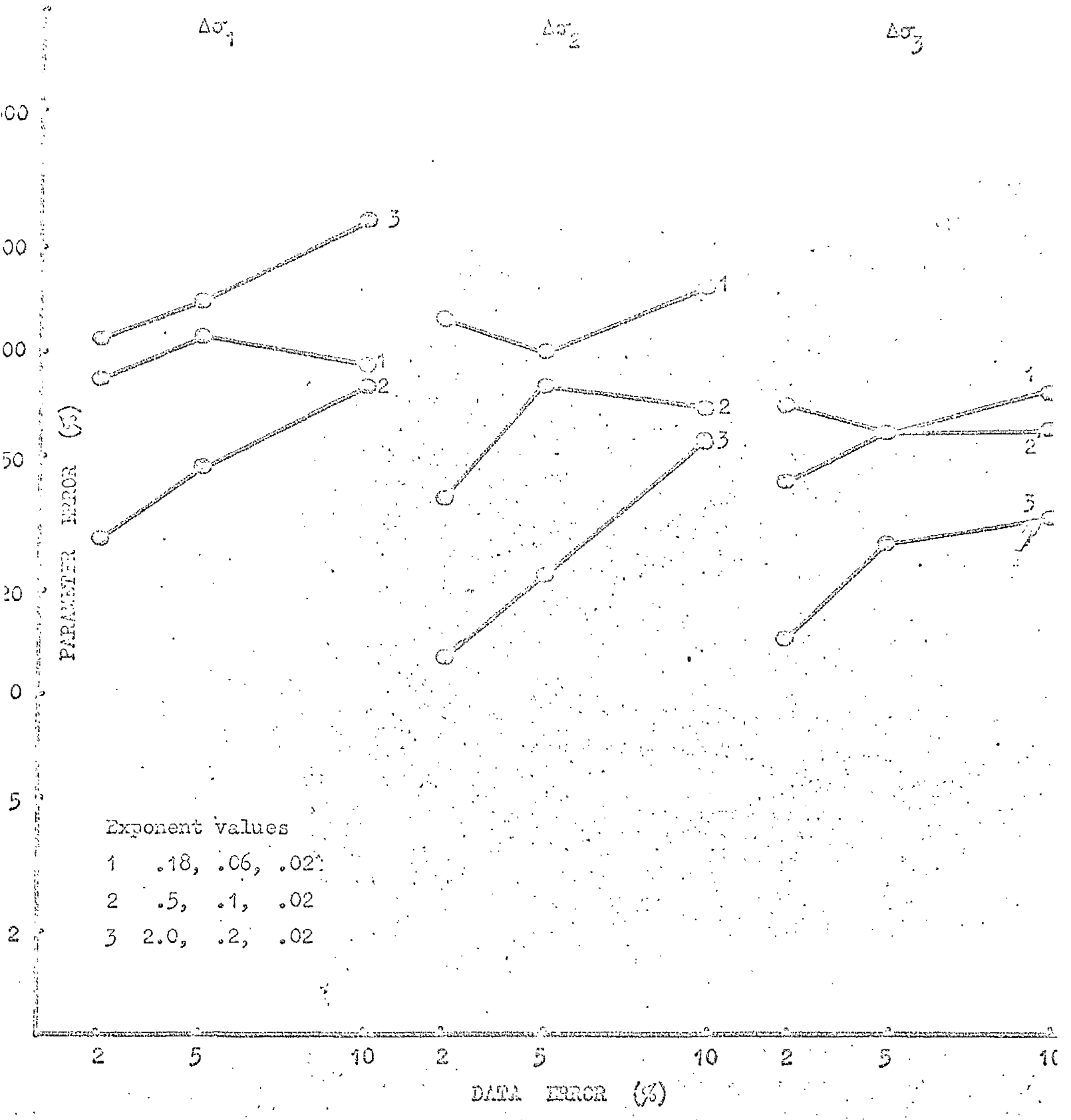


Fig. 5.1a Dependence of parameter errors on exponent ratio

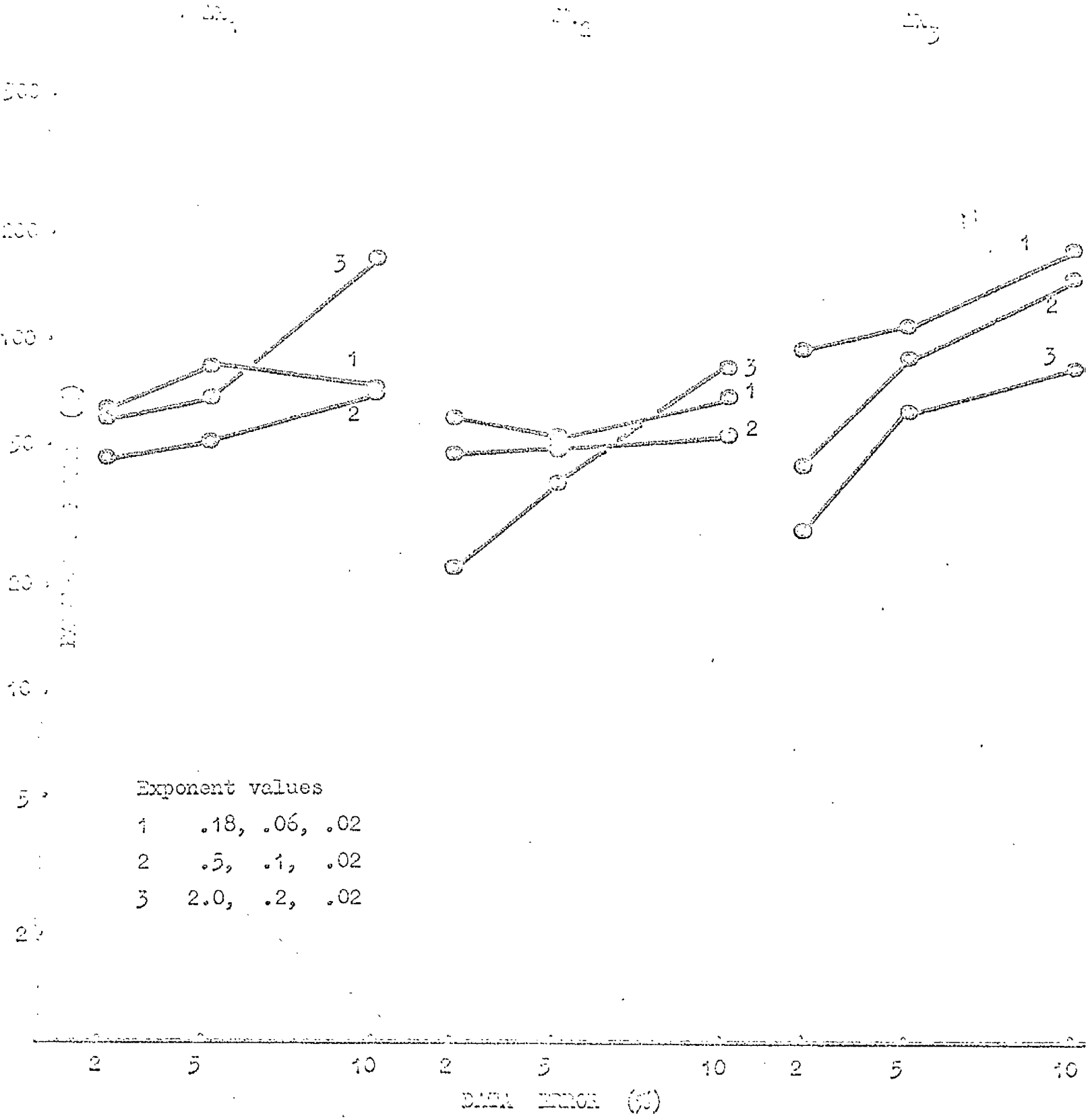


Fig. 5.1b Dependence of parameter errors on exponent ratio

TABLE 5.1.1

Dependence of Parameter Errors on Exponent Ratio (Detailed Amplitude Results)

(amplitudes 0.33; 20 data points)

Error %	Exponents	σ_1 Mean	σ_1 S.D.	σ_1 2xCoef of Var (%)	σ_2 Mean	σ_2 S.D.	σ_2 2xCoef of Var (%)	σ_3 Mean	σ_3 S.D.	σ_3 2xCoef of Var (%)
2	.18, .06, .02	0.2996	0.1286	85.84	0.3650	0.2380	130.41	0.3356	0.1252	74.61
5		0.2625	0.1512	115.20	0.4225	0.2167	102.58	0.2994	0.08975	59.95
10		0.3205	0.1462	91.23	0.2953	0.2382	161.32	0.3905	0.1519	79.84
2	.5, .1, .02	0.3409	0.04955	29.07	0.3046	0.05885	38.64	0.3623	0.07945	43.85
5		0.3441	0.08381	48.71	0.3679	0.1535	83.44	0.3504	0.1100	62.78
10		0.3278	0.1348	82.84	0.4078	0.1454	71.30	0.2795	0.08827	63.16
2	.2, .2, .02	0.4341	0.2440	112.41	0.3261	0.02122	13.01	0.3319	0.02576	15.52
5		0.4685	0.3406	145.40	0.3409	0.03944	23.13	0.2314	0.04692	29.19
10		0.2924	0.3642	249.11	0.3054	0.08697	56.95	0.3666	0.0624	34.04
2	.5, .05, .005	0.3389	0.03361	22.78	0.3018	0.1345	89.13	0.3540	0.1323	74.74
5		0.3738	0.09823	52.56	0.3802	0.1700	89.42	0.2787	0.1740	124.86
10		0.4326	0.3131	145.03	0.3777	0.1307	69.20	0.3464	0.1460	84.29

TABLE 5.1.2

Dependence of Parameter Errors on Exponent Ratio (Detailed Exponent Results)

(amplitudes 0.33; 20 data points)

Error %	Exponents	λ_1			λ_2			λ_3		
		Mean	S.D.	2xCoef of Var (%)	Mean	S.D.	2xCoef of Var (%)	Mean	S.D	2xCoef of Var (%)
2	.18, .06, .02	0.2163	0.07341	67.87	0.06447	0.02095	64.99	0.01833	0.009447	103.77
5		0.2151	0.09842	91.51	0.06912	0.01957	56.62	0.01484	0.009211	124.08
10		0.1600	0.06374	79.67	0.06876	0.02591	75.36	0.02953	0.03064	207.51
2	.5, .1, .02	0.5031	0.06292	25.01	0.1105	0.02861	51.78	0.02281	0.005544	48.61
5		0.5394	0.1465	54.77	0.1054	0.02807	53.26	0.02103	0.01052	100.04
10		0.5826	0.2263	77.68	0.1136	0.03374	59.45	0.01128	0.009878	175.14
2	.2, .2, .02	2.134	0.6801	63.73	0.2045	0.02527	24.64	0.02020	0.003258	32.35
5		2.098	0.7727	73.60	0.2033	0.04328	42.78	0.01877	0.013260	70.64
10		1.131	1.1071	189.38	0.2902	0.1340	92.35	0.02798	0.02622	93.70
2	.5, .05, .005	0.5006	0.08288	33.11	0.05285	0.02169	82.08	0.006779	0.006639	195.86
5		0.5136	0.1795	69.89	0.04373	0.02621	119.87	0.005914	0.006480	219.14
10		0.6314	0.5691	180.26	0.09586	0.05269	109.93	0.004992	0.006894	276.20

TABLE 5.2

Dependence of Parameter Errors on Amplitude Ratio

(exponents 0.5, 0.1, 0.02; 20 data points)

Amplitudes	Data Error	Parameter Error					
		$\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\sigma_3$	$\Delta\lambda_1$	$\Delta\lambda_2$	$\Delta\lambda_3$
.81, .16, .03	2%	2.81	39.88	200.78	6.13	41.91	228.47
.69, .23, .08		7.02	20.27	95.55	11.90	54.38	122.74
.33, .33, .33		29.07	38.64	43.85	25.01	51.78	48.61
.08, .23, .69		164.52	141.72	49.20	95.66	111.80	87.58
.03, .16, .81		216.18	99.02	18.57	61.23	61.50	31.14
.81, .16, .03	10%	18.81	83.83	220.12	49.53	113.99	214.66
.69, .23, .08		28.58	84.47	102.54	40.90	70.70	155.36
.33, .33, .33		82.84	71.30	63.16	77.68	59.45	175.14
.08, .23, .69		235.04	107.36	33.78	87.77	189.28	134.05
.03, .16, .81		245.62	182.87	36.16	56.08	316.62	98.17

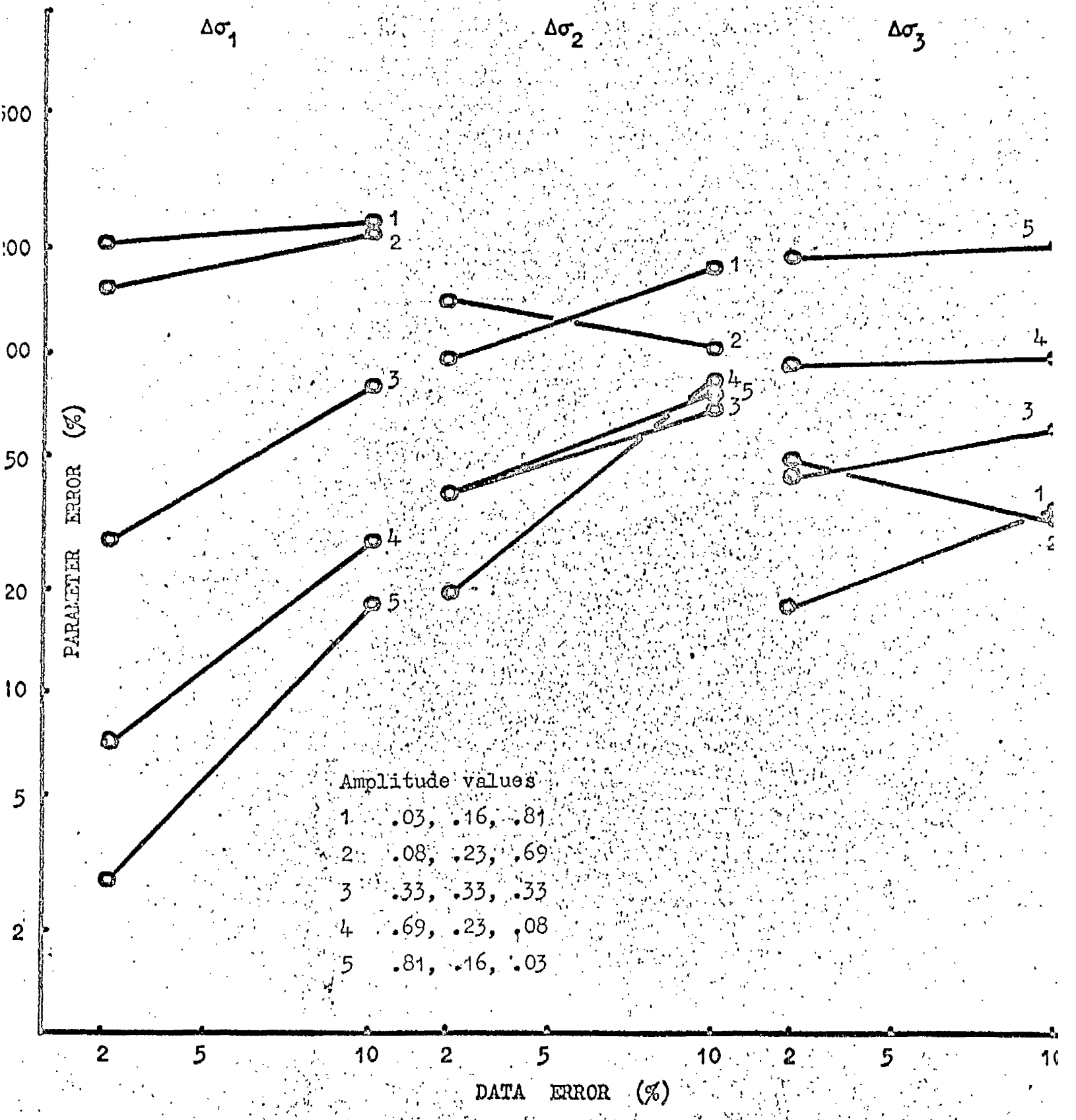


Fig. 5.2a Dependence of parameter errors on amplitude ratio

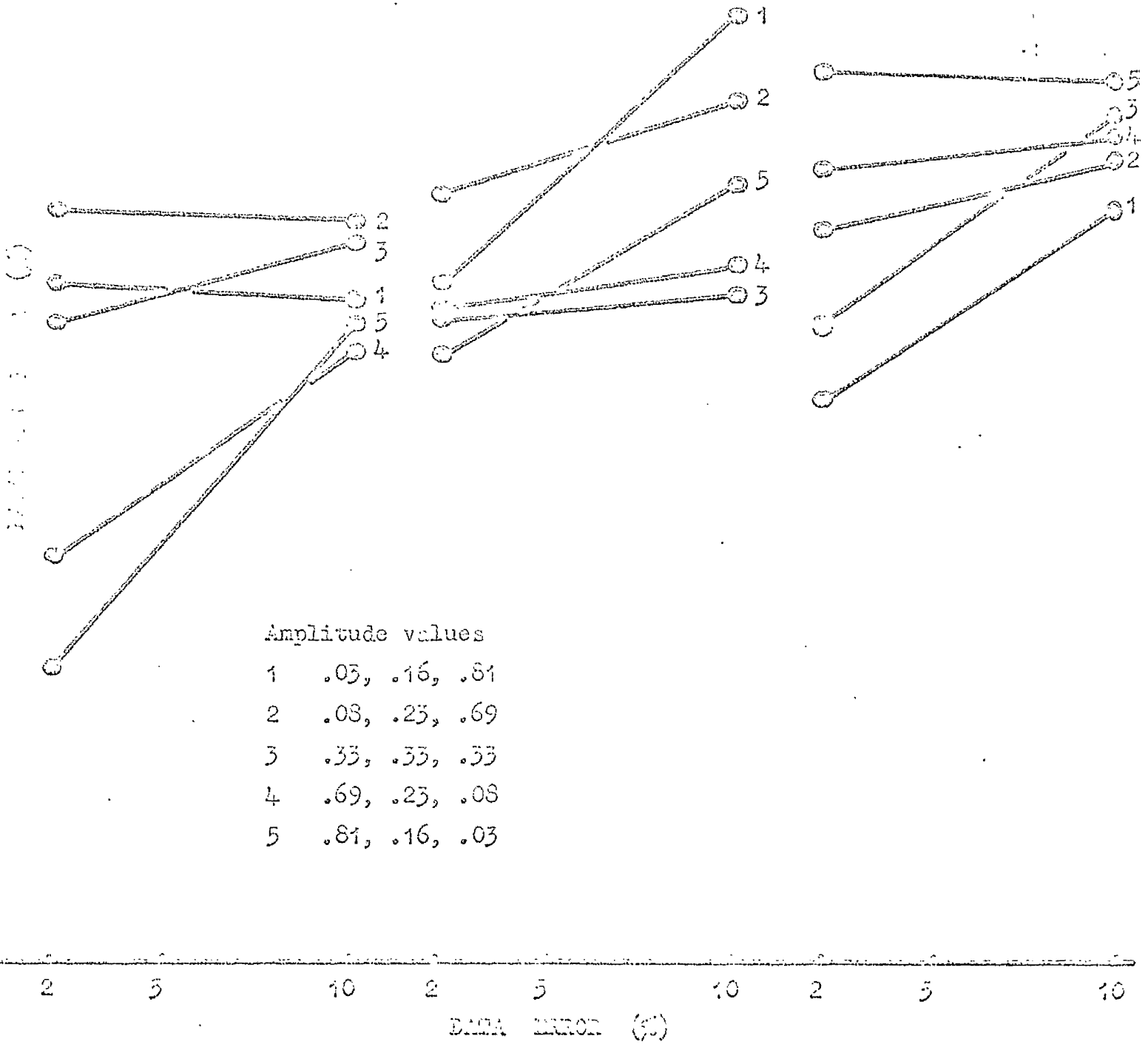


Fig. 5.2b Dependence of parameter errors on amplitude ratio

TABLE 5.2.1

Dependence of Parameter Errors on Amplitude Ratio (Detailed Amplitude Results)

(exponents 0.5, 0.1, 0.02; 20 data points)

Error %	Amplitude	σ_1 Mean	σ_1 S.D.	σ_1 2xCoef of Var (%)	σ_2 Mean	σ_2 S.D.	σ_2 2xCoef of Var (%)	σ_3 Mean	σ_3 S.D.	σ_3 2xCoef of Var (%)
2	.03, .16, .81	0.2954	0.03193	216.18	0.1695	0.08392	99.02	0.8007	0.07514	18.57
10		0.08501	0.1044	245.62	0.1782	0.1580	182.87	0.7766	0.1404	36.16
2	.08, .23, .69	0.1421	0.1169	164.52	0.2471	0.1751	141.72	0.6601	0.1624	49.20
10		0.09207	0.1082	235.04	0.2798	0.1341	107.36	0.6584	0.1112	33.78
2	.33, .33, .33	0.3409	0.04955	29.07	0.3046	0.05885	38.64	0.3623	0.07945	43.85
10		0.3278	0.1348	82.84	0.4078	0.1454	71.30	0.2795	0.08827	63.16
2	.69, .23, .08	0.6950	0.02441	7.02	0.2366	0.02399	20.27	0.07248	0.03463	95.55
10		0.6823	0.09752	28.58	0.3111	0.1314	84.47	0.07636	0.03915	102.54
2	.81, .16, .03	0.8117	0.01143	2.81	0.1606	0.03203	39.88	0.03066	0.03078	200.78
10		0.7891	0.07423	18.81	0.1950	0.08174	83.83	0.03299	0.03631	220.12

TABLE 5.2.2

Dependence of Parameter Errors on Amplitude Ratio (Detailed Exponent Results)

(exponents 0.5, 0.1, 0.02; 20 data points)

Error %	Amplitude	λ_1			λ_2			λ_3		
		Mean	S.D.	2xCoef of Var (%)	Mean	S.D.	2xCoef of Var (%)	Mean	S.D.	2xCoef of Var (%)
2	.03, .16, .81	0.5504	0.1685	61.23	0.1117	0.03435	61.50	0.01968	0.003064	31.14
10		0.5356	0.1502	56.08	0.1305	0.2066	316.62	0.01824	0.008953	98.17
2	.08, .23, .69	0.5279	0.2525	95.66	0.1281	0.07161	111.80	0.02118	0.009276	87.58
10		0.5571	0.2445	87.77	0.1138	0.1077	189.28	0.02114	0.01417	134.05
2	.33, .33, .33	0.5031	0.06292	25.01	0.1105	0.02861	51.78	0.02281	0.005544	48.61
10		0.5826	0.2263	77.68	0.1136	0.03374	59.45	0.01128	0.009878	175.14
2	.69, .23, .08	0.5042	0.03002	11.90	0.10057	0.02735	54.38	0.01719	0.01055	122.74
10		0.5759	0.1178	40.90	0.1156	0.04087	70.70	0.01707	0.01326	155.36
2	.81, .16, .03	0.4993	0.01531	6.13	0.09891	0.02082	41.91	0.01440	0.01645	228.47
10		0.5402	0.1338	49.53	0.1179	0.0672	113.99	0.01664	0.01786	214.66

TABLE 5.3

Dependence of Parameter Errors on Number of Data Points

(amplitudes 0.3; exponents 0.18, 0.06, 0.02)

Data Error	Number of Data Points	Parameter Error $\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\sigma_3$	$\Delta\lambda_1$	$\Delta\lambda_2$	$\Delta\lambda_3$
2%	60	46.16	54.40	29.43	17.51	39.96	92.48
	20	85.84	130.41	74.61	67.87	64.99	103.77
	10	95.98	100.44	110.83	173.16	235.85	196.29
10%	60	98.47	92.61	83.01	88.04	47.51	159.33
	20	91.23	161.23	79.84	79.67	75.36	207.51
	10	163.78	126.40	114.22	197.20	62.96	257.22

Additional Result (see Table 5.6)

(amplitude 0.69, 0.23, 0.08; exponents 0.5, 0.1, 0.02)

2%	60	1.71	17.23	65.84	1.73	16.21	117.21

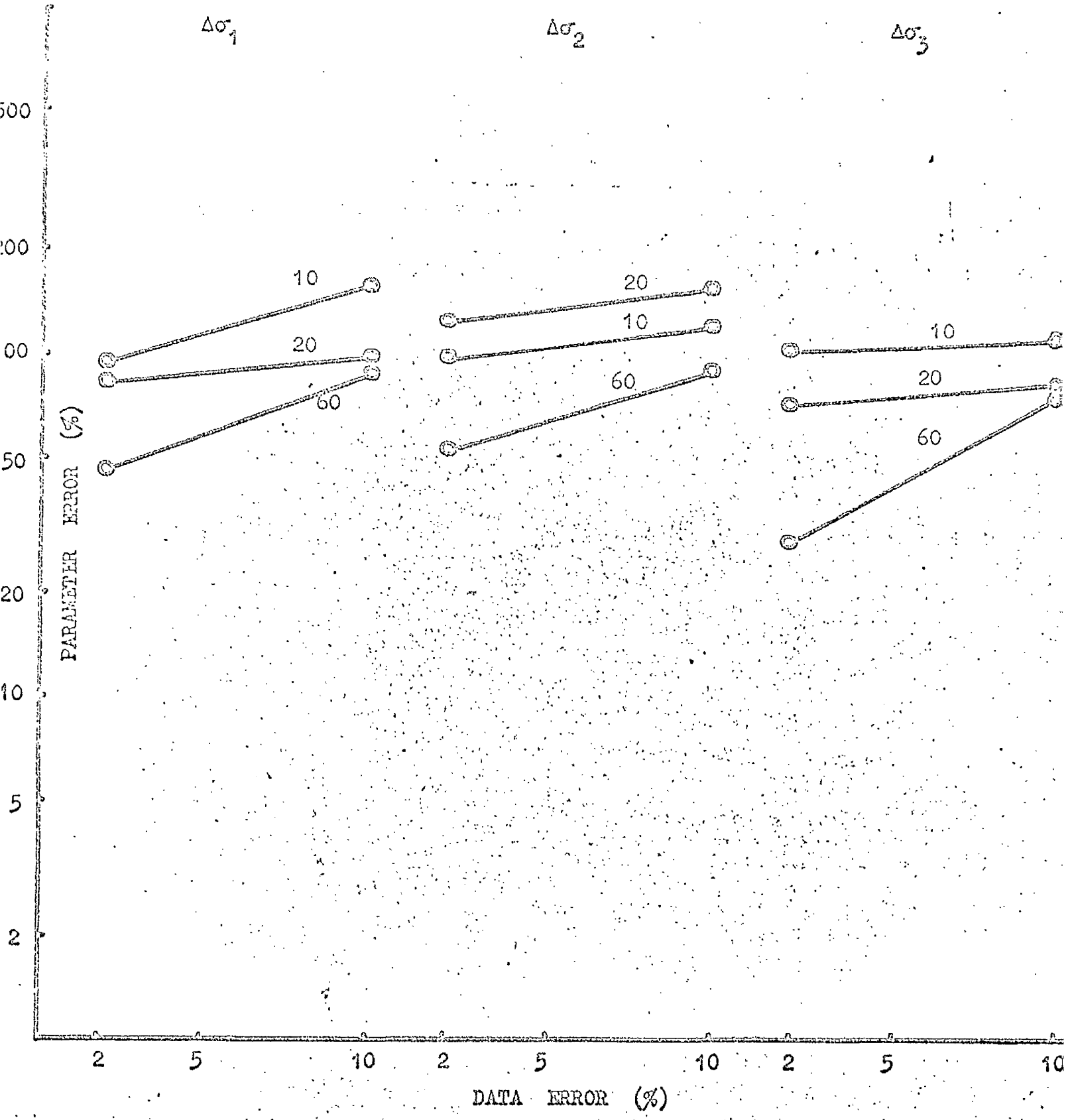


Fig. 5.3a Dependence of parameter errors on number of data points

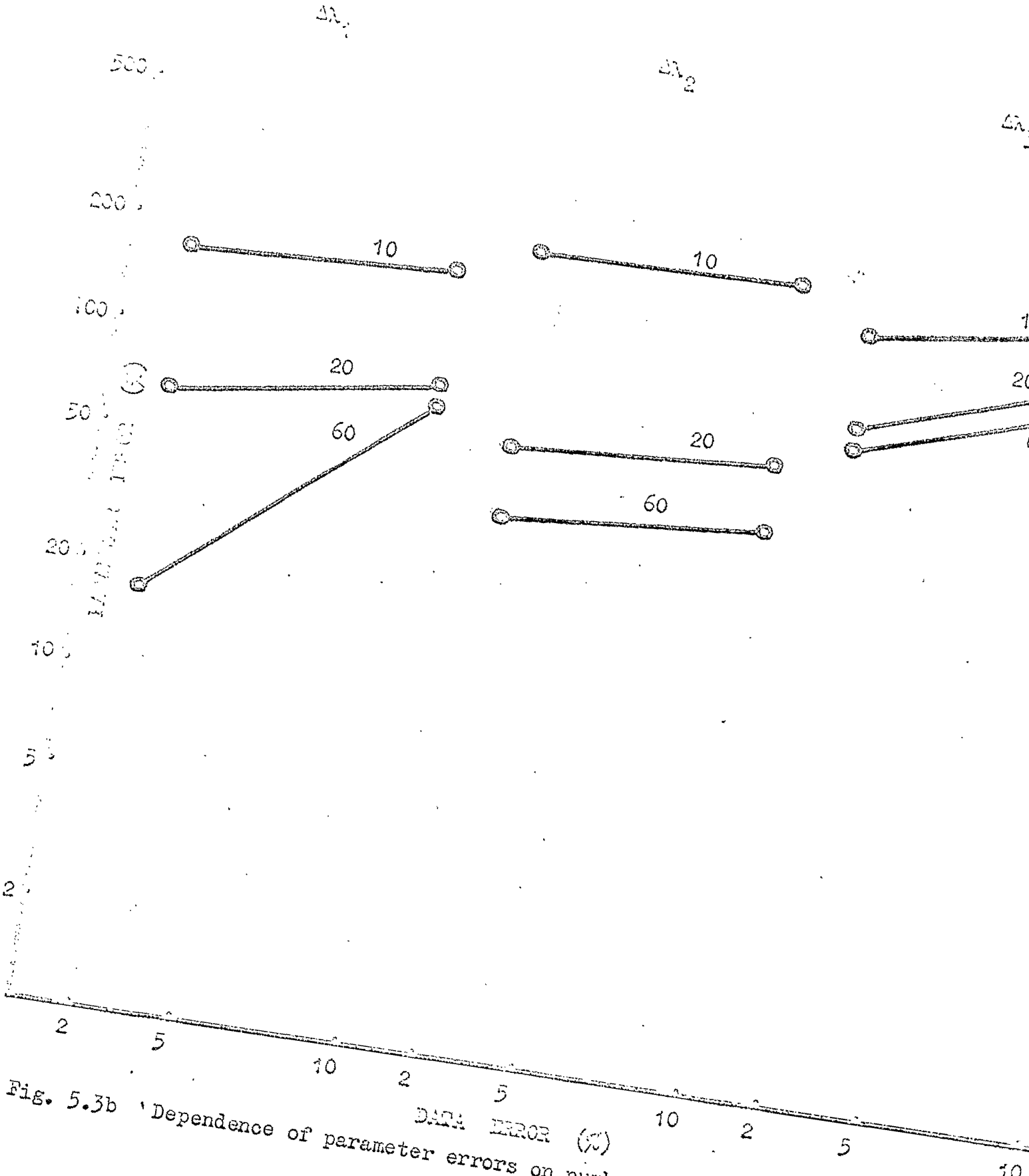


Fig. 5.3b Dependence of parameter errors on number of data points

TABLE 5.3.1

Dependence of Parameter Errors on Number of Data Points (Detailed Amplitude Results)

(amplitudes 0.3; exponents 0.18, 0.06, 0.02)

Error %	No. of Points	σ_1 Mean	σ_1 S.D.	σ_1 2xCoef of Var (%)	σ_2 Mean	σ_2 S.D.	σ_2 2xCoef of Var (%)	σ_3 Mean	σ_3 S.D.	σ_3 2xCoef of Var (%)
2	10	0.3540	0.1699	95.98	0.4902	0.2462	100.44	0.2528	0.1401	110.83
10		0.3650	0.2989	163.78	0.4677	0.2956	126.40	0.3311	0.1891	114.22
2	20	0.2996	0.1286	85.84	0.3650	0.2380	130.41	0.3356	0.1252	74.61
10		0.3205	0.1462	91.23	0.2923	0.2953	161.32	0.2382	0.1519	79.84
2	60	0.3093	0.07139	46.16	0.3400	0.09248	54.40	0.3406	0.05012	29.43
10		0.2563	0.1262	98.47	0.4360	0.2019	92.61	0.2862	0.1188	83.01

TABLE 5.3.2

Dependence of Parameter Errors on Number of Data Points (Detailed Exponent Results)

(amplitudes 0.3; exponents 0.18, 0.06, 0.02)

Error %	No. of Points	λ_1 Mean	λ_1 S.D.	λ_1 2xCoef of Var (%)	λ_2 Mean	λ_2 S.D.	λ_2 2xCoef of Var (%)	λ_3 Mean	λ_3 S.D.	λ_3 2xCoef of Var (%)
2	10	0.2795	0.2420	173.16	0.08111	0.09565	235.85	0.01080	0.01060	196.29
10		0.2647	0.2610	197.20	0.07528	0.02370	62.96	0.01625	0.02090	257.22
2	20	0.2163	0.07341	67.87	0.06447	0.02095	64.99	0.01833	0.009447	103.77
10		0.1600	0.06374	79.67	0.06876	0.02591	75.36	0.02953	0.03064	207.51
2	60	0.1850	0.0162	17.51	0.06425	0.01284	39.96	0.02290	0.01059	92.48
10		0.2060	0.09068	88.04	0.06709	0.01594	47.51	0.01323	0.01054	159.33

TABLE 5.4

Dependence of Parameter Errors on Sampling Frequency

(amplitudes 0.08, 0.23, 0.69; exponents 0.5, 0.1, 0.02; 20 data points)

Sampling Frequency	Data Error	Parameter Error $\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\sigma_3$	$\Delta\lambda_1$	$\Delta\lambda_2$	$\Delta\lambda_3$
Fixed	2%	164.52	141.72	49.20	95.66	111.80	87.58
Variable*		113.05	96.30	32.12	89.93	89.83	56.48
Fixed	10%	235.04	107.36	33.78	87.77	189.28	134.05
Variable*		202.60	114.10	22.87	65.83	129.75	91.91

* t = 0.2, 0.5, 0.7, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0

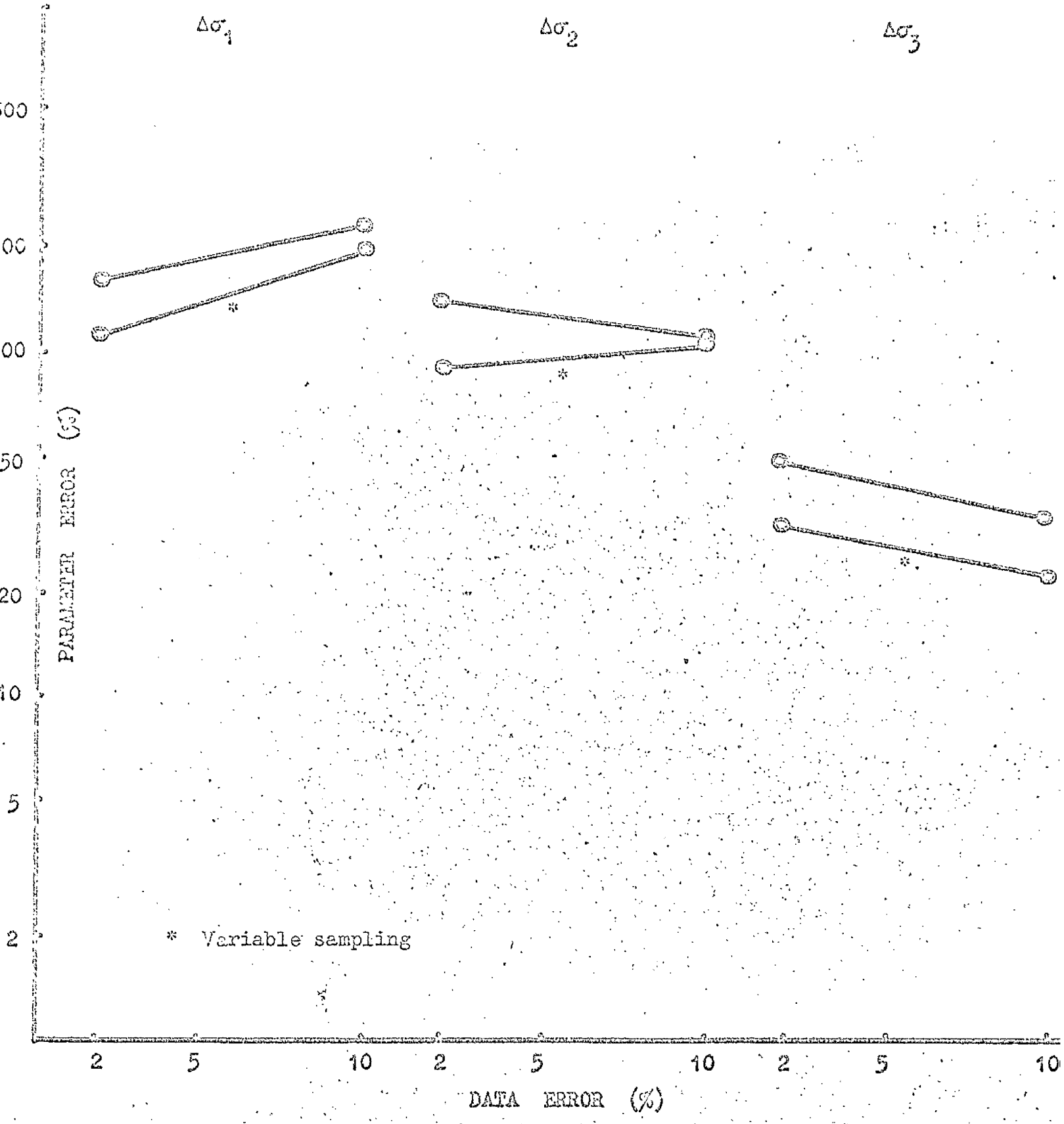


Fig. 5.4a Dependence of parameter errors on sampling frequency

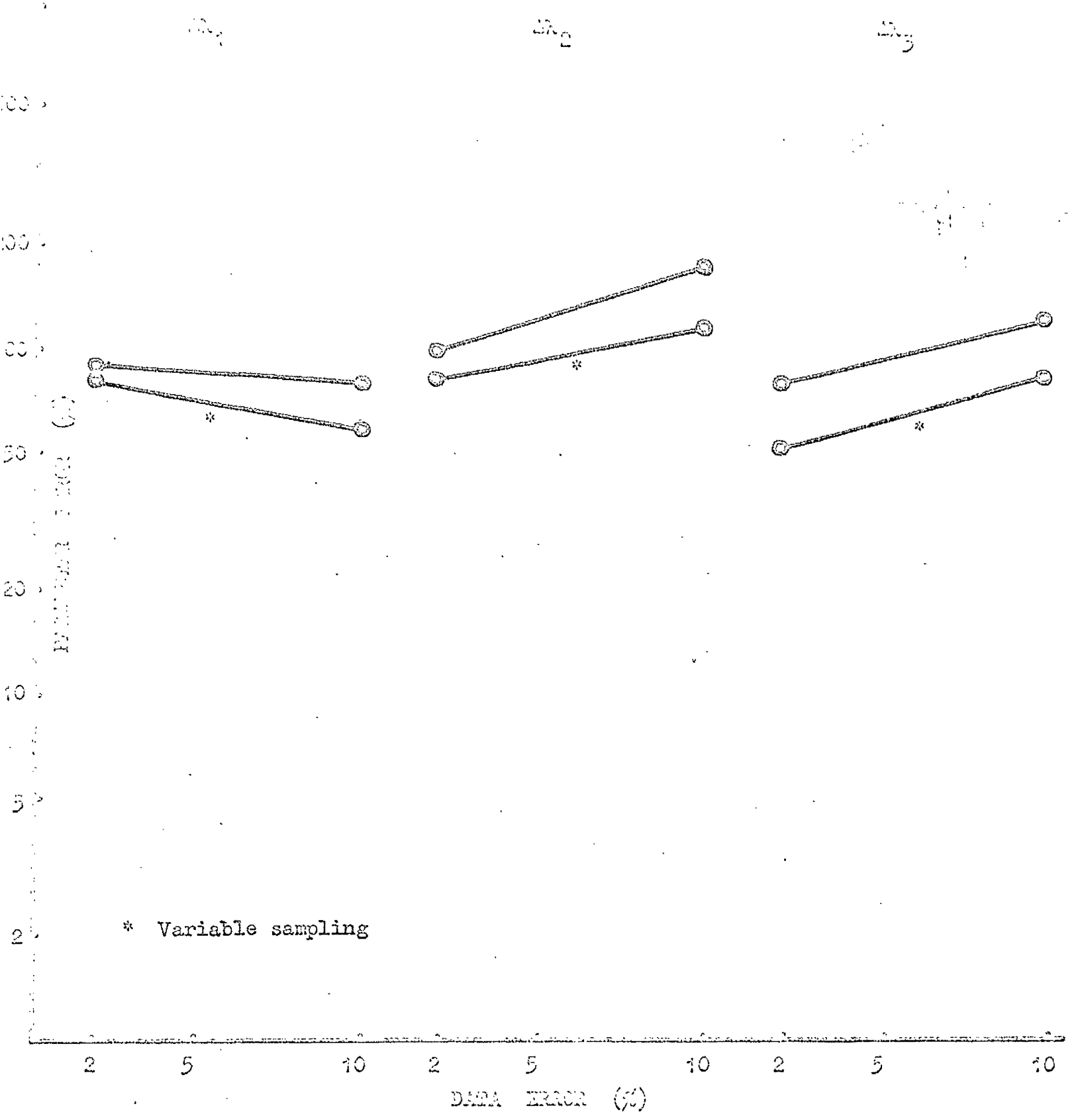


Fig. 5.4b Dependence of parameter errors on sampling frequency

TABLE 5.4.1

Dependence of Parameter Errors on Sampling Frequency (Detailed Amplitude Results)

(amplitudes 0.08, 0.23, 0.69; exponents 0.5, 0.1, 0.02; 20 data points)

Error %	σ_1			σ_2			σ_3		
	Mean	S.D.	2xCoef of Var (%)	Mean	S.D.	2xCoef of Var (%)	Mean	S.D.	2xCoef of Var (%)
2	0.1421	0.1169	164.52	0.2471	0.1751	141.72	0.6601	0.1624	49.20
10	0.09207	0.1082	235.04	0.2798	0.1341	107.36	0.6584	0.1112	33.78
2	0.08628	0.04877	113.05	0.2517	0.1212	96.30	0.6532	0.1049	32.12
10	0.09455	0.9578	202.60	0.2054	0.1172	114.10	0.6870	0.07854	22.87

TABLE 5.4.2

Dependence of Parameter Errors on Sampling Frequency (Detailed Exponent Results)

(amplitudes 0.08, 0.23, 0.69; exponents 0.5, 0.1, 0.02; 20 data points)

Error %	λ_1 Mean	λ_1 S.D.	λ_1 2xCoef of Var (%)	λ_2 Mean	λ_2 S.D.	λ_2 2xCoef of Var (%)	λ_3 Mean	λ_3 S.D.	λ_3 2xCoef of Var (%)
2	0.5279	0.2525	95.66	0.1281	0.07161	11.80	0.02118	0.009276	87.58
10	0.5571	0.2445	87.77	0.1138	0.1077	189.28	0.02114	0.01417	134.05
2	0.5393	0.2425	89.93	0.09974	0.04480	89.83	0.02029	0.00573	56.58
10	0.4551	0.1498	65.83	0.1120	0.07266	129.75	0.02213	0.01017	91.91

TABLE 5.5

Dependence of the Parameter Errors on Incomplete Collection of Data

(amplitudes 0.69, 0.23, 0.08; exponents 0.5, 0.1, 0.02; data error 2%)

	No. of Data Points	Parameter Error					
		$\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\sigma_3$	$\Delta\lambda_1$	$\Delta\lambda_2$	$\Delta\lambda_3$
	60	1.71	17.23	65.84	1.73	16.21	117.21
	50	3.08	38.85	150.88	3.98	31.42	182.53
	40	4.57	57.27	93.80	4.92	25.71	114.59
	30	9.50	19.65	91.37	6.71	55.38	71.84
	20	7.93	113.94	103.15	11.49	118.92	141.02
t = 0 - 13.32	60	6.91	26.61	120.04	4.03	47.02	155.11
t = 0 - 6.67		8.55	69.54	98.07	7.32	83.58	161.18

TABLE 5.5.1

Dependence of Parameter Errors on Incomplete Collection of Data (Detailed Amplitude Results)

(amplitudes 0.69, 0.23, 0.08; exponents 0.5, 0.1, 0.02; data error 2%)

No. of Points	σ_1 Mean	σ_1 S.D.	σ_1 2xCoef of Var (%)	σ_2 Mean	σ_2 S.D.	σ_2 2xCoef of Var (%)	σ_3 Mean	σ_3 S.D.	σ_3 2xCoef of Var (%)
60/60	0.6973	0.00597	1.71	0.2372	0.02044	17.23	0.06789	0.02235	65.84
50/60	0.7014	0.01079	3.08	0.2279	0.04427	38.85	0.06859	0.05175	150.88
40/60	0.6971	0.01596	4.57	0.2065	0.05912	57.27	0.09987	0.04684	93.80
30/60	0.6855	0.03257	9.50	0.2423	0.02381	19.65	0.07306	0.03338	91.37
20/60	0.7214	0.02861	7.93	0.1565	0.08916	113.94	0.1187	0.06122	103.15
0-13.32	0.6910	0.02386	6.91	0.2386	0.03175	26.61	0.07031	0.04220	120.04
0- 6.67	0.6916	0.02958	8.55	0.2038	0.07086	69.54	0.10229	0.05016	98.07

TABLE 5.5.2

Dependence of Parameter Errors on Incomplete Collection of Data (Detailed Exponent Results)

(amplitudes 0.69, 0.23, 0.08; exponents 0.5, 0.1, 0.02; data error 2%)

No. of Points	λ_1 Mean	λ_1 S.D.	λ_1 2xCoef of Var (%)	λ_2 Mean	λ_2 S.D.	λ_2 2xCoef of Var (%)	λ_3 Mean	λ_3 S.D.	λ_3 2xCoef of Var (%)
60/60	0.5010	0.004327	1.73	0.09664	0.07831	16.21	0.01551	0.00909	117.21
50/60	0.4870	0.009702	3.98	0.09420	0.01480	31.42	0.01683	0.01536	182.53
40/60	0.4983	0.01227	4.92	0.1023	0.01315	25.71	0.02611	0.02992	114.59
30/60	0.5035	0.01690	6.71	0.1071	0.02966	55.38	0.01793	0.006441	71.84
20/60	0.4796	0.02757	11.49	0.1009	0.0600	118.92	0.02350	0.01657	141.02
0-13.32	0.4994	0.01006	4.03	0.10039	0.02360	47.02	0.01595	0.01237	155.11
0- 6.67	0.4953	0.01813	7.32	0.09983	0.04172	83.58	0.02401	0.01935	161.18

TABLE 5.6

Dependence of Parameter Errors on the Definition of the Function by the Data

(amplitudes 0.69, 0.23, 0.08)

Result Number	Exponents	Error	No. of Data Points	Sampling Frequency	Parameter Error					
					$\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\sigma_3$	$\Delta\lambda_1$	$\Delta\lambda_2$	$\Delta\lambda_3$
1	1.0, 0.20, 0.05	2%	60	Equal	2.08	1.75	18.02	1.65	9.17	11.92
2	0.50, 0.10, 0.02	2%	60	Equal	1.71	17.23	65.84	1.75	16.21	117.21
3	1.0, 0.2, 0.05	2-11.6%*	20	Variable**	6.25	14.68	85.86	5.20	32.13	72.95
4	0.50, 0.10, 0.02	2%	20	Equal	7.02	20.27	95.55	11.90	54.38	122.74

* 2.1, 2.2, 2.3, 2.5, 2.7, 3.0, 3.3, 3.7, 4.0, 4.5, 5.0, 5.7, 6.3, 7.0, 7.6, 8.3, 9.6, 10.6, 11.6

** t = 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.5, 9.0, 11, 13, 15, 17, 19, 21, 23, 26, 29

TABLE 5.6.1

Dependence of Parameter Errors on Definition of Function by Data (Detailed Amplitude Results)

(amplitudes 0.69, 0.23, 0.08; exponents 1.0, 0.20, 0.05)

Error	No. of Points	Sampling Frequency	σ_1 Mean	σ_1 S.D.	σ_1 2xCoef of Var (%)	σ_2 Mean	σ_2 S.D.	σ_2 2xCoef of Var (%)	σ_3 Mean	σ_3 S.D.	σ_3 2xCoef of Var (%)
2%	60	Equal	0.6905	0.00719	2.08	0.2289	0.0020	1.75	0.07726	0.00696	18.02
Variable	20	Variable	0.7115	0.02223	6.25	0.2242	0.01646	14.68	0.06774	0.02908	85.86

TABLE 5.6.2

Dependence of Parameter Errors on Definition of Function by Data (Detailed Exponent Results)

(amplitudes 0.69, 0.23, 0.08; exponents 1.0, 0.2, 0.05)

Error	No. of Points	Sampling Frequency	λ_1 Mean	λ_1 S.D.	λ_1 2xCoef of Var (%)	λ_2 Mean	λ_2 S.D.	λ_2 2xCoef of Var (%)	λ_3 Mean	λ_3 S.D.	λ_3 2xCoef of Var (%)
2%	60	Equal	0.9932	0.00821	1.65	0.1998	0.00916	9.17	0.0500	0.00298	11.92
Variable	20	Variable	0.9796	0.02545	5.20	0.1853	0.02977	32.13	0.04400	0.01605	72.95

Tables and Figures of Chapter 7

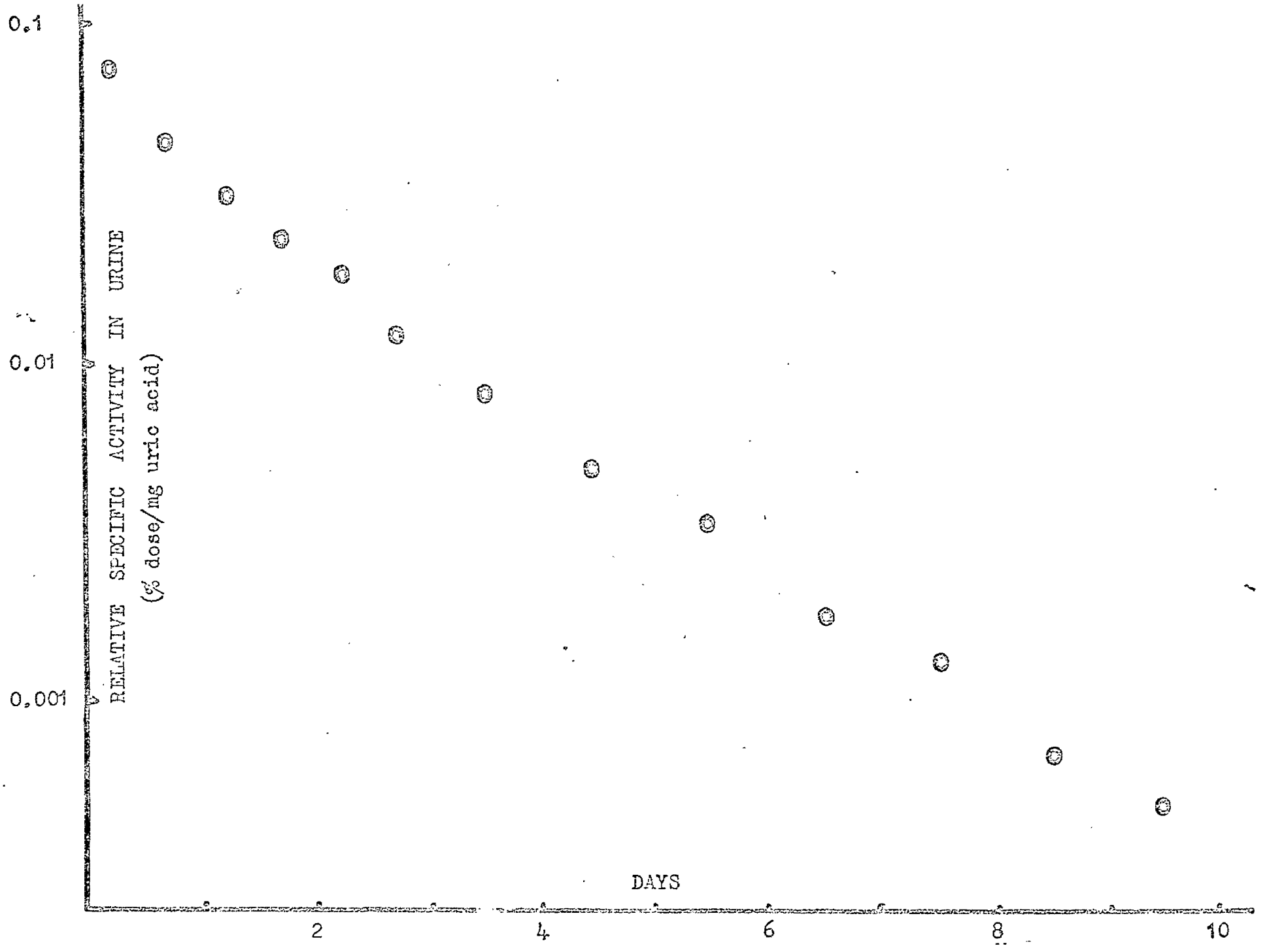


Fig. 7.1 An example of a radioactive uric acid urine concentration curve indicating a non-monoexponential clearance

TABLE 7.1

Number of Components Derived from Data

Patient Number	Berman all points in W.F. $(1/Y)$	Berman all points in W.F. $(1/Y^2)$	Gilles all points in W.F. $(1/Y^2)$	Gilles all points in W.F. $(1/Y)$	Gilles 1st point omitted W.F. $(1/Y)$	Diagnosis
3	1	1	1	1	1	Normal (Rheumatoid Arthritis)
11	1	2	1	1	1	Normal (Rheumatic Fever)
6	1	1	2	2*	1	Normal (Myocardial Infarct)
15	-	-	-	-	1	Normal (Ankylosing Spondilitis)
18	1	1	1	1	1	Gout on allopurinol
7	1	1	1	1*	2	Hypertension and Hyperuricaemia
10	2	2	2	2	2	Hypertension
4	1	1	1	1	1	Gout, non tophaceous and Hypertension
12	1	1	1	1	1	
17	1	1	1	1	1	Gout, non tophaceous
13	1	1	1	2	2	
1	2	1	2	2	2	
16	1	1	1	1	1	Gout, tophaceous and Hypertension
19	1	1	1	2*	1	
14	1	1	1	2	2	
2	2	2	2	2	2	
8	1	1	2	2	2	Gout, tophaceous
5	1	1	2	2*	1	
20	1	1	1	2*	1	
9	-	-	2	2	2	

* The number of components depends on the inclusion of the first data point

TABLE 7.2

Urine Uric Acid Concentration Data

Patient 1		Patient 2		Patient 3	
Days	% Dose	Days	% Dose	Days	% Dose
0.75	0.045500	1.25	0.0174100	0.75	0.065570
1.25	0.031850	1.75	0.0107100	1.25	0.049510
1.75	0.023900	2.25	0.0073100	1.75	0.031210
2.25	0.018500	2.75	0.0050290	2.25	0.022870
2.75	0.012630	3.50	0.0032940	2.75	0.018990
3.50	0.009220	4.50	0.0016720	3.50	0.013580
4.50	0.005260	5.50	0.0008133	5.50	0.004550
5.50	0.003640	6.50	0.0004348	9.50	0.000473
6.50	0.001965	7.50	0.0002331	10.50	0.000283
7.50	0.001450	8.50	0.0001415	0.25	0.088120
8.50	0.000763	9.50	0.0000818		
9.50	0.000551	10.50	0.0000513		
10.50	0.000226				
0.25	0.071900				

Patient 4		Patient 5		Patient 6	
Days	% Dose	Days	% Dose	Days	% Dose
0.75	0.057430	0.75	0.029160	0.75	0.039010
1.25	0.054630	1.25	0.024230	1.25	0.029600
1.75	0.036370	1.75	0.018050	1.75	0.025050
2.25	0.030680	2.25	0.019480	2.25	0.022280
2.75	0.020420	2.75	0.015370	2.75	0.016410
3.50	0.019180	3.50	0.013610	3.50	0.010270
4.50	0.012780	4.50	0.007108	4.50	0.005182
5.50	0.008545	5.50	0.005178	5.50	0.003418
6.50	0.007243	6.50	0.003970	6.50	0.002218
7.50	0.007045	7.50	0.003136	7.50	0.001365
8.50	0.002914	8.50	0.002237	8.50	0.000824
9.50	0.001990	9.50	0.001709	9.50	0.000578
10.50	0.001764	10.50	0.001453	10.50	0.000391
0.25	0.075010	0.25	0.042700	0.25	0.055930

TABLE 7.3

Patient 7	
Days	% Dose
0.75	0.042050
1.25	0.035340
1.75	0.029180
2.25	0.022200
2.75	0.022640
3.50	0.014930
4.50	0.011700
6.50	0.005615
7.50	0.003779
8.50	0.002246
9.50	0.001556
0.25	0.055920

Patient 8	
Days	% Dose
0.75	0.038150
1.25	0.026850
1.75	0.018350
2.25	0.014890
2.75	0.011540
3.50	0.007897
4.50	0.004943
5.50	0.003171
6.50	0.001752
7.50	0.001241
8.50	0.000656
9.50	0.000470
0.25	0.047660

Patient 9	
Days	% Dose
0.75	0.032050
1.25	0.025440
1.75	0.020830
2.25	0.013570
3.50	0.007910
4.50	0.004434
5.50	0.003570
6.50	0.002636
7.50	0.001946
8.50	0.001443
9.50	0.001313
10.50	0.001004

Patient 10	
Days	% Dose
0.75	0.054620
1.25	0.044980
1.75	0.034990
2.25	0.028020
2.75	0.022630
3.50	0.015810
4.50	0.011120
5.50	0.007711
6.50	0.005024
7.50	0.003525
8.50	0.002398
9.50	0.001753
10.50	0.001195
0.25	0.072290

Patient 11	
Days	% Dose
0.75	0.046470
1.25	0.037660
1.75	0.026610
2.25	0.016160
2.75	0.011290
3.50	0.008260
4.50	0.003611
5.50	0.001908
6.50	0.000878
7.50	0.000437
8.50	0.000241
9.50	0.000169
10.50	0.000115
0.25	0.089710

Patient 12	
Days	% Dose
0.75	0.049290
1.25	0.043270
1.75	0.035610
2.25	0.024400
2.75	0.019610
3.50	0.017370
4.50	0.010790
5.50	0.007000
6.50	0.004626
7.50	0.003209
8.50	0.002467
9.50	0.001551
10.50	0.001016
0.25	0.053210

TABLE 7.4

Patient 13

Days	% Dose
0.667	0.040220
1.167	0.031600
1.667	0.026830
2.167	0.023280
2.667	0.018100
3.417	0.011950
4.417	0.008425
5.417	0.006253
6.417	0.004163
7.417	0.002843
8.417	0.001765
9.417	0.001192
10.417	0.000886

Patient 14

Days	% Dose
0.667	0.037770
1.167	0.032850
1.667	0.027510
2.167	0.021590
2.667	0.015910
3.417	0.011860
4.417	0.008318
5.417	0.005481
6.417	0.003751
7.417	0.002695
8.417	0.001563
9.417	0.001021
10.417	0.000714

Patient 15

Days	% Dose
1.25	0.041950
1.75	0.027500
2.25	0.018640
2.75	0.014600
3.50	0.008891
4.50	0.003544
5.50	0.001837
6.50	0.000958
7.50	0.000624
8.50	0.000394
9.50	0.000208
10.50	0.000125

Patient 16

Days	% Dose
0.75	0.032990
1.25	0.029880
1.75	0.022280
2.25	0.017720
2.75	0.015790
3.50	0.013150
4.50	0.008230
5.50	0.005757
6.50	0.004155
7.50	0.003230
8.50	0.002488
9.50	0.001709
10.50	0.000889
0.25	0.041370

Patient 17

Days	% Dose
0.75	0.050890
1.25	0.041990
1.75	0.029510
2.25	0.025640
2.75	0.019350
3.50	0.012710
4.50	0.007648
5.50	0.004405
6.50	0.003353
7.50	0.002060
8.50	0.001242
9.50	0.000829
10.50	0.000505
0.25	0.068370

Patient 18

Days	% Dose
0.75	0.079590
1.25	0.062120
1.75	0.066110
2.25	0.045360
2.75	0.041560
3.50	0.031000
4.50	0.024080
5.50	0.019050
6.50	0.012740
7.50	0.010020
8.50	0.007518
9.50	0.006179
10.50	0.003921
0.25	0.070770

TABLE 7.5

Patient 19		Patient 20	
Days	% Dose	Days	% Dose
0.75	0.026220	0.75	0.027575
1.25	0.025420	1.25	0.025345
1.75	0.020380	1.75	0.017860
2.25	0.016850	2.25	0.015235
2.75	0.014160	2.75	0.012150
3.50	0.011560	3.50	0.008502
4.50	0.007831	4.50	0.004651
5.50	0.005857	5.50	0.002690
6.50	0.004395	6.50	0.001704
7.50	0.002811	7.50	0.001052
8.50	0.002038	8.50	0.000757
9.50	0.001529	9.50	0.000556
10.50	0.001164	10.50	0.000347
0.25	0.029120	0.25	0.033885

TABLE 7.6

F-ratio Test Results with Berman Programme (all data points)

Pt.	No. of Pts	Two Compartments "SIGMA"*	One Compartment "SIGMA"	F-ratio	Probability
G1	14	6.4122901E-8	1.1578701E-7	1.805	5%
G2	13	7.9964317E-10	6.5368903E-9	8.175	1%
G3	10	2.1091440E-8	3.6889936E-8	1.749	5%
G4	14	4.6239607E-6	5.8125793E-6	1.257	5%
G5	14	1.6660182E-6	2.2873779E-6	1.372	5%
G6	14	9.3396518E-8	1.6832652E-7	1.802	5%
G7	12	9.3465851E-7	1.0029647E-6	1.101	5%
G8	13	5.7631623E-8	1.2539461E-7	2.175	5%
G9	13	2.2195629E-6	2.7367210E-6	1.333	5%
G10	14	5.5411694E-8	5.6383394E-7	10.175	1%
G11	14	9.5183111E-9	3.6897343E-8	3.876	1%
G12	14	6.5239576E-7	5.3932766E-7	0.806	5%
G13	13	1.5804219E-7	1.6222692E-7	1.026	5%
G14	13	1.5075033E-7	1.0584067E-9	0.007	5%
G15	13	1.2348969E-8	6.9062269E-8	5.59	1%
G16	14	7.1371646E-7	7.3283622E-7	1.026	5%
G17	14	7.7190240E-8	1.4708377E-7	1.905	5%
G18	14	1.449095E-5	1.0350523E-5	0.714	5%
G19	14	1.0120193E-5	3.0513828E-7	0.03	5%
G20	14	8.0514641E-8	1.3452185E-7	1.671	5%

* "SIGMA" is the summation of the weighted least squares

TABLE 7.7

F-ratio Test Results Using Gilles Programme

Patient	All Data Points Probability of F	1st Data Point Omitted Probability of F
1	3.57×10^{-5}	2.83×10^{-3}
2	3.12×10^{-5}	1.72×10^{-4}
3	2.38×10^{-1}	1.63×10^{-1}
4	1.28×10^{-1}	2.14×10^{-1}
5	3.21×10^{-2}	6.23×10^{-1}
6	1.29×10^{-2}	2.93×10^{-1}
7	1.66×10^{-1}	1.16×10^{-2}
8	2.19×10^{-2}	4.50×10^{-4}
9	1.55×10^{-3}	1.55×10^{-3}
10	1.11×10^{-4}	1.07×10^{-3}
11		
12	8.44×10^{-2}	4.05×10^{-1}
13	5.36×10^{-2}	5.36×10^{-2}
14	4.56×10^{-2}	4.56×10^{-2}
15	1.14×10^{-4}	3.30×10^{-1}
16		
17		
18		2.56×10^{-1}
19	6.22×10^{-3}	
20	3.45×10^{-2}	

TABLE 7.8

Total Exchangeable Uric Acid Pool and Turnover Data

Patient Number	Total Exchangeable Pool (mg)	Turnover Rate (Day^{-1})	Turnover Rate (mg/day)	Diagnosis
3	992 ± 37	.607 ± .023	602	Normal (Rheumatoid Arthritis)
11	1081 ± 41	.735 ± .026	795	Normal (Rheumatic Fever)
6*	1650 ± 47	.508 ± .014	838	Normal (Myocardial Infarct)
15	1019 ± 26	.709 ± .013	723	Normal (Ankylosing Spondilitis)
18	1031 ± 36	.305 ± .010	314	Gout on allopurinol
7	1742 ± 52	.373 ± .011	650	Hypertension and Hyperuricaemia
10	1362 ± 18	.450 ± .006	552	Hypertension
4	1284 ± 64	.394 ± .019	506	Gout, non tophaceous and Hypertension
12	1569 ± 49	.393 ± .012	616	
17	1324 ± 22	.494 ± .008	659	Gout, non tophaceous
13**	1928 ± 34	.400 ± .007	771	
1	1388 ± 20	.733 ± .024	789	
16	2282 ± 53	.361 ± .008	824	Gout, tophaceous and Hypertension
19*	2933 ± 71	.320 ± .008	939	
14**	1943 ± 35	.413 ± .007	802	
2	1948 ± 23	.961 ± .027	1542	
8	1933 ± 49	.576 ± .022	1000	Gout, tophaceous
5	2471 ± 138	.562 ± .267	861	
20*	2475 ± 68	.464 ± .012	1149	
9	3199 ± 1477	.480 ± .051	948	

* Owing to excessive parameter error estimates, pool and turnover estimates were obtained from the single exponential analysis. These patients would have a single pool if the first point were omitted.

** Owing to excessive parameter error estimates, pool and turnover estimates were obtained from a single exponential analysis.

TABLE 7.9

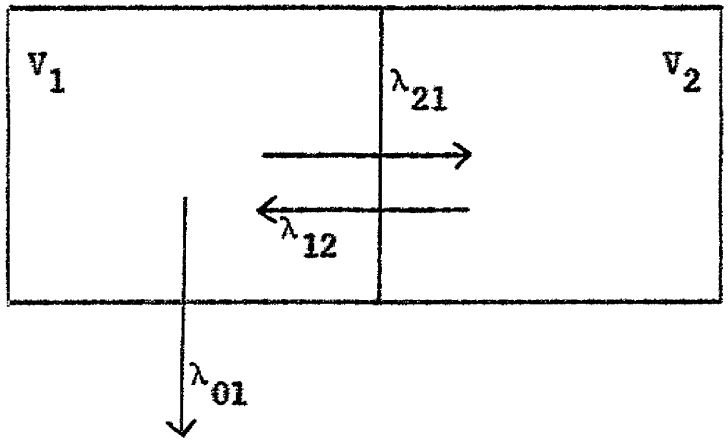
Summary of Total Exchangeable Pools and Turnover Rates

	Number in Group	Total Exchangeable Pool (mg)		Turnover Rate (mg/day)	
		Average	Range	Average	Range
Normals	4	1186	992 - 1650	740	723 - 838
Gout on allopurinol	1	1031	-	314	-
Hypertension	2	1552	1362, 1742	601	552, 650
Gout, non tophaceous	5	1499	1284 - 1928	668	506 - 789
Gout, tophaceous	8	2398	1933 - 3199	1008	802 - 1542

TABLE 7.10

Two Compartment Model

Pool Sizes, Turnover and Transfer Rates



Patient	Pool Size (V_1) (mg)	Pool Size (V_2) (mg)	Turnover Rate ($\lambda_{01}.V_1$) mg/day	Transfer Rate between Compartments ($\lambda_{12}V_2.\lambda_{21}V_1$) mg/day
10	1227 ± 17	135 ± 15	522 ± 11	77 ± 21
8	1736 ± 66	197 ± 64	1000 ± 55	148 ± 114
1	1076 ± 35	312 ± 32	789 ± 38	390 ± 101
2	1606 ± 46	342 ± 35	1542 ± 70	329 ± 76
9	1975 ± 208	1224 ± 1283	948 ± 115	195 ± 39

TABLE 7.11

Serum Uric Acid Concentration Data

Patient 7		Patient 8		Patient 10	
Days	% Dose*	Days	% Dose	Days	% Dose
0.06	0.07158	0.06	0.06213	0.15	0.07767
0.15	0.06427	0.23	0.04962	0.31	0.06404
0.31	0.06043	0.33	0.04579	0.49	0.05872
0.48	0.05460	0.50	0.04215	0.56	0.04671
0.94	0.04189	0.97	0.03140	1.30	0.03526
1.23	0.03899	1.27	0.02699	1.48	0.03702
1.46	0.03293	1.5	0.02441	1.97	0.02941
1.94	0.03169	2.0	0.02030	2.28	0.02334
2.23	0.02700	2.5	0.01368	2.49	0.02193
2.5	0.02239	3.0	0.01120	2.94	0.01728
2.96	0.01982	3.23	0.009128	3.24	0.01500
3.25	0.01798	4.0	0.006237	3.52	0.01323
3.54	0.01407	5.0	0.004520	3.95	0.01146
4.04	0.01296	5.97	0.002268	4.96	0.007927
5.00	0.009413	7.0	0.001804	6.92	0.004273
5.98	0.005722			7.92	0.003119
6.96	0.004287			9.42	0.001877
8.10	0.002422			9.94	0.001584
9.05	0.001494				
9.96	0.001400				

* % of C-14 administered/mg uric acid

TABLE 7.12

Serum Uric Acid Concentration Data

Patient 11		Patient 12		Patient 13	
Days	% Dose	Days	% Dose	Days	% Dose
0.08	0.08876	0.06	0.09860	0.08	0.05199
0.15	0.07840	0.15	0.07529	0.18	0.04644
0.31	0.0658	0.33	0.06610	0.32	0.04324
0.50	0.04807	0.52	0.05697	0.49	0.04367
0.96	0.03972	0.98	0.04618	0.92	0.03287
1.31	0.02487	1.29	0.04027	1.18	0.02781
1.42	0.02487	1.48	0.03569	1.42	0.02664
1.97	0.01725	1.98	0.02893	1.92	0.01844
2.29	0.01130	2.26	0.02590	2.17	0.01679
2.49	0.009404	2.50	0.02335	2.42	0.01611
2.94	0.006626	3.03	0.01848	2.92	0.01284
3.25	0.005759	3.27	0.01683	3.21	0.01148
3.52	0.003855	3.48	0.01542	3.44	0.01181
4.96	0.00102	3.98	0.01283	3.95	0.007807
		4.98	0.008319	4.92	0.004039
		5.98	0.006307	6.05	0.004054
		6.99	0.004492	7.04	0.002342
		7.91	0.003011	8.04	0.001941
		8.98	0.002166	8.96	0.001641
		9.97	0.001655	10.00	0.001379

* % of C-14 administered/mg uric acid

TABLE 7.13

Serum Uric Acid Concentration Data

Patient 14		Patient 15		Patient 16	
Days	% Dose*	Days	% Dose	Days	% Dose
0.08	0.05169	0.10	0.1015	0.10	0.04757
0.18	0.04526	0.19	0.09143	0.19	0.04213
0.32	0.04191	0.35	0.07474	0.35	0.03681
0.49	0.03930	0.47	0.06938	0.47	0.03968
0.95	0.03171	0.99	0.04084	0.99	0.02753
1.18	0.02899	1.33	0.03283	1.33	0.02695
1.42	0.02530	1.50	0.02996	1.50	0.02748
2.17	0.01753	1.97	0.02105	1.97	0.01957
2.42	0.01523	2.25	0.01637	2.25	0.01946
2.92	0.01420	2.50	0.01417	2.50	0.01926
3.18	0.01224	2.97	0.01155	2.96	0.01505
3.44	0.01394	3.21	0.01373	3.21	0.01446
3.95	0.01053	3.53	0.008024	3.53	0.01454
4.92	0.006917	3.96	0.005405	3.96	0.01097
6.05	0.005866	5.04	0.003605	5.04	0.008529
7.04	0.003608	6.03	0.002041	6.03	0.006032
8.04	0.002473	7.01	0.001426	7.01	0.004285
8.96	0.003508	8.01	0.001124	8.01	0.003100
10.00	0.002545	9.07	0.0009203	9.07	0.002233
		10.31	0.0008304	10.31	0.001903
		11.08	0.0006261	11.08	0.001701

* % of C-14 administered/mg uric acid

TABLE 7.14

Serum Uric Acid Concentration Data

Patient 17		Patient 18		Patient 19	
Days	% Dose	Days	% Dose	Days	% Dose
0.12	0.08629	0.07	0.09076	0.07	0.04027
0.35	0.06097	0.26	0.07101	0.26	0.03218
0.49	0.05688	0.46	0.06212	0.46	0.03156
0.96	0.04041	0.96	0.05508	0.96	0.02538
1.29	0.03185	1.21	0.04849	1.21	0.02124
1.96	0.02089	1.47	0.04667	1.47	0.01881
2.24	0.02032	1.96	0.04120	1.96	0.01719
2.42	0.01944	2.22	0.03696	2.22	0.01604
2.96	0.01427	2.46	0.03430	2.46	0.01418
3.24	0.01274	3.0	0.03147	3.0	0.01249
3.52	0.01017	3.21	0.02849	3.21	0.01164
3.96	0.007666	3.49	0.02643	3.49	0.008667
5.00	0.005682	3.94	0.02391	3.92	0.008990
5.98	0.003504	4.97	0.01964	4.97	0.006401
6.99	0.002336	5.96	0.01414	5.96	0.004801
7.99	0.001348	6.94	0.009351	6.94	0.004092
8.96	0.0009426	7.95	0.007159	7.95	0.002660
10.08	0.0007185	8.99	0.005503	8.99	0.001744
11.00	0.0005749	9.97	0.004274	9.97	0.001408
		10.93	0.002438	10.93	0.001172

* % of C-14 administered/mg uric acid

TABLE 7.15

Serum Uric Acid Concentration Data

Patient 20

Days	% Dose*
0.04	0.04649
0.12	0.03642
0.29	0.03000
0.46	0.02534
0.96	0.01859
1.33	0.01525
1.46	0.01444
1.96	0.01209
2.27	0.01056
2.52	0.009088
2.98	0.007210
3.23	0.006583
3.46	0.005626
4.00	0.004412
4.96	0.002590
6.04	0.001571
6.50	0.001372
6.98	0.0009907
7.46	0.0008105
7.96	0.0005502
8.44	0.0004516
8.98	0.0004509
9.98	0.0003449

* % of C-14 administered/mg uric acid

TABLE 7.16

Comparison of Pool Size and Turnover Estimated from Urine and Serum

Patient Number	Pool Size (mg)		Turnover (mg/day)	
	Urine	Serum	Urine	Serum
11	1081	975	795	906
18	1031	1474	314	392
10	1362	1446	552	665
7	1742	1455	650	611
12	1569	1570	616	631
16	2282	2689	824	789
19	2933	3092	939	1014
17	1324	1804	659	837
13	1928	2421	771	968
14	1943	3044	802	893
8	1933	1668	1000	938
20	2475	3084	1149	1545
9	3199	4006	948	1735
	Standard Error of Estimate = 12.3% on serum measurement		Standard Error of Estimate = 19.6% on serum measurement	

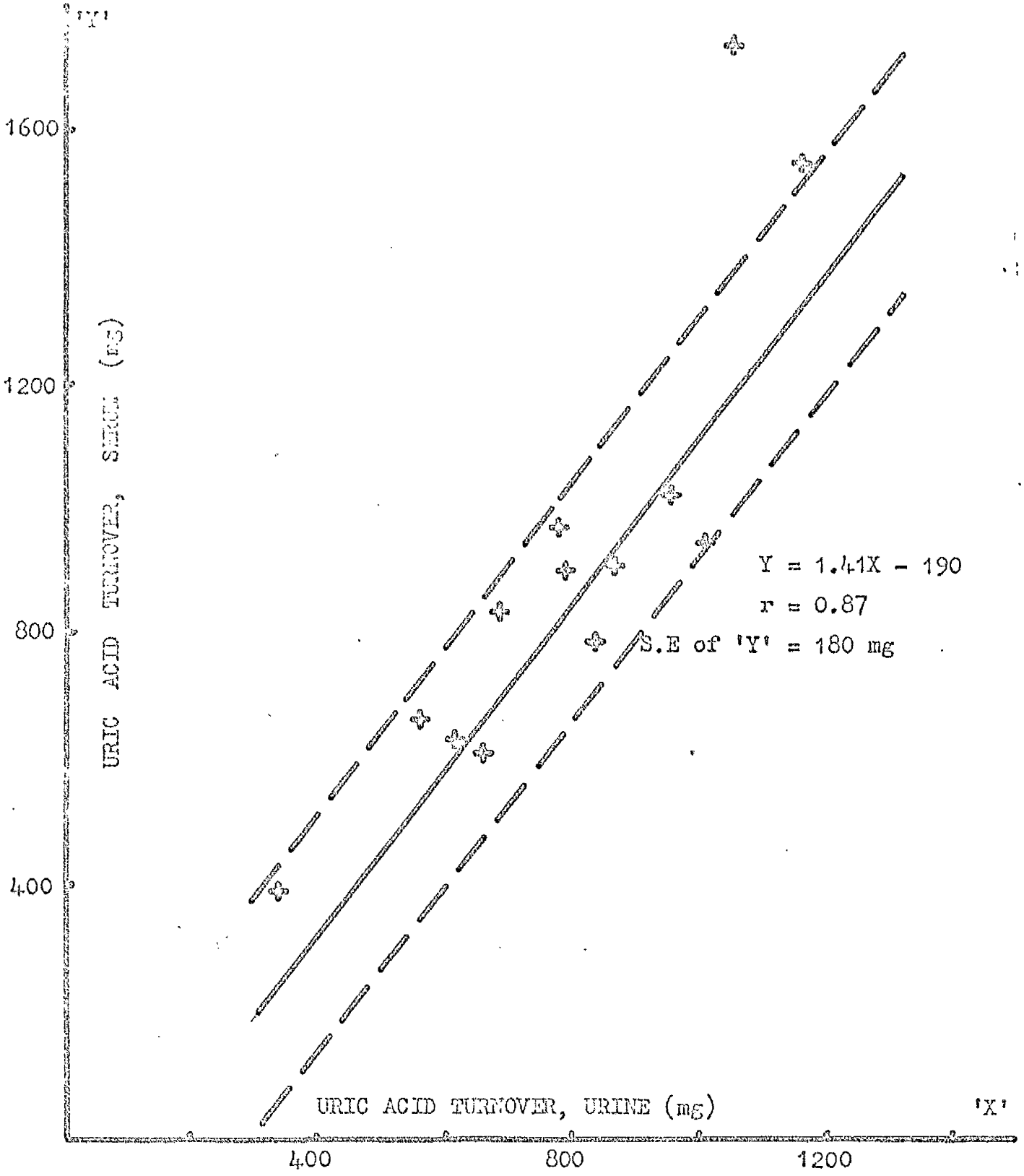


Fig. 7.2 Correlation between turnover of uric acid estimation from the urine (using all data), and the serum (using data from days 2 - 9 inclusive).

Tables and Figures of Chapter 8

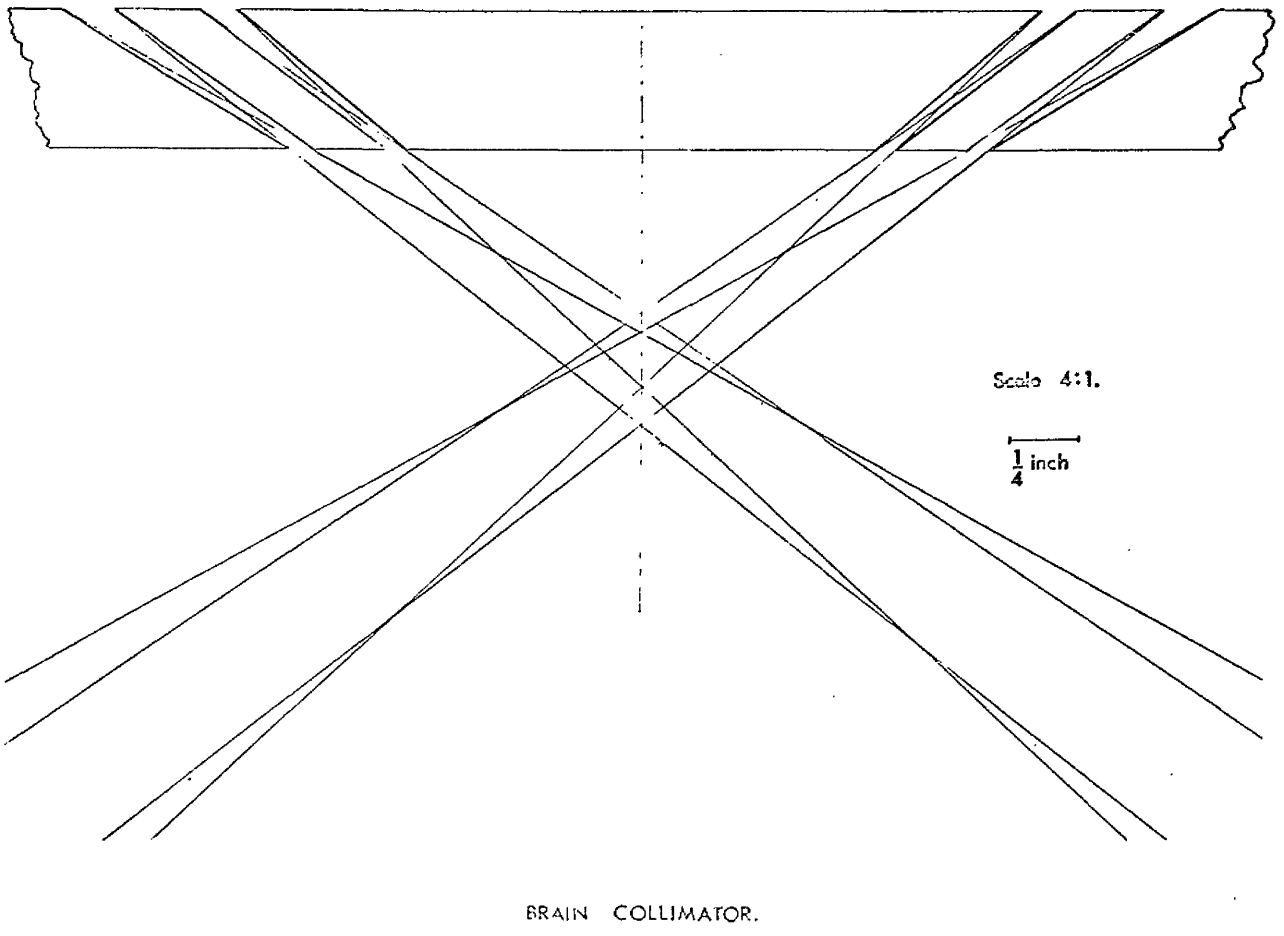


Fig. 8.1 Diagrammatic Representation of the depth focu,sing collimator

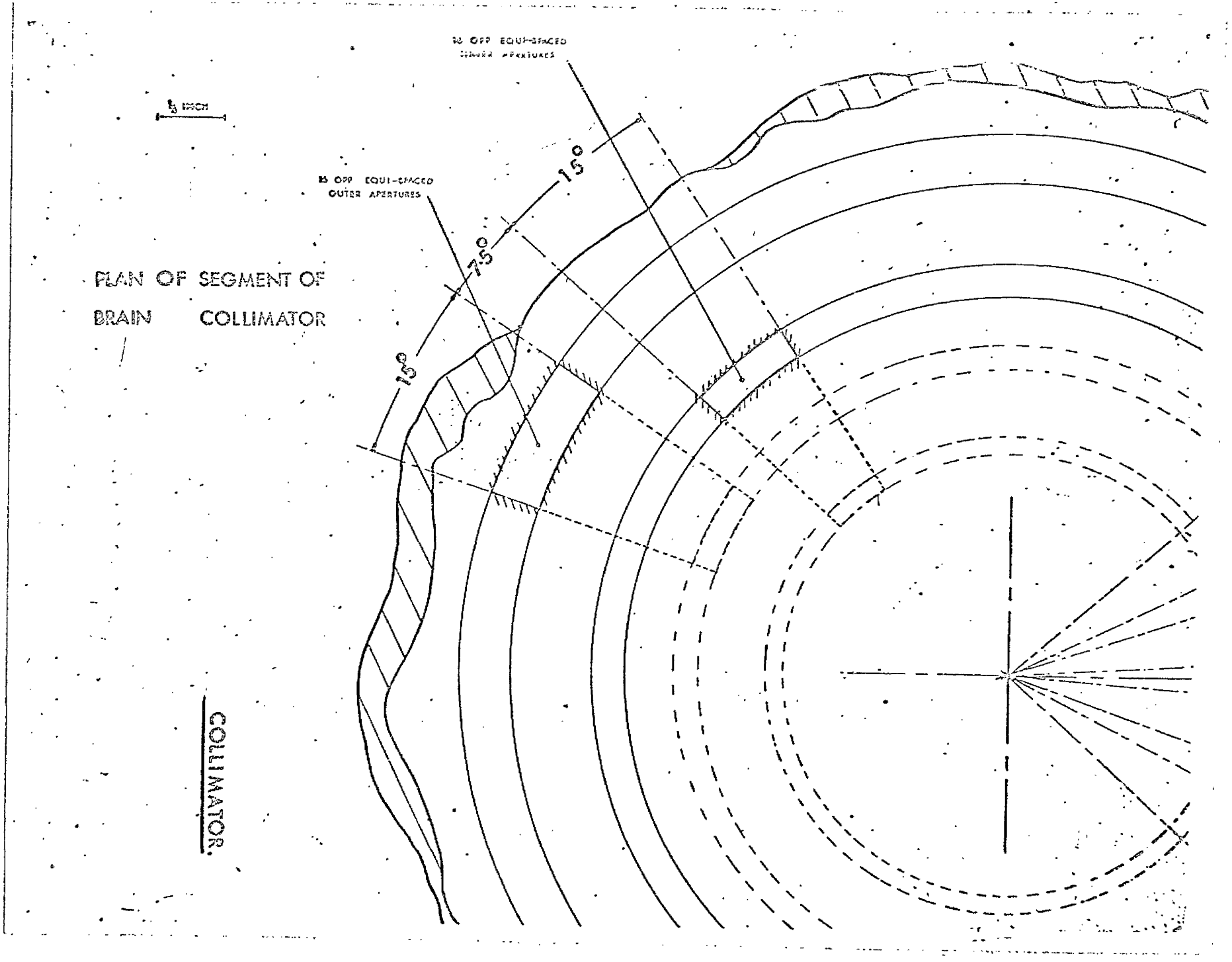


Figure 8.3 Mechanical Dimensions of Depth Focusing Collimator

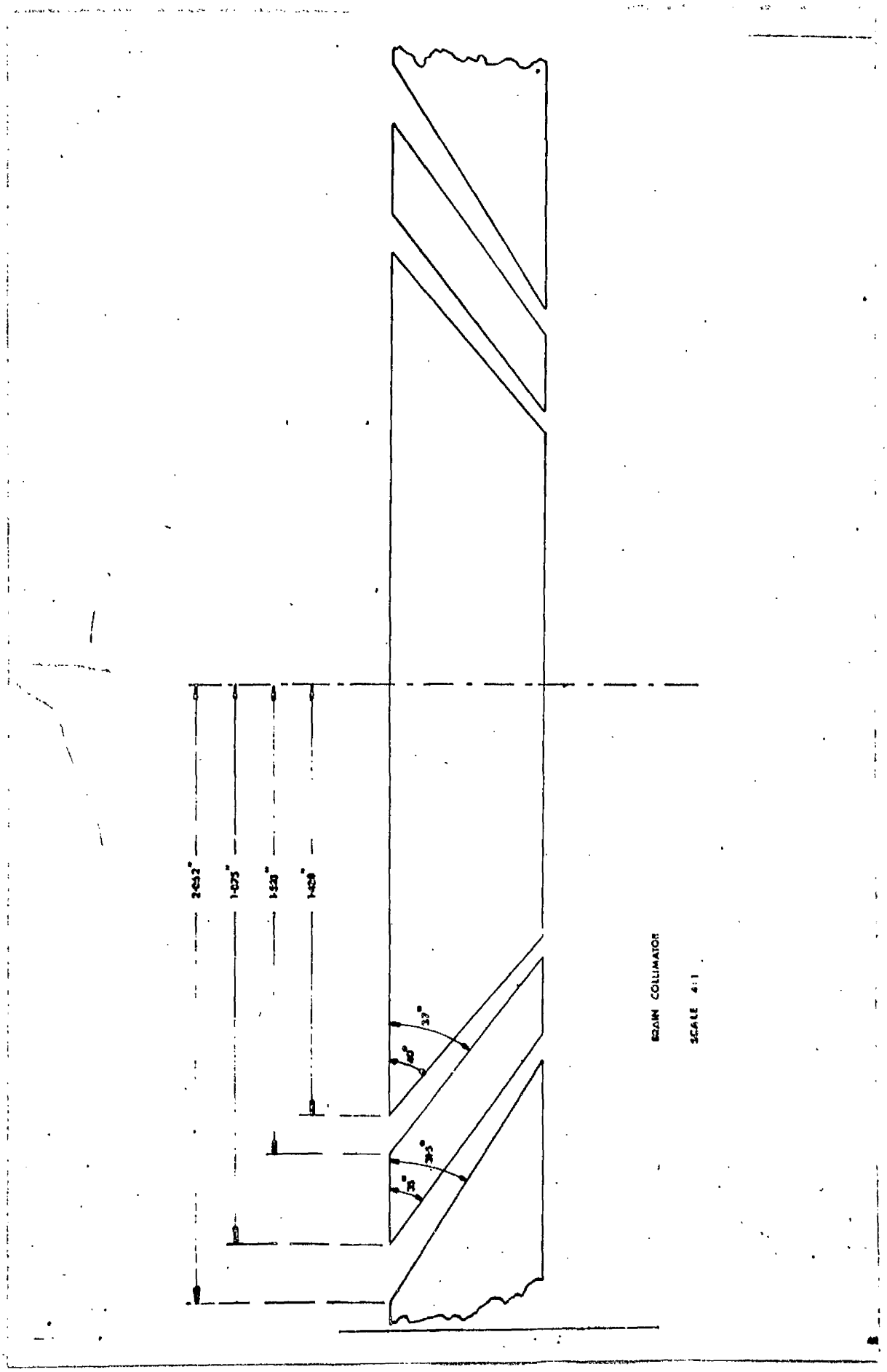


Figure 8.2 Mechanical Dimensions of Depth Focusing Collimator

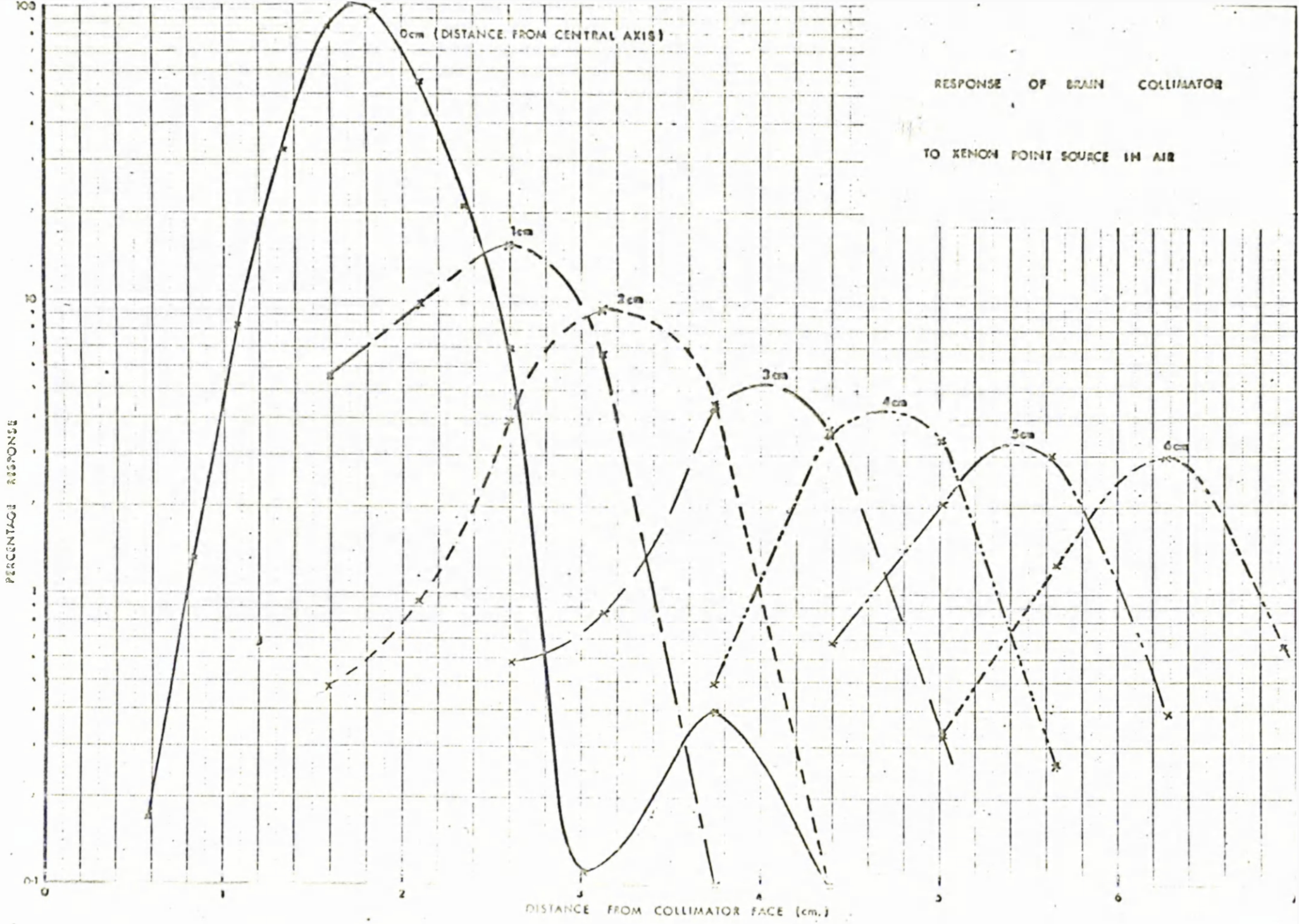


Figure 8.4 Response of Brain Collimator

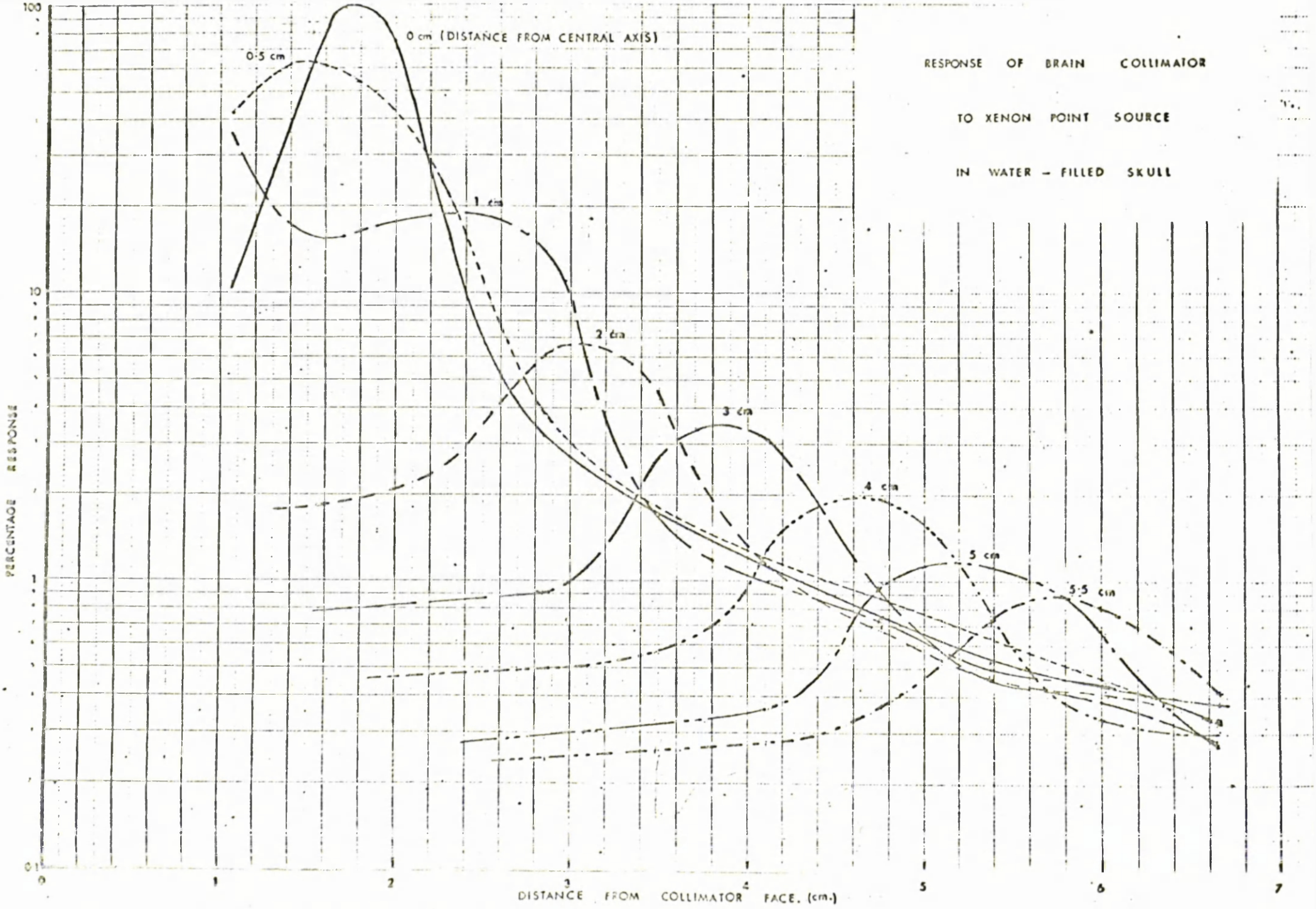


Figure 8.5 Response of Brain Collimator

TABLE 8.1a

Annular Area of Brain Sections

(annular width 0.1 cm)

DEPTH 1.317 cm

Radius (cm) 'r'	Chord Length (cm) 'C'	Segment Half Angle $\theta/2$ ($\sin^{-1}C/2r$)	Total Segment Half Angle $\sum \frac{\theta}{2}$	Arc Length (cm) $\frac{\pi r}{90}\sum \frac{\theta}{2}$ 'a'	Annular Area (sq.cm) (0.1a) 'A'
1.0					0.628
2.0					1.256

TABLE 8.1b

Annular Area of Brain Sections

(annular width 0.1 cm)

DEPTH 1.635 cm

Radius (cm) 'r'	Chord Length (cm) 'C'	Segment Half Angle	Total Segment Half Angle	Arc Length (cm) 'a'	Annular Area (sq.cm) 'A'
2.0	0.5	7.18			
	0.6	8.63			
	1.3	18.97	83.85	5.86	0.586
	2.6	40.54			
	0.6	8.63			
3.0	1.4	13.50			
	0.8	7.65	43.29	4.533	0.453
	1.5	14.47			
4.0	0.5	3.57	3.57	0.489	0.050

TABLE 8.1c

Annular Area of Brain Sections

(annular width 0.1 cm)

DEPTH 1.952 cm

Radius (cm) 'r'	Chord Length (cm) 'C'	Segment Half Angle	Total Segment Half Angle	Arc Length (cm) 'a'	Annular Area (sq.cm) 'A'
2.0	1.8	26.75			
	1.3	18.97			
	0.7	10.08	122.67	8.563	0.856
	2.0	30.00			
	2.4	36.87			
3.0	0.7	6.70			
	0.9	8.63	49.85	5.220	0.522
	0.7	6.70			
	2.8	27.82			
4.0	0.6	48.58			
	1.2	8.63	72.43	10.113	1.011
	2.1	15.22			
5.0	2.0	11.53	11.53	2.012	0.201

TABLE 8.1d

Annular Area of Brain Sections

(annular width 0.1 cm)

DEPTH 2.270 cm

Radius (cm) 'r'	Chord Length (cm) 'C'	Segment Half Angle	Total Segment Half Angle	Arc Length (cm) 'a'	Annular Area (sq.cm) 'A'
2.0	2.0	30.00			
	1.5	22.03			
	3.75	69.63	159.07	11.105	1.111
	0.4	5.74			
	2.1	31.67			
3.0	0.7	6.70			
	1.4	13.50	88.56	9.274	0.927
	3.9	40.54			
	2.8	27.82			
4.0	1.9	13.77			
	1.0	7.18			
	1.4	10.08	36.05	5.033	0.503
	0.7	5.02			
5.0	1.2	6.89			
	0.7	4.01			
	1.5	8.63	31.03	5.415	0.542
	1.5	8.63			
	0.5	2.87			

TABLE 8.1e

Annular Area of Brain Sections

(annular width 0.1 cm)

DEPTH 2.587 cm

Radius (cm) 'r'	Chord Length (cm) 'C'	Segment Half Angle	Total Segment Half Angle	Arc Length (cm) 'a'	Annular Area (sq.cm) 'A'
2.0	2.0	30.00			
	1.3	23.58			
	1.6	23.58			
	3.6	64.15	171.80	11.943	1.194
	1.0	14.47			
	2.55	39.60			
3.0	1.0	9.59			
	4.25	45.10			
	0.8	7.66	101.12	10.589	1.059
	3.1	31.11			
	0.8	7.66			
4.0	3.6	26.75			
	3.0	21.25			
	0.3	2.15	56.61	7.904	0.790
	0.9	6.46			
5.0	1.7	9.78			
	2.3	13.30	39.94	6.970	0.697
	2.9	16.86			

TABLE 8.1f

Annular Area of Brain Sections

(annular width 0.1 cm)

DEPTH 3.222 cm

Radius (cm) 'r'	Chord Length (cm) 'C'	Segment Half Angle	Total Segment Half Angle	Arc Length (cm) 'a'	Annular Area (sq.cm) 'A'
2.0	1.1	15.96			
	1.7	25.15			
	1.5	22.03	136.19	9.507	0.951
	2.15	32.51			
	2.6	40.54			
3.0	1.3	12.52			
	3.0	30.00			
	1.5	14.47	118.28		
	2.0	19.48		12.386	1.239
	4.0	41.81			
4.0	1.5	10.81			
	0.4	2.86			
	1.3	9.35	66.45	9.278	0.928
	5.5	43.43			
5.0	0.7	4.01			
	2.6	15.07	41.41	7.227	0.723
	3.8	22.33			

TABLE 8.1g

Annular Area of Brain Sections

(annular width 0.1 cm)

DEPTH 4.492 cm

Radius (cm) 'r'	Chord Length (cm) 'C'	Segment Half Angle	Total Segment Half Angle	Arc Length (cm) 'a'	Annular Area (sq.cm) 'A'
2.0	3.97	83.00			
	1.7	25.15			
	2.0	30.00	148.23	10.348	1.035
	0.7	10.08			
3.0	0.8	7.66			
	2.6	25.67			
	1.3	12.32	113.65	11.901	1.190
	2.4	23.58			
	4.2	44.42			
4.0	0.8	5.74			
	4.0	30.00			
	1.7	6.45	79.61	11.115	1.112
	4.7	35.99			
	0.2	1.43			
5.0	0.6	3.44			
	0.6	3.44	46.76	8.161	0.816
	6.1	37.59			
	0.4	2.29			

TABLE 8.1h

Annular Area of Brain Sections

(annular width 0.1 cm)

DEPTH 5.762 cm

Radius (cm) 'r'	Chord Length (cm) 'C'	Segment Half Angle	Total Segment Half Angle	Arc Length (cm) 'a'	Annular Area (sq.cm) 'A'
1.5	3.0	90.00			
	0.8	15.46	105.46	5.521	0.552
2.0	3.1	50.81			
	0.8	11.54	120.56	8.416	0.842
	3.4	58.21			
3.0	2.7	26.74			
	2.5	24.63	94.33	9.878	0.988
	1.0	9.60			
	3.3	33.36			
4.0	2.0	14.47			
	1.1	7.9			
	1.3	9.29	56.94	7.950	8.795
	1.6	11.54			
	1.9	13.74			
5.0	0.8	4.58			
	0.8	4.58	46.55	8.124	0.812
	4.9	29.34			
	1.4	8.05			

TABLE 8.11

Annular Area of Brain Sections

(annular width 0.1 cm)

DEPTH 6.397 cm

Radius (cm) 'r'	Chord Length (cm) 'C'	Segment Half Angle	Total Segment Half Angle	Arc Length (cm) 'a'	Annular Area (sq.cm) 'A'
1.5	3.0	90.00			
	2.0	41.81	131.81	6.901	0.690
2.0	4.0	90.00			
	1.9	28.36	118.36	8.263	0.826
3.0	3.7	38.07			
	1.1	8.63	77.74	8.140	0.814
	1.5	10.56			
	2.1	20.48			
4.0	0.5	3.58			
	0.75	5.38			
	3.1	22.80	57.77	8.066	0.807
	1.7	12.27			
	1.9	13.74			
5.0	0.4	2.29			
	1.6	9.2	29.39	5.129	0.513
	0.7	4.01			
	2.4	13.89			

TABLE 8.2a

Annular Response of Brain Sections

(annular width 0.1 cm)

DEPTH 1.317 cm

Radius (cm) 'r'	Annular Area (sq.cm) 'A'	Response (%) 'R'	Annular Response 'R x A'
0.05	0.0314	32.0	1.004
0.15	0.0942	41.0	3.862
0.25	0.1570	47.0	7.379
0.35	0.2199	51.7	11.368
0.45	0.2827	55.5	15.689
0.55	0.3455	54.5	18.829
0.65	0.4084	43.0	17.561
0.75	0.4712	34.5	16.256
0.85	0.5340	27.0	14.418
0.95	0.5969	21.7	12.952
1.05	0.6597	17.5	11.544
1.15	0.7225	14.2	10.259
1.25	0.7853	11.3	8.873
1.35	0.8482	9.0	7.633
1.45	0.9110	6.7	6.103
2.0	1.256	1.77	2.223
3.0	0.0	.76	0.0
4.0	0.0	.44	0.0
5.0	0.0	.24	0.0

TABLE 8.2b

Annular Response of Brain Sections

(annular width 0.1 cm)

DEPTH 1.635 cm

Radius (cm) 'r'	Annular Area (sq.cm) 'A'	Response (%) 'R'	Annular Response 'R x A'
0.05	0.0314	84.2	2.643
0.15	0.0942	82.2	7.743
0.25	0.1570	79.2	12.434
0.35	0.2199	75.0	16.492
0.45	0.2827	67.5	19.082
0.55	0.3455	51.0	17.620
0.65	0.4084	37.5	15.315
0.75	0.4712	28.5	13.429
0.85	0.5340	22.5	12.015
0.95	0.5969	17.5	10.445
1.05	0.6597	13.5	8.905
1.15	0.7225	10.7	7.730
1.25	0.7853	8.5	6.675
1.35	0.8482	7.0	5.937
1.45	0.9110	5.7	5.192
2.0	0.5860	1.87	1.095
3.0	0.4530	.78	0.353
4.0	0.0500	.45	0.022
5.0	0.0	.25	0.0

TABLE 8.2c

Annular Response of Brain Sections

(annular width 0.1 cm)

DEPTH 1.952 cm

Radius (cm) 'r'	Annular Area (sq.cm) 'A'	Response (%) 'R'	Annular Response 'R x A'
0.05	0.0314	83.0	2.61
0.15	0.0942	79.0	7.441
0.25	0.1570	72.0	11.304
0.35	0.2199	61.0	13.413
0.45	0.2827	49.5	13.993
0.55	0.3455	40.5	13.992
0.65	0.4084	33.0	13.477
0.75	0.4712	27.2	12.816
0.85	0.5340	22.7	12.121
0.95	0.5969	19.0	11.341
1.05	0.6597	16.0	10.555
1.15	0.7225	13.5	9.753
1.25	0.7853	11.2	8.795
1.35	0.8482	9.2	7.803
1.45	0.9110	7.7	7.014
2.0	0.8560	2.05	1.754
3.0	0.5220	0.81	0.422
4.0	1.0110	0.46	0.465
5.0	0.2010	0.26	0.052

TABLE 8.2d

Annular Response of Brain Sections

(annular width 0.1 cm)

DEPTH 2.270 cm

Radius (cm) 'r'	Annular Area (sq.cm) 'A'	Response (%) 'R'	Annular Response 'R x A'
0.05	0.0314	20.7	0.65
0.15	0.0942	22.2	2.091
0.25	0.1570	23.1	3.626
0.35	0.2199	23.7	5.211
0.45	0.2827	24.0	6.784
0.55	0.3455	24.0	8.292
0.65	0.4084	23.7	9.679
0.75	0.4712	23.0	10.837
0.85	0.5340	21.9	11.694
0.95	0.5969	20.2	12.057
1.05	0.6597	16.6	10.951
1.15	0.7225	13.0	9.392
1.25	0.7853	10.3	8.088
1.35	0.8482	8.3	7.040
1.45	0.9110	6.7	6.103
2.0	1.1110	2.5	2.777
3.0	0.9270	0.84	0.778
4.0	0.5030	0.47	0.236
5.0	0.5420	0.27	0.146

TABLE 8.2e

Annular Response of Brain Sections

(annular width 0.1 cm)

DEPTH 2.587 cm

Radius (cm) 'r'	Annular Area (sq.cm) 'A'	Response (%) 'R'	Annular Response 'R x A'
0.05	0.0314	5.75	0.81
0.15	0.0942	6.05	0.5699
0.25	0.1570	6.45	1.012
0.35	0.2199	6.9	1.517
0.45	0.2827	7.5	2.120
0.55	0.3455	8.8	3.040
0.65	0.4084	11.3	4.614
0.75	0.4712	15.4	7.256
0.85	0.5340	17.2	9.184
0.95	0.5969	18.0	10.744
1.05	0.6597	17.7	11.676
1.15	0.7225	16.2	11.704
1.25	0.7853	13.8	10.837
1.35	0.8482	11.6	9.839
1.45	0.9110	9.9	9.018
2.0	1.1940	4.1	4.895
3.0	1.0590	.88	0.931
4.0	0.7900	.49	0.387
5.0	0.0970	.28	0.195

TABLE 8.2£

Annular Response of Brain Sections

(annular width 0.1 cm)

DEPTH 3.222 cm

Radius (cm) 'r'	Annular Area (sq.cm) 'A'	Response (%) 'R'	Annular Response 'R x A'
0.05	0.0314	2.2	0.07
0.15	0.0942	2.21	0.2031
0.25	0.1570	2.23	0.350
0.35	0.2199	2.25	0.494
0.45	0.2827	2.28	0.644
0.55	0.3455	2.37	0.818
0.65	0.4084	2.49	1.016
0.75	0.4712	2.67	1.258
0.85	0.5340	2.90	1.548
0.95	0.5969	3.22	1.922
1.05	0.6597	3.60	2.374
1.15	0.7225	4.00	2.890
1.25	0.7853	4.37	3.431
1.35	0.8482	4.77	4.045
1.45	0.9110	5.15	4.691
2.0	0.9510	6.30	5.991
3.0	1.2390	1.36	1.685
4.0	0.9280	0.52	0.482
5.0	0.7230	0.31	0.224

TABLE 8.2g

Annular Response of Brain Sections

(annular width 0.1 cm)

DEPTH 4.492 cm

Radius (cm) 'r'	Annular Area (sq.cm) 'A'	Response (%) 'R'	Annular Response 'R x A'
0.05	0.0314	0.92	0.0028
0.15	0.0942	0.96	0.0904
0.25	0.1570	0.99	0.155
0.35	0.2199	1.00	0.219
0.45	0.2827	1.00	0.282
0.55	0.3455	0.98	0.338
0.65	0.4084	0.95	0.387
0.75	0.4712	0.91	0.428
0.85	0.5340	0.86	0.459
0.95	0.5969	0.82	0.489
1.05	0.6597	0.78	0.514
1.15	0.7225	0.75	0.541
1.25	0.7853	0.73	0.573
1.35	0.8482	0.71	0.602
1.45	0.9110	0.7	0.637
2.0	1.0350	0.8	0.828
3.0	1.1900	1.6	1.904
4.0	1.1120	1.9	2.112
5.0	0.8160	0.52	0.424

TABLE 8.2h

Annular Response of Brain Sections

(annular width 0.1 cm)

DEPTH 5.762

Radius (cm) 'r'	Annular Area (sq.cm) 'A'	Response (%) 'R'	Annular Response 'R x A'
0.05	0.0314	0.48	0.0015
0.15	0.0942	0.495	0.0466
0.25	0.1570	0.507	0.079
0.35	0.2199	0.515	0.113
0.45	0.2827	0.520	0.147
0.55	0.3455	0.515	0.177
0.65	0.4084	0.505	0.206
0.75	0.4712	0.490	0.230
0.85	0.5340	0.475	0.253
0.95	0.5969	0.465	0.277
1.05	0.6597	0.457	0.301
1.15	0.7225	0.45	0.325
1.25	0.7853	0.447	0.351
1.35	0.8482	0.442	0.374
1.45	0.9110	0.437	0.398
2.0	0.8420	0.42	0.353
3.0	0.9880	0.41	0.405
4.0	0.7950	0.40	0.318
5.0	0.8120	0.92	0.747
1.5	0.5520	0.435	0.240

TABLE 8.2i

Annular Response of Brain Sections

(annular width 0.1 cm)

DEPTH 6.397 cm

Radius (cm) 'r'	Annular Area (sq.cm) 'A'	Response (%) 'R'	Annular Response 'R x A'
0.05	0.0314	0.395	0.0012
0.15	0.0942	0.39	0.0367
0.25	0.1570	0.385	0.060
0.35	0.2199	0.382	0.084
0.45	0.2827	0.380	0.107
0.55	0.3455		
0.65	0.4084		
0.75	0.4712		
0.85	0.5340		
0.95	0.5969		
1.05	0.6597		
1.15	0.7225		
1.25	0.7853		
1.35	0.8482		
1.45	0.9110		
2.0	0.8260	0.37	0.305
3.0	0.8140	0.32	0.260
4.0	0.8070	0.30	0.242
5.0	0.5130	0.35	0.179
1.5	0.6900	0.38	0.262

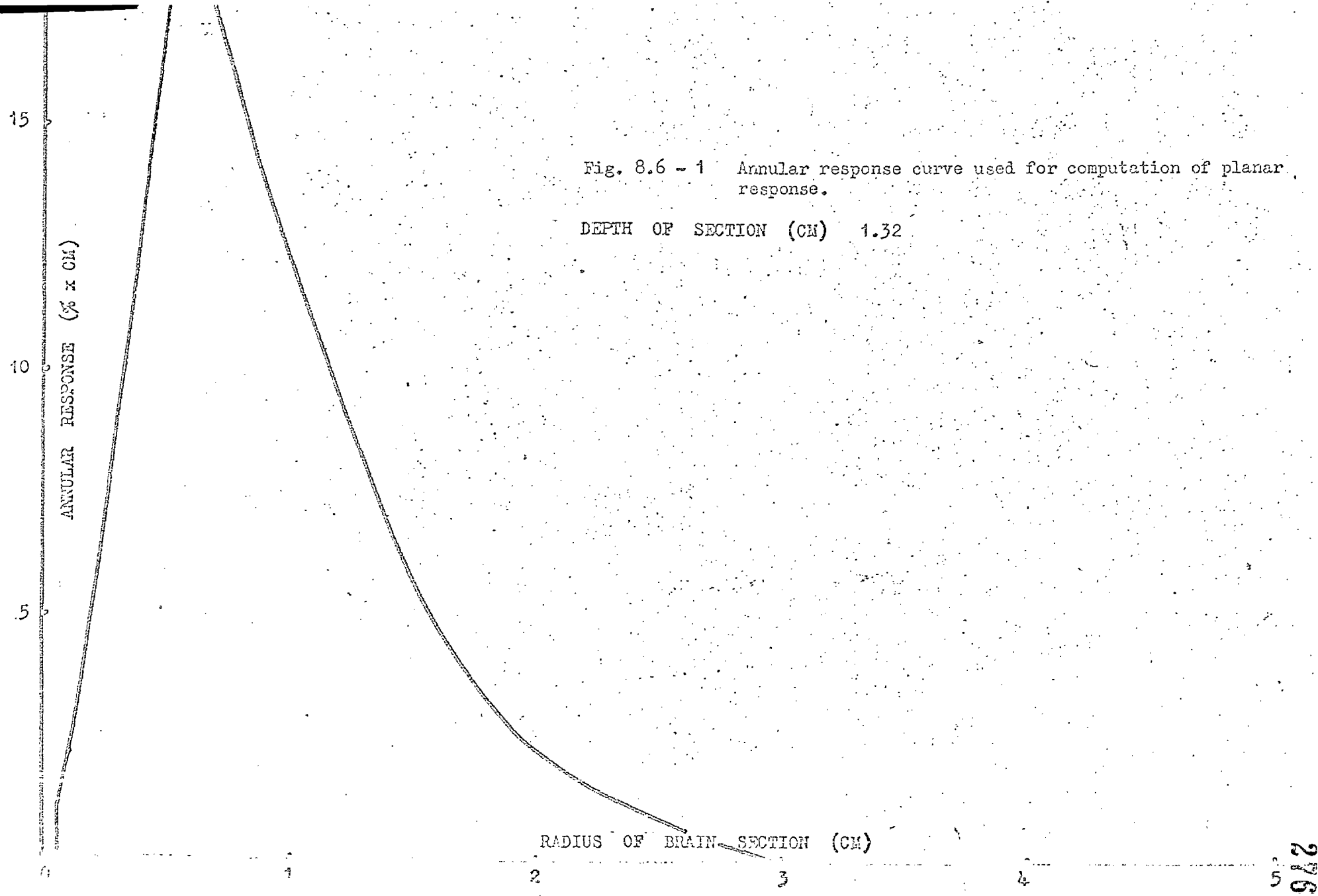


Fig. 8.6 - 1 Annular response curve used for computation of planar response.

DEPTH OF SECTION (CM) 1.32

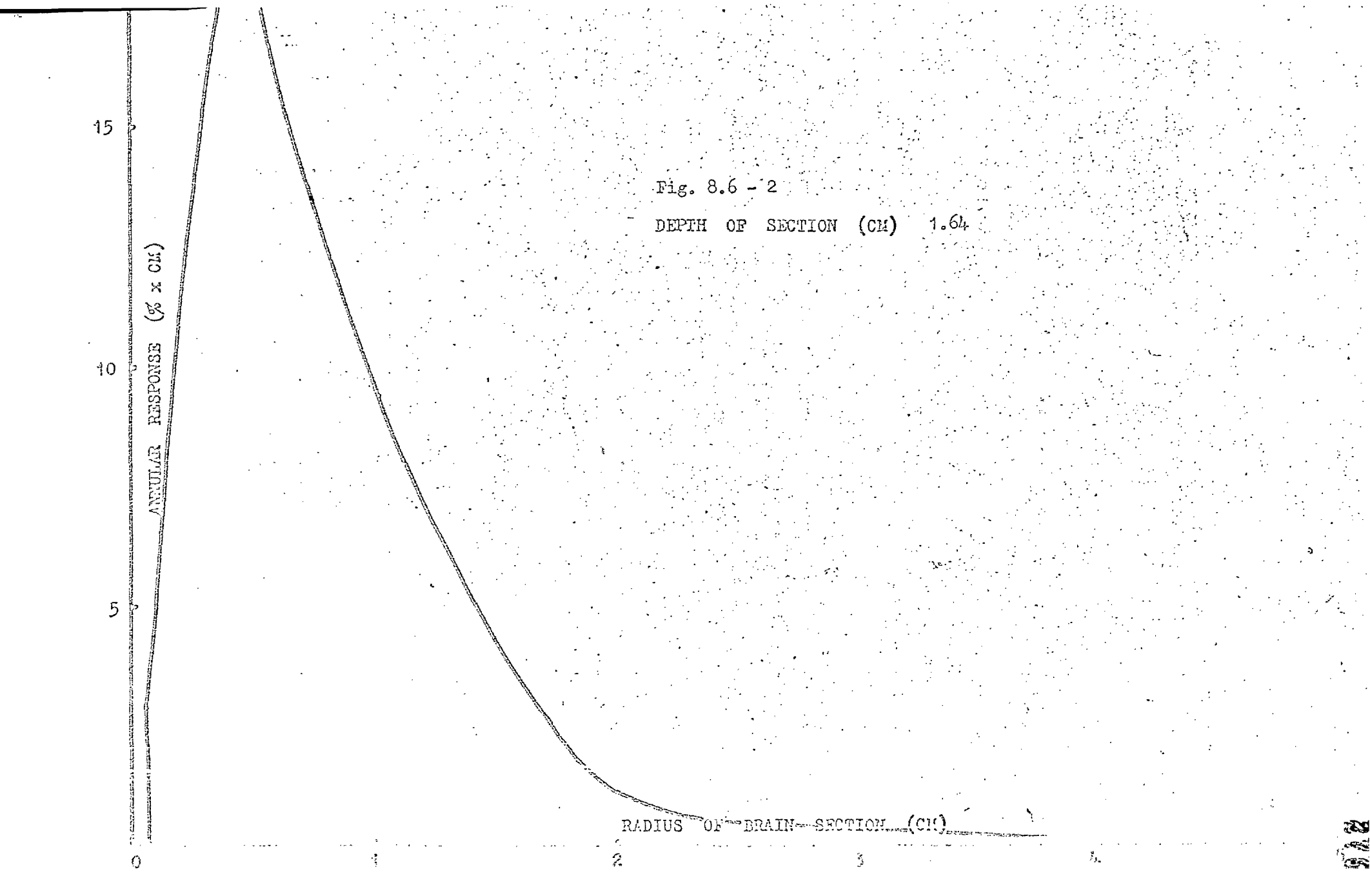


Fig. 8.6 - 2

DEPTH OF SECTION (CM) 1.64

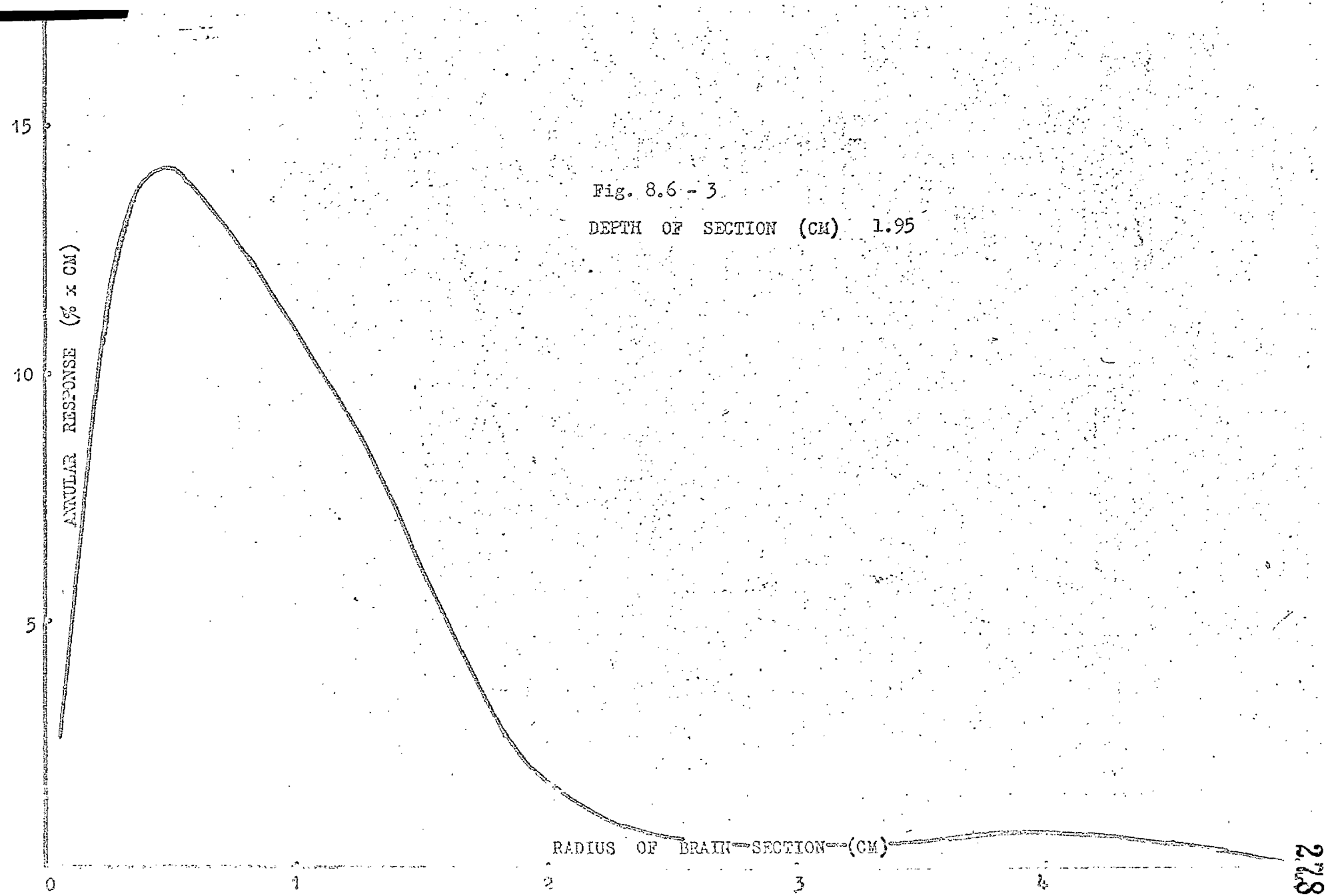


Fig. 8.6 - 3

DEPTH OF SECTION (CM) 1.95

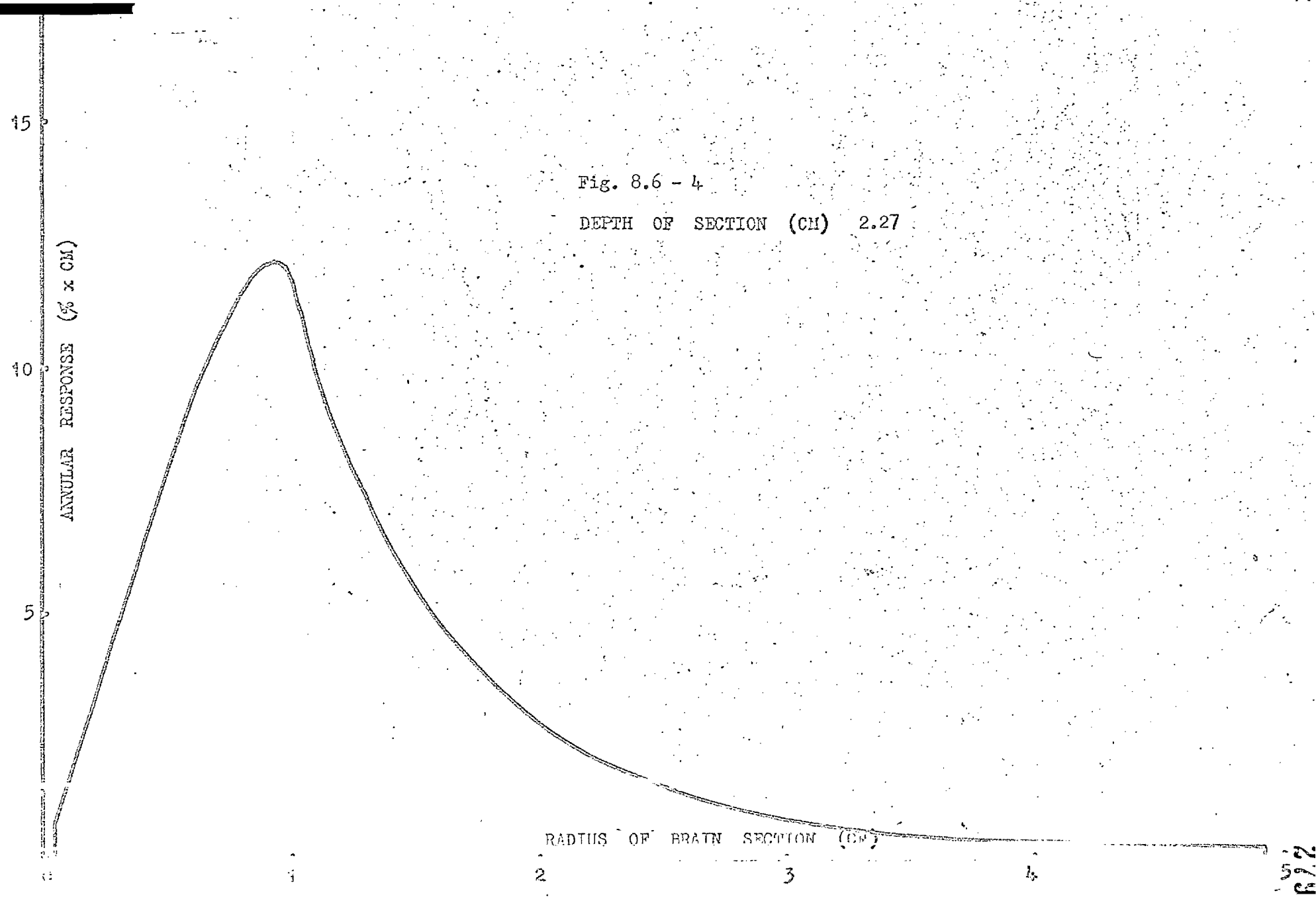


Fig. 8.6 - 4

DEPTH OF SECTION (CM) 2.27

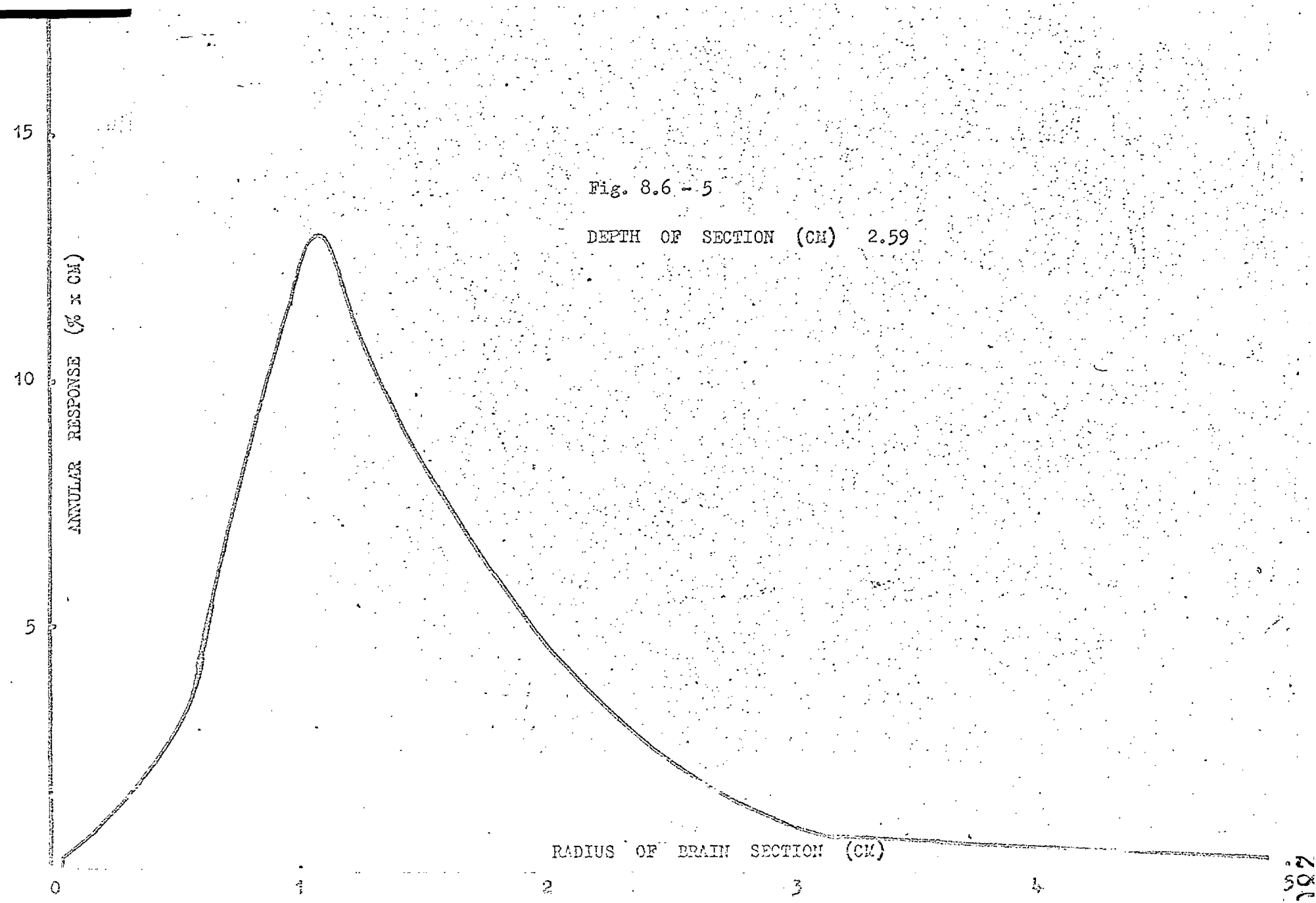


Fig. 8.6 - 5

DEPTH OF SECTION (CM) 2.59

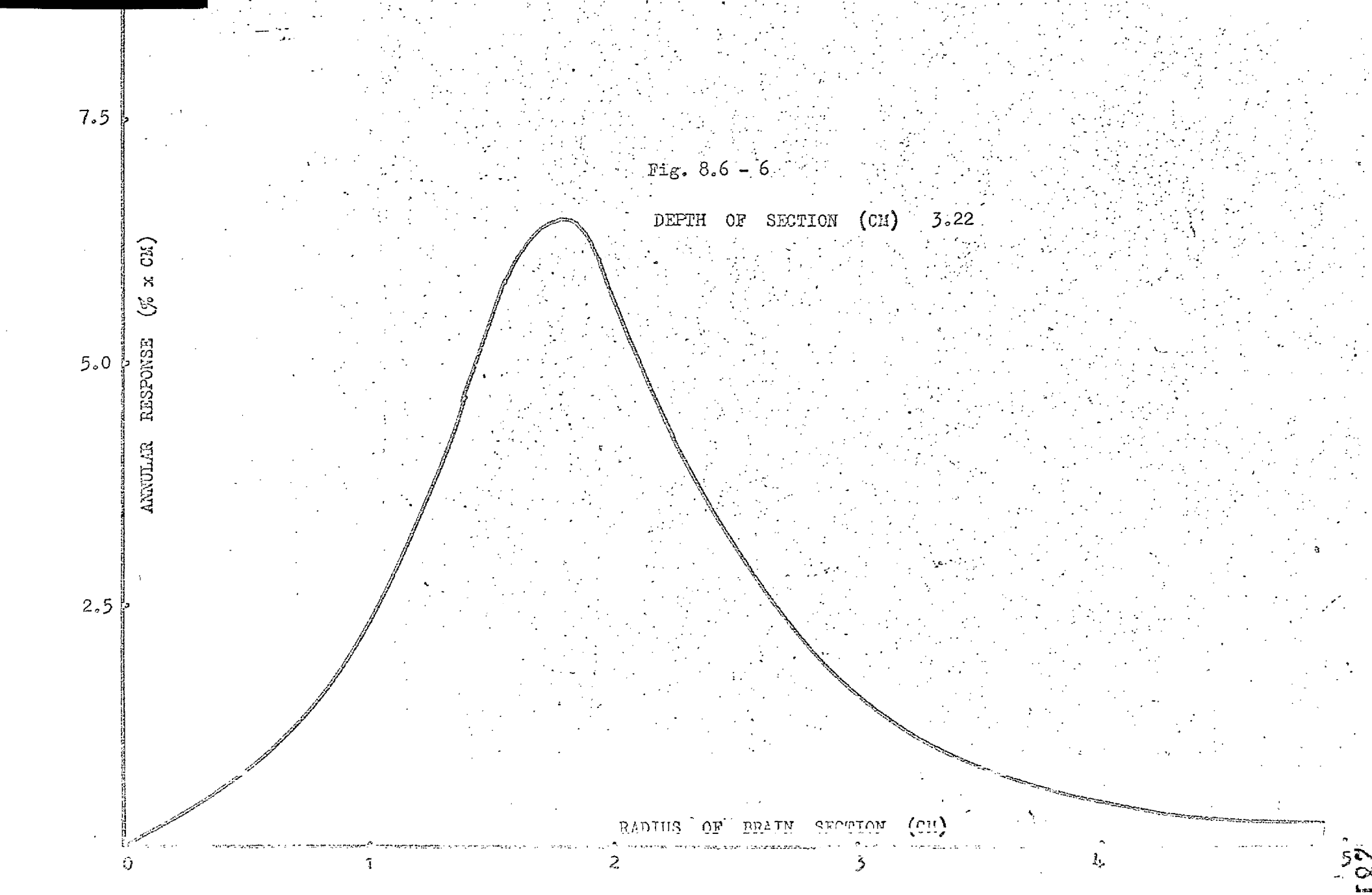


Fig. 8.6 - 6
DEPTH OF SECTION (CM) 3.22
ANNULAR RESPONSE (% x CM)
7.5
5.0
2.5
0
1
2
3
4
5
RADIUS OF BRAIN SECTION (CM)

Fig. 8.6 - 7

DEPTH OF SECTION (CM) 4.49

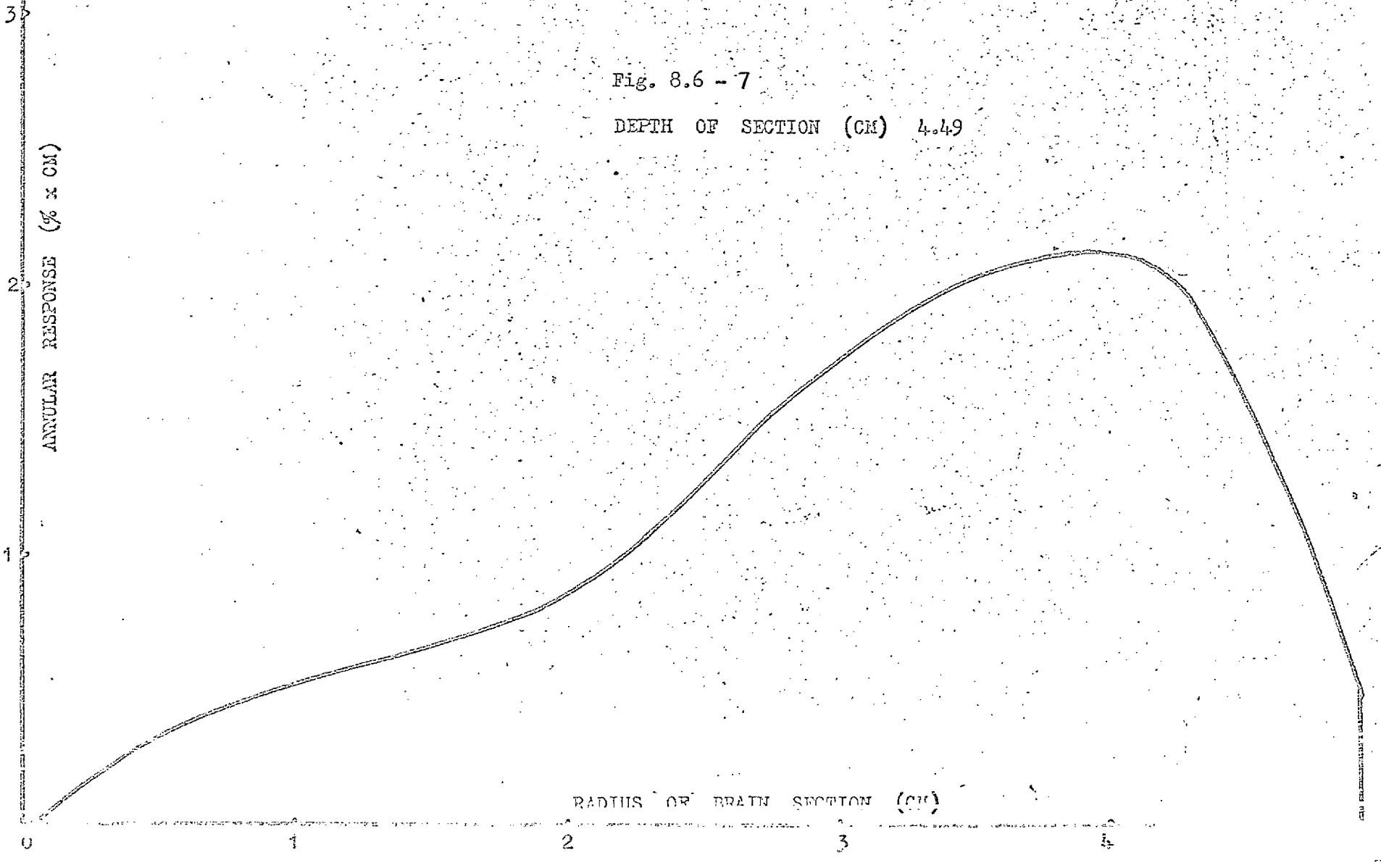


Fig. 8.6 - 8

DEPTH OF SECTION (cm) 5.76

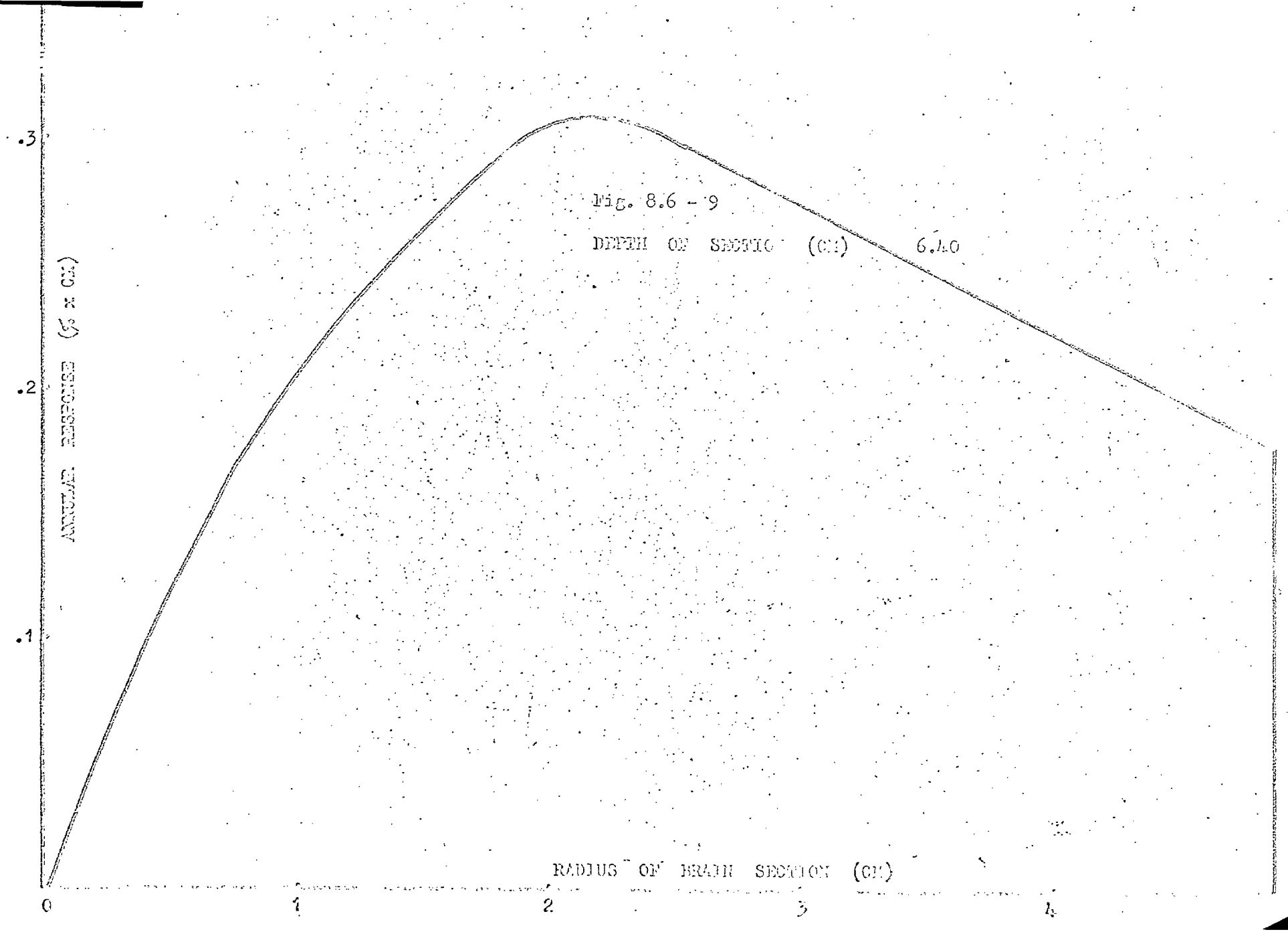


Fig. 8.6 - 9

DEPTH OF SECTION (cm) 6.40

TABLE 8.3

Planar Response of Brain Sections

for Planes of Various Radii

Depth cm	Radius 1.0 cm	1.5 cm	2.0 cm	3.5 cm	5.0 cm
1.317	12.02	16.46	18.290	19.270	19.270
1.635	12.78	16.24	17.590	18.30	18.30
1.952	11.25	15.62	17.58	18.51	19.230
2.270	7.08	11.25	13.26	15.13	15.470
2.587	4.070	9.57	12.92	15.78	16.340
3.222	00835	2.62	5.66	9.69	10.32
4.492	0.288	0.578	00944	3.09	5.732
5.762	0.143	0.268	0.419	0.995	1.873
6.397	0.110	0.227	0.372	0.806	1.1316

TABLE 8.4

Annular Response of Grey Matter in Brain Sections

Relative to Total Tissue Response

Depth (cm)	Radius (cm) (r)	Response (%) (R)	Area of Grey Tissue (A_g)	Tissue Total Area (A_T)	($R.A_g$)	($R.A_T$)
	1	0.40	0	2.68	0	1.072
5.762	2	0.38	3.9	12.54	1.480	4.765
	3	0.37	7.3	17.38	2.70	6.343
	4	0.38	11.2	21.80	4.26	8.175
	5	0.57	12.0	16.80	6.84	9.576
	5.5	0.79	10.0	13.90	7.90	10.981
	1	0.68	0.6	6.30	0.41	4.284
4.492	2	0.62	4.6	12.50	2.85	7.750
	3	0.78	9.0	18.8	7.02	14.664
	4	1.92	13.8	23.3	26.50	44.736
	5	1.00	11.8	15.7	11.80	15.700
	5.5	0.39	9.7	12.8	3.78	4.923
	1	2.45		6.3	5.39	15.435
3.222	2	5.60	3.8	12.6	21.28	70.560
	3	2.00	10.0	18.8	20.0	37.600
	4	0.54	11.4	15.5	6.16	8.370
	5	0.32	8.2	10.0	2.62	3.200
	5.5	0.66	4.5	5.4	2.97	3.564
	1	17.80	4.3	6.3	76.54	112.140
2.270	2	4.30	9.3	12.6	39.99	54.140
	3	0.88	8.6	9.4	7.57	8.272
	4	0.49	2.6	2.9	1.27	1.421
	1	18.70	4.9	6.3	91.63	117.800
1.952	2	2.65	14.0	14.2	37.10	37.630
	3	0.87	3.3	3.5	2.87	3.045
	4	0.48	0.1	0.1	0.05	0.048
	0.5	41.00	2.2	2.2	90.20	90.20
1.635	1.0	17.20	4.4	4.4	75.68	75.68
	1.5	6.20	1.9	1.95	11.78	12.09
	2.0	2.20	1.5	1.50	3.30	3.30
	2.5	0.80	0.7	0.75	0.56	0.60

TABLE 8.5

Planar and Volume Response of Grey Matter

Relative to Total Tissue Response

Planar Response

Depth (cms) (t)	Grey Tissue R_g $R_g = \int R.A_g.dr$	All Tissue R_T $R_T = \int R.A_T.dr$
5.762	19.08	36.96
4.492	45.54	80.91
3.222	65.52	105.20
2.286	79.36	99.84
1.968	95.46	103.20
1.635	34.22	34.22

Relative Volume Response of Grey Tissue/Non-Grey Tissue

Grey Tissue: $\int R_g.dt = 226.0$

Total Brain Tissue: $\int R_T.dt = 334.5$

Ratio of response of grey tissue to that of non-grey tissue = 2.14

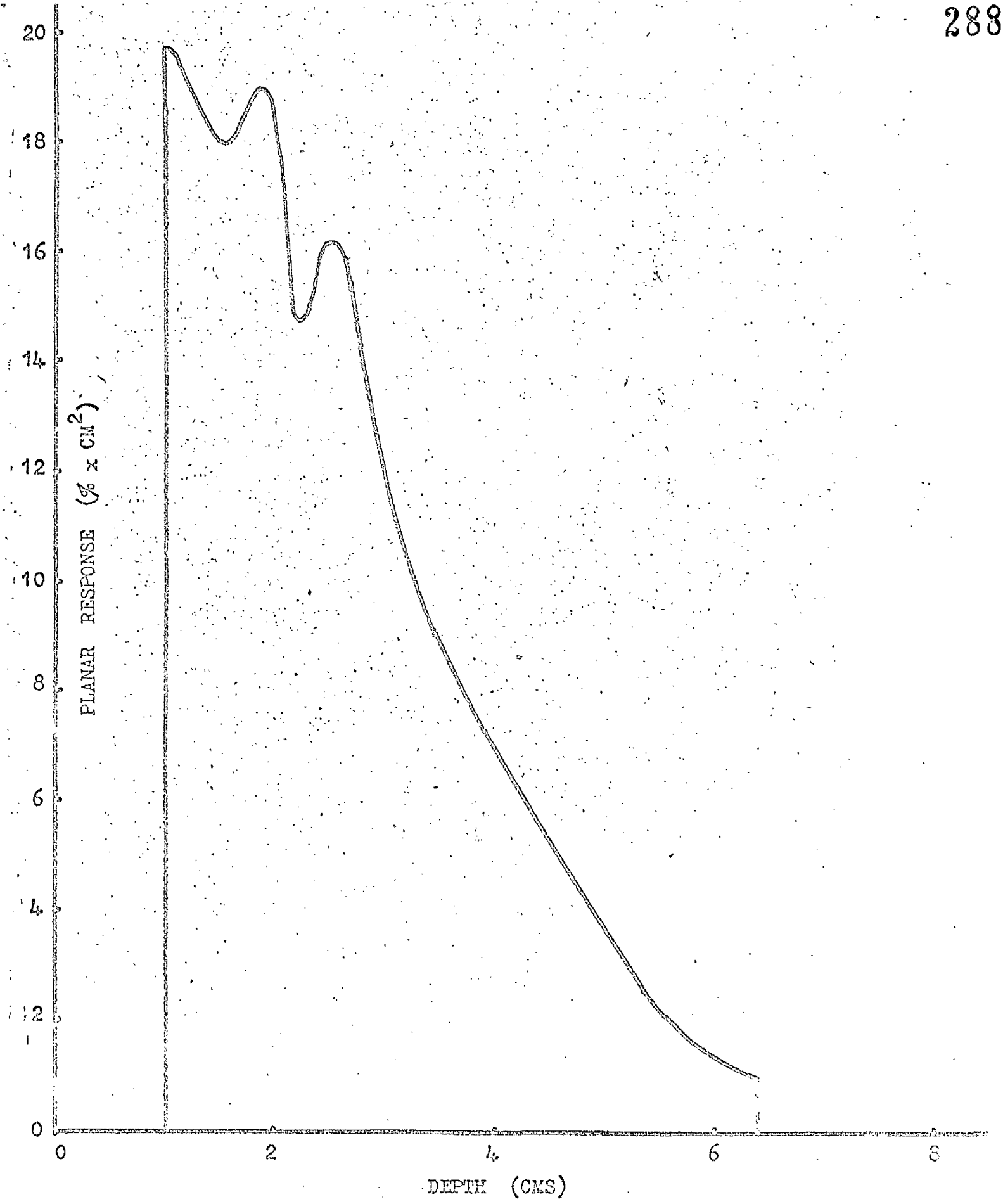


Fig. 8.7 - 1 Variation in total collimator response with depth below brain surfac

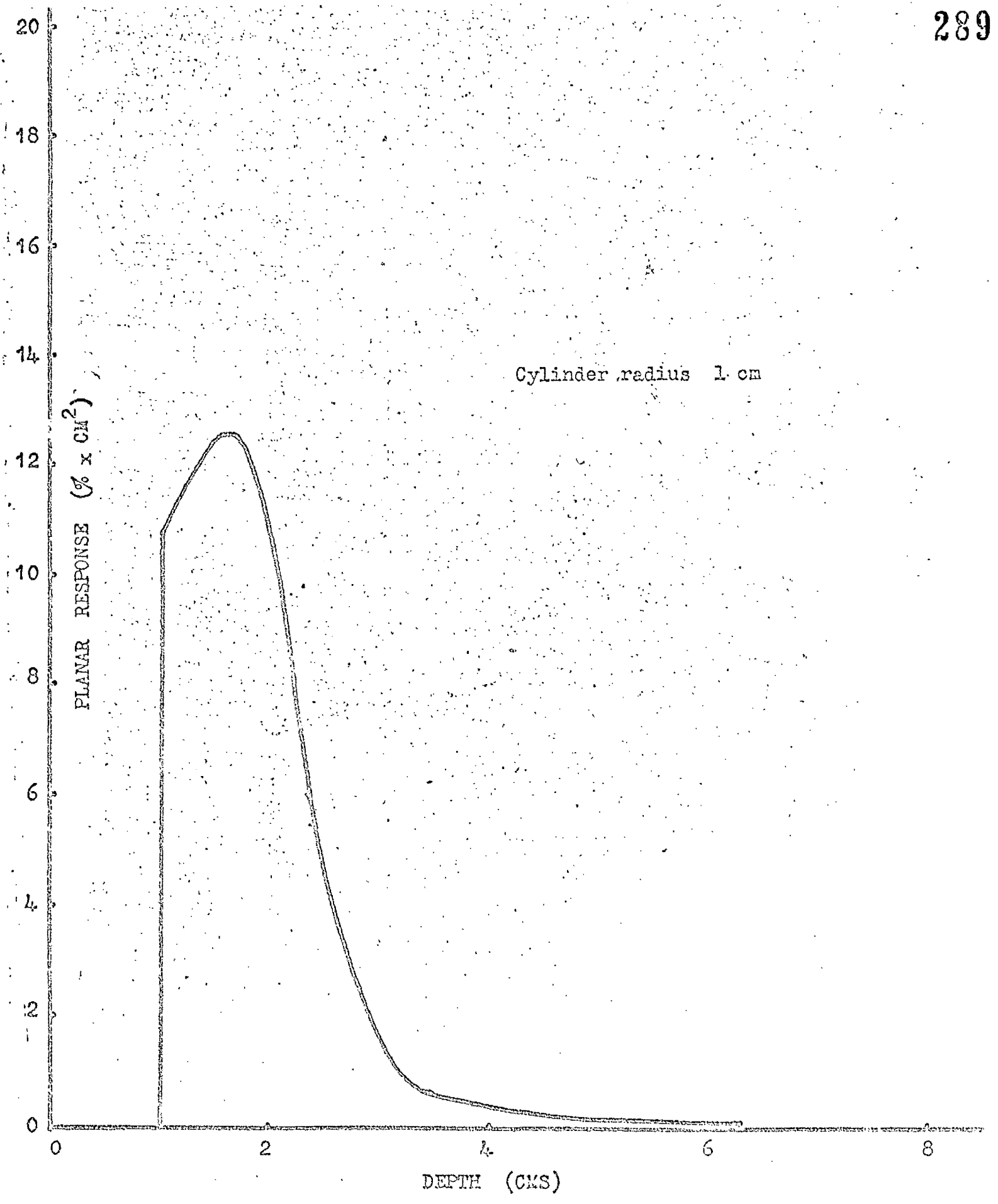


Fig. 8.7 - 2 Planar response curve used for computation of volume response for a cylinder of tissue of fixed radius.

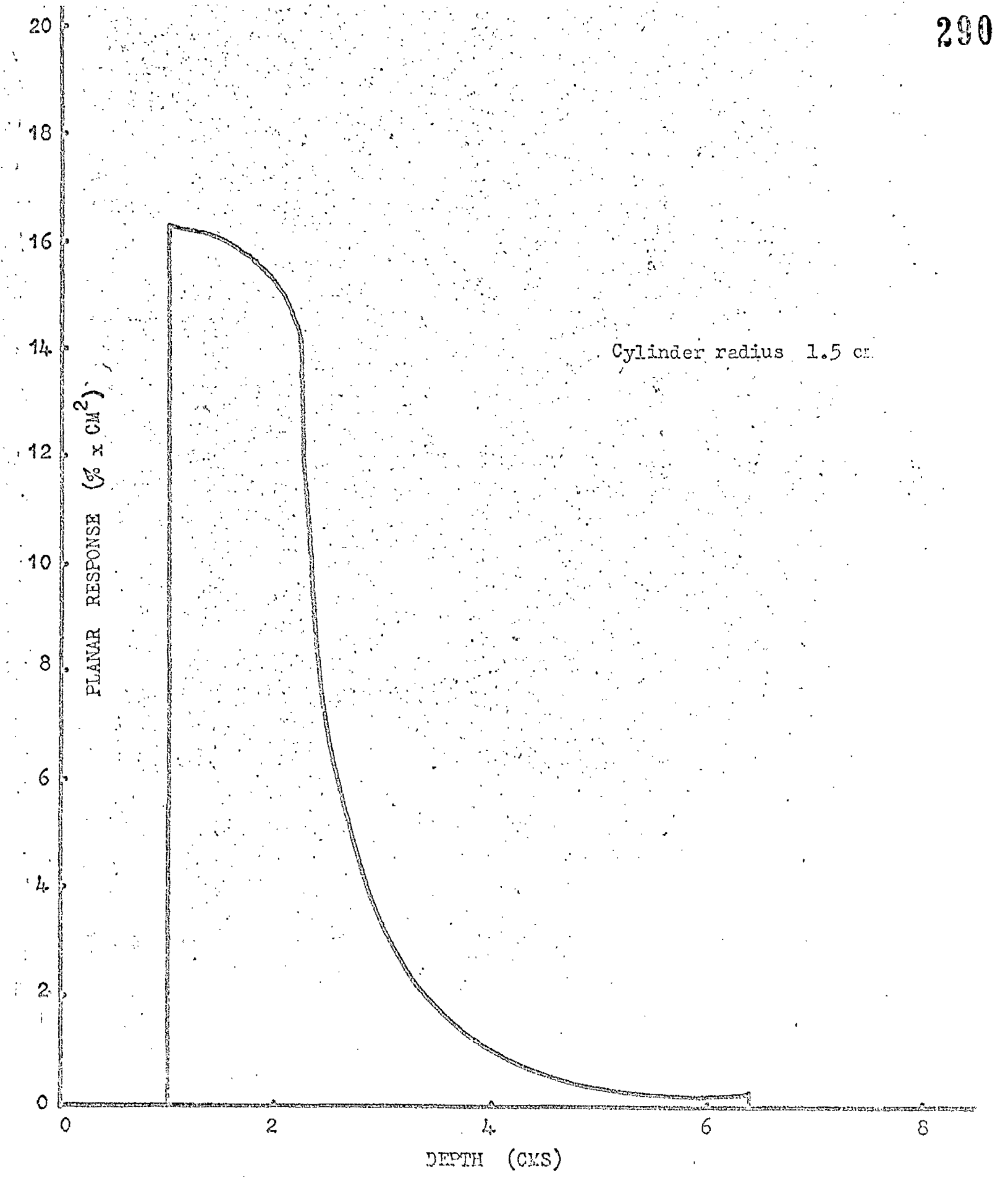

Cylinder radius 1.5 cm
PLANAR RESPONSE (% x CM²)
DEPTH (CMS)
0
2
4
6
8
10
12
14
16
18
20

Fig. 8.7 - 3

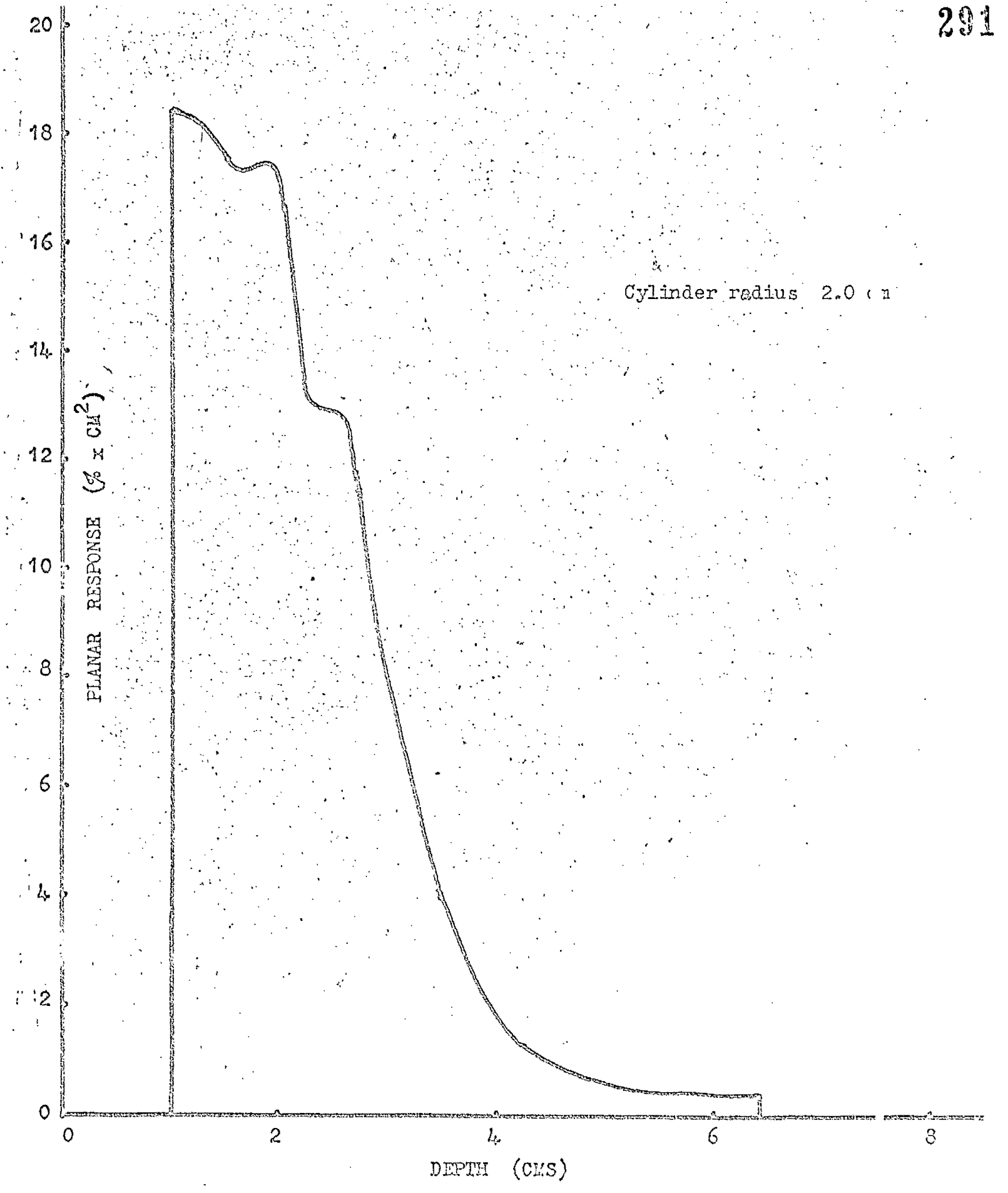

Cylinder radius 2.0 cm
PLANAR RESPONSE (% x CM^2)
DEPTH (CMS)
0
2
4
6
8
0
2
4
6
8
10
12
14
16
18
20

Fig. 8.7 - 4

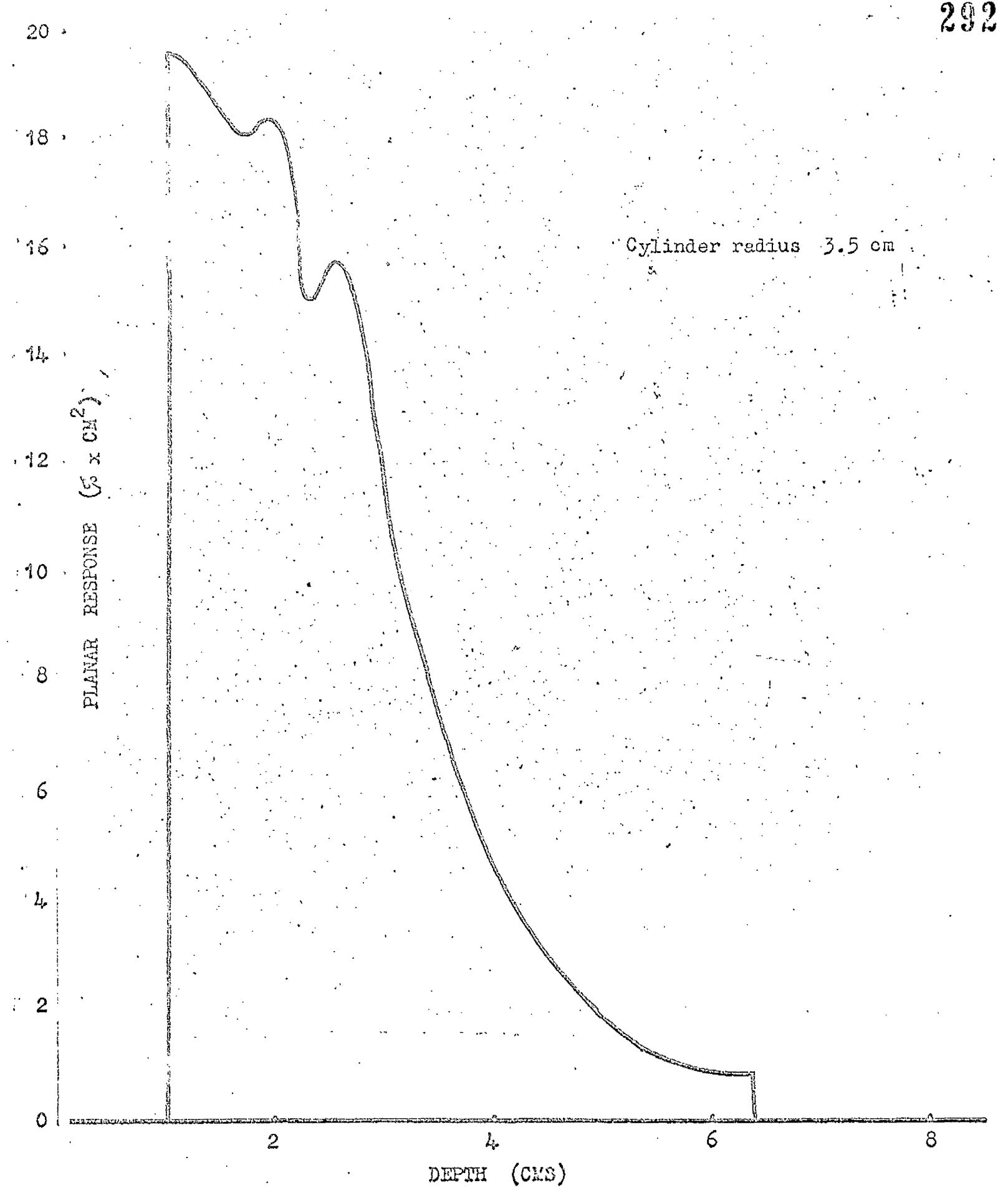

Cylinder radius 3.5 cm
PLANAR RESPONSE (Cs x CM²)
DEPTH (CMS)
0
2
4
6
8
10
12
14
16
18
20
2
4
6
8

Fig. 8.7 - 5

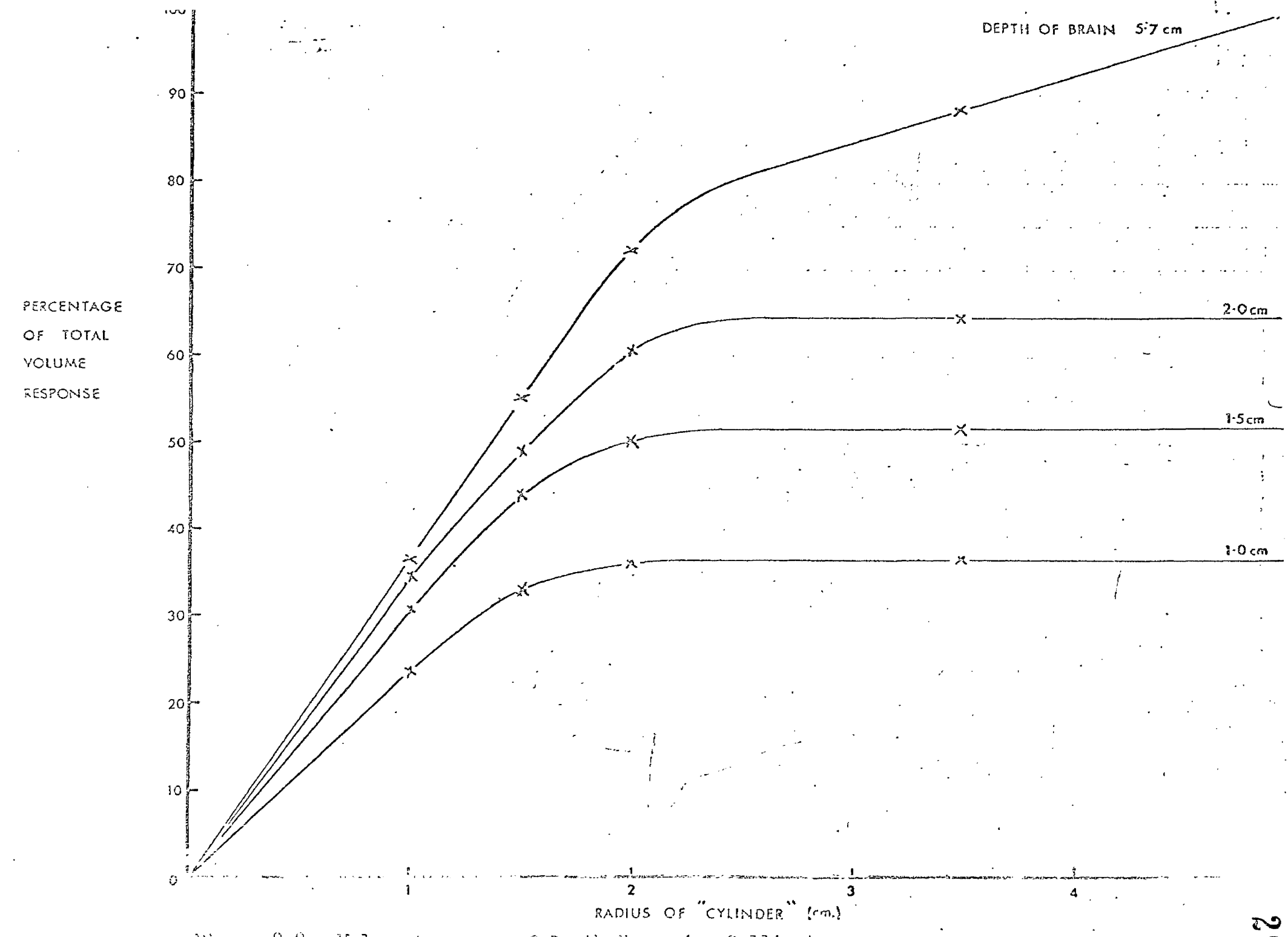


Figure 8.8 Volume Response of Depth Focussing Collimator

TABLE 8.6

Computer Derived Parameter and Parameter Error Estimates

Obtained from Brain Radioactivity Clearance Data

Patient Name	σ_1	σ_2	λ_1*	λ_2*	$\Delta\sigma_1$	$\Delta\sigma_2$	$\Delta\lambda_1$	$\Delta\lambda_2$
Innes	22.71	47.03	.1557	.0108	.091	.039	.186	.085
Grierson	18.85	24.16	.0081	.0493	.745	.610	1.09	.400
Scott 1	26.34	73.97	0	.0783	.067	.224	.110	0
Scott 2	0	65.99	.0744	.0416	0	.013	∞	.013
Murray	12.15	50.87	.1000	.0077	.213	.057	.391	.140
Jackson 2	4.875	60.90	.0988	.0191	.753	.071	.989	.058
Bell 1	39.81	41.96	.1132	.0189	.044	.039	.089	.034
Bell 2	1.855	70.94	.1096	.0289	2.12	.049	1.03	.034
Hutcheson 1	4.662	68.34	.1411	.0138	2.23	.158	1.72	.394
Jackson 1	4.455	69.19	.5279	.0158	.568	.009	.856	.024
McGregor	8.837	59.59	.1202	.0171	.476	.077	.381	.100
Hutcheson 2	38.79	26.90	.0362	.0111	2.29	3.34	1.01	3.28
Greer 2	45.10	27.87	.0669	.0157	.733	1.20	.499	1.24
Greer 1	27.02	53.74	.2772	.0423	.134	.074	.182	.094
Beaver 1	17.25	24.54	.1996	.0356	.831	.603	.599	.826

* Exponents are in units of fraction/5 seconds

TABLE 8.7

Detailed Summary of Clinical Cerebral Flow Results

	pCO_2 (mm Hg)	pCO_2 Correction Factor	Normalised Initial Slope (min^{-1})	Corrected Cortical Blood Flow (ml/min/gm)
Greer 1	56.0	0.682	1.450	.8891
Greer 2	30.0	1.230	.568	.627
Beaver	42.0	0.917	1.239	1.020
Innes	27.5	1.280	.696	.799
F.R.	40.0	0.960	-**	.770
Hutcheson 1	28.0	1.270	.263	.302
Hutcheson 2	74.0*	0.526	.311	.147
Bell 1	46.0	0.833	.778	.582
Bell 2	34.5	1.110	.371	.372
Scott 1	29.5	1.240	.693	.774
Scott 2	19.6*	1.390	.499	.616
Jackson 1	32.0	1.180	.561	.593
Jackson 2	22.0*	1.370	.300	.356
McGregor	28.5	1.260	.365	.415
Murray	25.0	1.330	.306	.365
Grierson	35.0	1.100	.374	.447

* Correction factor contains excessive error

** See text

TABLE 8.8

Summary of Clinical Results and Diagnoses

Patient Name	Diagnosis	Cortical Blood Flow (Corrected to pCO_2 of 38) (ml/min/gm)	
Greer 1	Normal	.889	
Greer 2		.627	
Beaver		1.020	
Innes		.779	
F.R.		.770	
		Mean .82	S.D. ± .146
Hutcheson	Right Carotid Aneurysm - Marked Spasm	.302	
Grierson	Basilar Artery Aneurysm - Mild Spasm	.447	
McGregor	Migraine	.415	
Murray	Left Hemisphere Tumour - Hydrocephalus	.365	
Scott	Right Carotid and Left Middle Cerebral Aneurysm	.774	
Jackson	Parkinsonism	.593	
Bell 1	Right Parietal Tumour - Glioblastoma Multiforme	.582	
Bell 2	Right Parietal Tumour - Glioblastoma Multiforme	.372	
		Mean .48	S.D. ± .156