KINETICS OF EXPERIMENTAL TUMOUR PROCESSES

N.M. Emanuel

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by

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In order to make this volume available as economically and as rapidly as possible the author's typescript has been reproduced in its original form. This method unfortunately has its typographical limitations but it is hoped that they in no way distract the reader.

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FOREWORD TO THE ENGLISH EDITION

Cancer research has for a long time, and for various reasons, attracted the attention of chemists, physicians and mathematicians, but the greatest contributions have come from physicians and biologists.

In-depth studies of oncological problems in various scientific disciplines have acquired international importance.

In the USSR, investigations of kinetics and physico-chemical (especially free radical) mechanisms of carcinogenesis, tumour growth, and the molecular biology of chemotherapy, are very well developed. Advances have been made in techniques for objectively assessing cancer treatment statistically with the aid of computers. This research parallels that of the Chester Beatty Research Institute in London, the Paterson Research Laboratory at the Christie Hospital in Manchester and in many similar institutions.

This monograph was published in the USSR in 1977. In the following three years, Soviet research paid great attention to the fundamental principles of creating new effective antitumour drugs. The basic laws of pharmacokinetics, the mathematical investigations of the quantum kinetics of antitumour activity and drug structures, and the results of biochemical and biophysical investigations underlie these principles.

A survey of these investigations was presented in the author's lecture 'Physical, Biochemical and Biophysical Bases for Creation of New Effective Anticancer Agents' at the 28th Congress of the International Union of Pure and Applied Chemistry (IUPAC) in Helsinki in August 1979. The lecture was published in the journal *Pure and Applied Chemistry* and constitutes an addition to this monograph.

I hope that the publication of the English edition of the monograph will be welcomed by our colleagues and will assist our mutual efforts for the solution of the most important current problems in oncology.

Professor N. M. Emanuel
Member of the Academy of Science

FOREWORD

Clinical reactions are subject to the laws of kinetics and thus kinetic studies are of great importance in many fields of natural science. While physical kinetics and chemical kinetics have already become independent scientific fields, the kinetics of biological processes are less well understood. However, knowledge of molecular mechanisms and of the general quantitative principles of the development of biological processes with time is essential for further progress of biology and medicine.

In the past two decades this has become so obvious that mathematicians, physicists, chemists, biologists and physicians from many countries have joined forces to solve biological and medical problems.

Cancer is one of the most formidable problems of humanity; any advance here is of extreme importance. Systematic studies of the kinetic principles and molecular (mostly free radical) mechanisms of malignant growth, as well as the search for rational principles of approach to the creation of efficient anticancer drugs were started at the Institute of Chemical Physics in the USSR in 1957.

These problems are investigated now by a team of researchers at the Department of Kinetics of Chemical and Biological Processes in the Institute of Chemical Physics in the USSR.

Numerous studies on the kinetics and mechanisms of tumour growth have yielded results of theoretical and practical value. Several chemotherapeutic drugs proposed by this Department have been clinically effective.

This monograph develops the principles of the kinetics of experimental malignant growth. The molecular mechanisms of chemotherapy and certain problems of carcinogenesis and biochemistry are discussed.

The first chapter is theoretical. It introduces the notion of a true average kinetic curve for tumour growth and discusses the principles for obtaining experimental kinetic curves. Various analytical functions for the approximation of experimental kinetic curves are given. These functions are obtained by integration of the population growth equation under certain simple assumptions about the time dependence of the specific rate of growth. Attention is given to a large set of exponential and power functions, and Gompertz, Bertalanffy and logistic functions, the dependences for curves with an extremum (polynomial exponents), etc.

These dependences are widely used in describing kinetic curves that have to be applied in experiments. Simple equations for the kinetic survival curves are also given. The procedures of analytical functions by means of their linearization are described. This section is included for convenience of readers in order to save them from the need to resort to special handbooks on regression analysis.

A whole section is devoted to quantitative criteria used in estimating the effectiveness of antitumour drugs. Many are proposed for the first time. It is expected that the use of these criteria in specific experimental research will show the expediency and advantages of their application, in particular in standardizing the experiments conducted in different laboratories.

The second chapter contains experimental data on tumour growth from the USSR and elsewhere. The general treatment of the experimental results and plots, using the analytical functions given in the first chapter, is described. This seems to be the first systematic survey of data on the kinetics of tumour growth. This chapter contains valuable reference material on over 40 tumour models.

The third chapter deals with kinetic analysis of the results obtained by various treatments of tumours (chemotherapy, surgery and combined treatment). Drug effectiveness is estimated with the same kinetic criterion — the effectiveness coefficient.

The fourth chapter is a survey of pharmacokinetic methods of major regularities connected with general problems of cancer chemotherapy, in particular with kinetic analysis of the behaviour of certain antitumour drugs in the body.

The molecular mechanisms of chemotherapy discussed in the fifth chapter are considered only from the standpoint of the biological action of the new antitumour drugs developed at the Department of the Kinetics of Chemical and Biological Processes of the Institute of Chemical Physics. These are inhibitors-antioxidants (phenol compounds), alkylnitrosoureas and diazoketones. The alkylnitrosoureas are of particular interest because of their interaction with nucleic acids. In studying the mechanism of the action of diazoketones, attention was paid to their effect on biosynthesis. The inhibitors-antioxidants were considered in terms of the free-radical mechanisms of their action.

In the last fifteen years the role of free radicals in the mechanisms of carcinogenesis and tumour growth have become one of the most important developing trends in the biophysics of cancer; this field is still at the stage of phenomenological description. The kinetic approach allowed the solution of many problems and enabled the finding of certain general patterns (e.g. the change in content of free radical species during the initial period of tumour growth, the step-wise nature of changes in the free radical content during chemical carcinogenesis, etc.). The limiting factor for the development of these studies is the absence of methods for identification of the individual nature of paramagnetic species exhibiting EPR signals. These problems are discussed in the sixth chapter.

The seventh chapter deals with the kinetic analysis of biochemical shifts occurring in tumour tissues. It is suggested that knowledge of the quantitative kinetic parameters characteristic of these shifts and of various therapeutic treatment effects can help in developing methods of strictly controlled therapy.

The last chapter contains a survey of the present data on disturbance of structure and biosynthesis of informational macromolecules occurring in tumour growth. These are new fields for effective application of kinetic methods; their discussion in the monograph seems to be expedient.

The author wishes to thank his colleagues of the Department of Kinetics of Chemical and Biological Processes for their participation in the development of this new field of oncology — the physico-chemical study of cancer. The author also expresses his thanks to V.M. Andreev, G.N. Bogdanov, V.A. Gor'kov, N.P. Konovalova, V.I. Naidich, V.V. Suchchenko, L.S. Ter-Vartanyan, I.N. Todorov, O.K. Shiyataya for help in writing this monograph and also to V.N. Varfolomeev and L.P. Zaikova for preparation of the figures.

CONTENTS

CHAPTER 1

THE KINETICS OF TUMOUR GROWTH

Theoretical and experimental studies of tumour growth and of the accompanying biochemical and biophysical changes are concerned with general patterns, the nature and mechanism of a process, the setting up of criteria for evaluation of the effectiveness of therapeutic drugs, and with a rational search for new principles in the prophylaxis, diagnosis, and medical treatment of cancer.

Kinetics, as a science, deals with the development of various (physical, chemical and biological) processes in time: it is of particular value for investigating these problems. Tumour growth progresses regularly in time; kinetic studies have become important in experimental and clinical oncology. A formal mathematical approach, the creation of tumour growth models and kinetic analysis of these results concerning the rules for development of malignant processes and the mechanisms of drug action are required. Mathematical description of the kinetic regularities yields numerous parameters that can be used to model these processes and computer techniques can be used to evaluate the vast amount of data generated.

1. KINETIC CURVES

Kinetic curves are the most usual means of representing the results of kinetic studies. Besides the two definitions: 'kinetics' and 'kinetic curve', a general one, namely 'dynamics', is often used in biology and medicine.

A kinetic curve is a graphical representation of changes in a certain value, F, characteristic of the process development in time. This value denotes any measurable property of the system studied and the result of measurements is given as a numerical value corresponding to each fixed moment of time.

Values of a different mathematical nature are used in plotting kinetic curves. Certain values continuously change with time (are continuous time functions), others can change only discretely (discrete time functions). The volume, diameter, area or weight of a solid tumour can serve as an example of continuous time functions of tumour development. A discrete function is, for instance, the change in number, N, of tumour cells. It will obviously be represented by integer numbers only. Naturally, the discrete number of changes in N will be important only for low N values.

Kinetic studies of tumour growth and the effect of various drugs reveal a great

diversity of kinetic functions (exponentially decreasing or increasing curves, curves which pass through a minimum or maximum, and curves going from minimum to maximum extremes). These graphs reflect the phenomena of tumour growth inhibition, regressions, recurrences, etc. Figure 1 presents kinetic curves obtained in the study of experimental tumour processes. The common S-shaped kinetic curve is taken as a standard and treatments giving different results are considered.

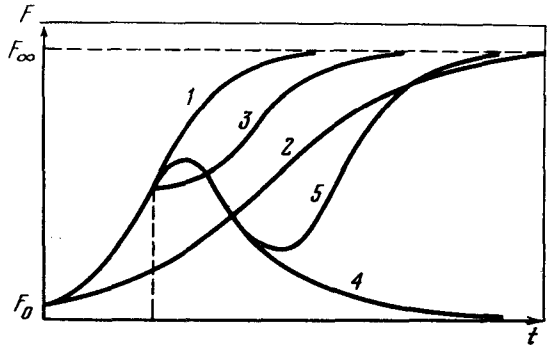

Fig. 1 Types of kinetic curves for tumour growth. 1 — Control; 2 — Inhibition after early therapy; 3-5 — Treatment of a developed process with inhibition effects (3), with complete (4) and partial (5) regressions; F — Tumour size (F_0 — initial, F_∞ — attainable limit).

Experimentally, kinetic curves are obtained by means of many data from a large number of animals. However, kinetic curves can be plotted for individual animals as well: the shape will then be prone to individual fluctuations during the experiment. Tumour growth in another animal will not give exactly the same kinetic curve, even if the experimental conditions are exactly the same. This lack of reproducibility cannot be helped; it is due to the variable proliferation of tumour cells: different animals are not genetically identical and their individual physiology can differ widely, resulting in large deviations from mean values.

Figure 2 presents kinetic curves for individual animals. These curves show the random nature of tumour growth. Each point in an individual kinetic curve is defined by its time coordinate, t, and a relevant value, F, characteristic of tumour growth. All kinetic curves have a natural original point $(0, F_0)$ corresponding to an initial F value at the time of tumour transplantation when $t = 0$. Each individual curve also has its final point (τ, F_τ) at the death of the animal. Death can be caused by the tumour, as a result of toxic therapy, or old age in cases of successful chemotherapy. Therefore a group of animals must be used for the study of tumour growth. Tumour models are amenable techniques for use in large groups of animals; the results are reproducible and are suitable for statistical analysis. Mathematical models help in planning experiments and analyzing the data obtained (Kramer, 1948; Nalimov, 1971; Malenvo, 1975).

The theory of random processes helps to construct the model (Prokhorov and Rozanov, 1973; Feller, 1967; Grenander, 1961). We may imagine an infinite number of all potentially possible kinetic curves for tumour growth. Each of these curves is denoted by ω so that the functions $F(t)$ and the set Ω of all ω have the same value. Each curve originates at a point $(0, F_{0\omega})$ and ends at a point $(\tau_\omega, F_{\tau\omega})$. Let the subset B in set Ω be probability $P\{B\}$.

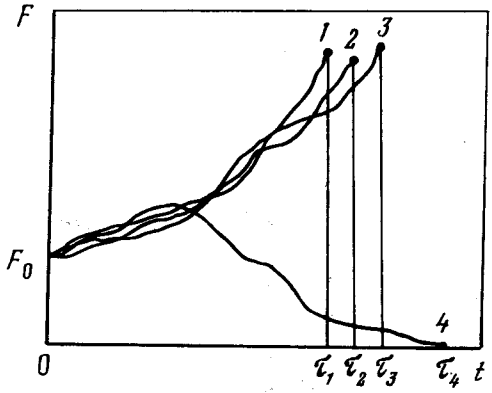

Fig. 2 Examples of kinetic curves for tumour growth in individual animals. 1-3 — Control; 4 — Regression as a result of treatment; τ_i ($i = 1, 2, 3, 4$) — time of death.

Denote by Ω_t the set of all ω that correspond to curve F_ω still present to the time t, i.e. those for which $\tau_\omega \geq t$. It will be seen that $P\{\Omega_t\}$ is the probability of survival at time t. This probability $v(t)$ is:

$$v(t) = P\{\Omega_t\} \tag{1}$$

The curve $v(t)$ is the true survival curve. Obviously $v(0) = 1$, and $v(\infty) = 0$. Consider two procedures of averaging the individual kinetic curves.

1. Introduce the notion 'true average kinetic curve' $\mu(t)$. Define $\mu(t)$ for each moment of time as the mathematical expectation of the value F_ω

$$\mu(t) = MF_\omega(t) = \int_{\Omega_t} F_\omega(t) \frac{P\{d\omega\}}{P\{\Omega_t\}} = \int_{\Omega_t} F_\omega(t) \frac{P\{d\omega\}}{v(t)}, \tag{2}$$

where M is the mathematical expectation symbol, and integration is made over set Ω_t.

Another equivalent expresssion can also be given for curve $\mu(t)$. Estimate for any t the distribution function of the random value F as the probability that $F_\omega(t) \leq F$. Then

$$P_t(F) = P\{F_\omega(t) \leq F\} = \int \frac{P\{d\omega\}}{P\{\Omega_t\}}, \tag{3}$$

with integration over the set of all $\omega \in \Omega_t$ such that $F_\omega(t) \leq F$. The equation

$$\mu(t) = \int_{-\infty}^{+\infty} F dP_t(F) \tag{4}$$

is then valid. The true dispersion of F is defined as

$$\sigma_t^2 = M[F - \mu(t)]^2 = \int_{-\infty}^{+\infty} [F - \mu(t)]^2 dP_t(F).$$

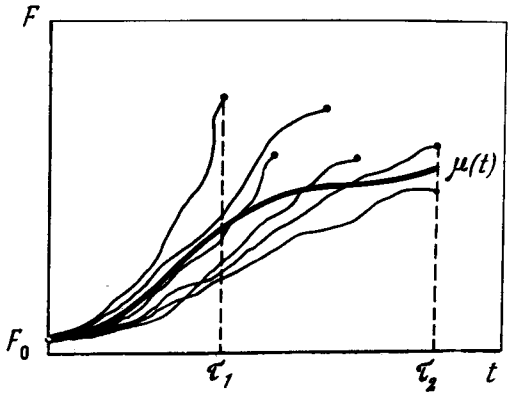

Fig. 3 Plotting of kinetic curves for $\mu(t)$ from the mean arithmetic values of F.

Figure 3 shows the position of curve $\mu(t)$ in the set of individual curves $F_\omega(t)$.

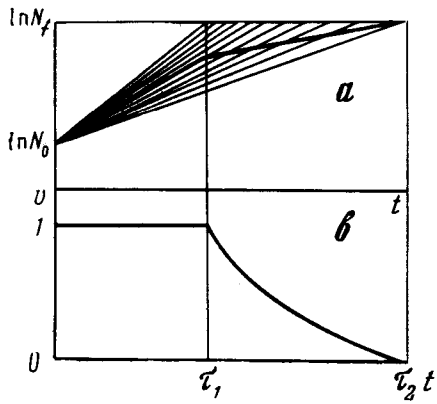

Fig. 4 Averaging of kinetic curves for exponentially growing tumours (a) and the relevant survival curve (b).

Take a simple example of the definitions (1-4) (Fig. 4a). Assume an entity of animals each having a number of tumour cells increasing strictly exponentially, $N = N_0 \exp(\phi t)$. The same number of tumour cells, N_0, has been transplanted to all animals at $t = 0$. Let the exponential factor differ for different animals, the possible values ϕ being distributed uniformly in the order $\phi_1 \leq \phi \leq \phi_2$.

Assume that the death of animals occurs at a certain value $N = N_f$, independently of ϕ. For this case index ω can be identified with the value ϕ. F_ϕ will be chosen as

$$F_\phi = \ln N = \ln [N_0 \exp(\phi t)] = \phi t + \ln N_0.$$

Every individual curve is plotted only up to time

$$\tau_\phi = \frac{1}{\phi} \ln (N_f/N_o).$$

The probability that ϕ will fall within the range $(\phi, \phi + d\phi)$ is:

(1) at $\phi < \phi_1$ $P\{d\phi\} = 0$;

(2) at $\phi_1 < \phi < \phi_2$ $P\{d\phi\} = \frac{d\phi}{(\phi_2 - \phi_1)}$;

(3) at $\phi > \phi_2$ $P\{d\phi\} = 0$.

Over the time range

$$0 \le t \le \tau_1 = \frac{1}{\phi_2} \ln \frac{N_f}{N_o}$$

all F_ϕ curves are defined. Consequently Ω_t coincides here with the whole range $\phi_1 \le \phi \le \phi_2$, and thus $P\{\Omega_t\} = 1$. Over the time range

$$\tau_1 \le t \le \tau_2 = \frac{1}{\phi_1} \ln \frac{N_f}{N_o}$$

only a part of the curves is defined. Here Ω_t coincides with the range

$$\phi_1 \le \phi \le \frac{1}{t} \ln \frac{N_f}{N_o}$$

and therefore

$$P\{\Omega_t\} = \frac{1}{\phi_2 - \phi_1} \left(\frac{1}{t} \ln \frac{N_f}{N_o} - \phi_1 \right).$$

The kinetic curves are not defined for $t > \tau_2$, and here $P\{\Omega_t\} = 0$.

Thus the survival curve $v(t)$ plotted using Eq. (1) consists of three segments of different analytical shapes (see Fig. 4b). Its descent represents an hyperbola.

Calculate (t) by Eq. (2). At $0 \le t \le \tau_1$,

$$\mu(t) = \int_{\phi_1}^{\phi_2} (\phi t + \ln N_o) \frac{d\phi}{\phi_2 - \phi_1} = \frac{\phi_1 + \phi_2}{2} t + \ln N_o$$

At $\tau_1 \le t \le \tau_2$,

$$\mu(t) = \int_{\phi_1}^{\frac{1}{t} \ln (Nf/N_o)} (\phi t + \ln N_o) - \frac{d\phi}{\phi_2 - \phi_1} \cdot \frac{\phi_2 - \phi_1}{\frac{1}{t} \ln \frac{Nf}{N_o} - \phi_1}$$

$$= \frac{1}{2} \left(\phi_1 t + \ln \frac{N_f}{N_o} \right) + \ln N_o.$$

At $t > \tau_2$, $\mu(t)$ is not evaluated.

Thus $\mu(t)$ represents a broken line consisting of two straight segments, though all individual $F_\phi(t)$ represent straight lines. This example suggests that generally the shape of the curve $\mu(t)$ will probably be very different from the shapes of individual curves $F_\omega(t)$ over the time range corresponding to the death of the animals. The curves for $\mu(t)$ and individual $F_\omega(t)$ certainly can differ not only in the death section, but also throughout the range of tumour growth. An example of this can be found in Comfort (1965).

Now calculate $P_t(F)$, using the definitions

$$F_1(t) = \phi_1 t + \ln N_0,$$
$$F_2(t) = \phi_2 t + \ln N_0.$$

Eq. (3) yields the expressions for $0 \le t \le \tau_1$

$$P_t(F) = \begin{cases} 0 & \text{for } F \le F_1(t) \\ \dfrac{F - F_1(t)}{F_2(t) - F_2(t)} & \text{for } F_1(t) \le F \le F_2(t) \\ 1 & \text{for } F \ge F_2(t) \end{cases}$$

and at $\tau_1 \le t \le \tau_2$

$$P_t(F) = \begin{cases} 0 & \text{for } F \le F_1(t) \\ \dfrac{F - F_1(t)}{\ln N_f - F_1(t)} & \text{for } F_1(t) \le F \le \ln N_f \\ 1 & \text{for } F \ge \ln N_f \end{cases}$$

The function $P_t(F)$ is not determined for $t > \tau_2$.

The $P_t(F)$ probability distributions enable calculation of $\mu(t)$ using Eq. (4). The function obtained coincides with $\mu(t)$ calculated by Eq. (2).

A more satisfactory model of tumour cell population growth is the 'branching process', or the process of 'birth-death', It starts with a number, $N = N_0$, of tumour cells and is characterized by parameters representing the probabilities of division and decay of a cell in unit time. Such a model can provide a precise solution (Prokhorov and Rozanov, 1973; Feller, 1967; Beili, 1970; Bartlett, 1958; Karlin, 1971; Harris, T.E., 1963). At high N values, N can be considered as continuously changing with time. The process 'birth-death' then becomes a 'diffusion process' (Venttsel, 1975). The theory of diffusion processes is well developed (Ito and McKean, 1965).

The 'birth-death' models are representative of the kinetic patterns of population growth of pathogenic micro-organisms in animals where growth begins with a small number of cells (Shortley and Wilkins, 1965). In certain cases these models might also be used to represent tumour growth kinetics. The practical value of these and other detailed mathematical models in experimental oncology depends on how well they represent the factors controlling tumour growth. The most important of these factors are:

(a) The heterogeneity of the tumour cell population which is more like a series of interacting subpopulations with different biological properties. This heterogeneity can increase with tumour growth due to the appearance of mutant cells, metastases, etc.

(b) The lack of synchrony of internal physiology of different cells, even in a uniform subpopulation.

(c) The necessity of allowing for the time-dependent interaction of tumour cells with host systems responsible for feeding the tumour, systems creating anti-tumour immunity (specific and nonspecific), and systems controlling the proliferation of normal cells.

Examples of mathematical population growth models allowing for such factors can be found in ecology, demography, and the theory of evolution (Moran, 1962; Eigen, 1973; Crow and Kimura, 1970). However, the creation of mathematical models for tumour growth that would find wide application and would adequately reflect the proliferation mechanisms of tumour cells needs further investigation.

2. Take a group of individual kinetic curves (Figure 5b) that were presented earlier in Figure 3. Every curve terminates at a time τ_ω corresponding to the death of the animal. However, these curves can be rearranged in such a way that a fraction of an individual animal life-span after tumour transplantation $\theta = t/\tau_\omega$ would be chosen as an independent dimensionless variable. Then all kinetic curves will originate at $\theta = 0$ and terminate at $\theta = 1$. As a result of this procedure the curves will transform as shown in Figure 5a. We can now define the true average kinetic curve for the set of curves in Figure 5a as the curve for mathematical expectation of F for each θ:

$$\mu(\theta) = M(F) = \int_\Omega F_\omega(\theta) P(d\omega)$$

To define the true average life-span of animals as the mathematical expectation of individual life-span τ_ω:

$$\tau = \int_\Omega \tau_\omega P(d\omega)$$

Let function $\mu(\theta)$ correspond to the time $t = \theta\tau$. Thus the true average kinetic curve will be defined as the function $\mu = \mu(t/\tau)$ that terminates at $t = \tau$ (Fig. 5b).

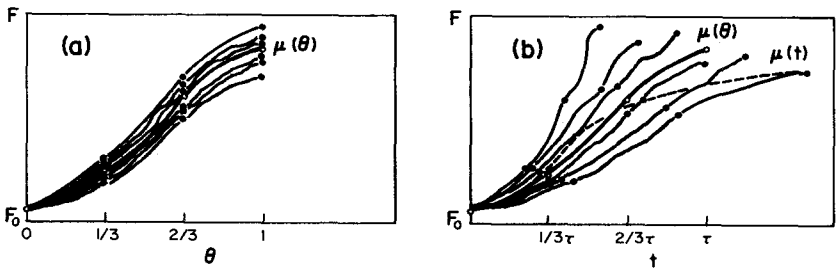

Fig. 5 Plotting the mean kinetic curve for time relative to the animal's life-span

Consider the example of a family of semilogarithmic anamorphoses of the exponential kinetic curves in Figure 4. The curve for mathematical expectation (the semilogarithmic anamorphosis of the true average kinetic curve) will then be a straight line instead of a broken line within the range $t = 0$ to $t = \tau$, i.e. to the mean lifespan of tumour-bearing animals (compare the straight line 2 with the broken line 1 in Figure 6a). Passing from semilogarithmic anamorphoses to kinetic curves we obtain accordingly two types (3 and 4) of mathematical expectation curves (Figure 6b). With both averaging procedures the kinetic curves virtually coincide up to time $t = \tau_1$ (time of death of the first animal) and then the curves diverge. This divergence might appear to be immaterial, if the time interval between τ_1 and τ_2 (death time of the last animal) is small.

At the same time it will be noted that curve 4 belongs to the same family of curves as do the individual kinetic curves, and in this sense it describes better than curve 3 the tumour growth kinetics, just as does the curve $\mu(\theta)$ in Fig. 5b.

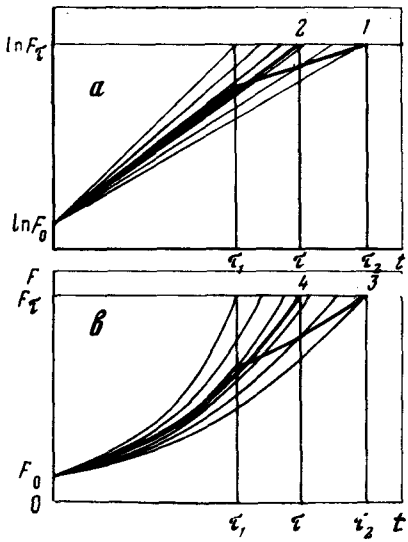

Fig. 6 Comparison of two averaging procedures for exponential kinetic curves

2. EXPERIMENTAL POINT AND THE EXPERIMENTAL KINETIC CURVE

In practice the individual kinetic curve $F_j(t)$ referring to an animal with index j is recorded not in full, but only for a finite number of points or only for one point if measurement necessitates slaughter of the animal. Thus the experimental data obtained for a group of Q animals represent a set of n separate measurements of F made at various m times. These measurements are represented in Table 1.

TABLE 1 Sample of Experimental Data

Time of measurement	Index j 1	2 ... j ... Q	Number of measurement
t_1	F_{11}	F_{12} ... F_{1j} ... F_{1Q}	n_1
t_2	F_{21}	F_{22} ... F_{2j} ... F_{2Q}	n_2
...	...	... ...	...
t_i	F_{i1}	F_{i2} ... F_{ij} ... F_{iQ}	n_i
...	...	... ...	...
t_m	F_{m1}	F_{m2} ... F_{mj} ... F_{mQ}	n_m
Number of measurement	ν_1	ν_2 ... ν_j ... ν_Q	n

Certain F_{ij} values may be absent if relevant measurements were not made. The number of all measurements is

$$n = \sum_{j=1}^{Q} \nu_j = \sum_{i=1}^{m} n_i \ .$$

The pairs of numbers (t_i, F_{ij}) that can be plotted in co-ordinates (t,F) are referred to as experimental points. The times t_i must not be considered as random, since they are usually fixed by the investigators, whereas F_{ij} represent random values.

Generally, the values F_{ij} representing measurements for the same animal j made at various times t_i are stochastically dependent. The value F_{ij} for the same time t_i can be considered as stochastically independent, as measurements refer to different animals.

As stated before, the probability distribution for the random value F_{t_i} is $P_{t_i}(F)$. The distribution $P_{t_i}(F)$ is characterized first of all by its mathematical expectation $\mu(t_i)$ and by dispersion δ_i^2. The following sample characteristics are calculated to estimate these values:

1) Sample means

$$\overline{F}_i = \frac{1}{n_i} \sum_{j=1}^{n_i} F_{ij}$$

that can serve as estimates for $\mu(t_i)$, since $MF_i = \mu(t_i)$;

2) Sample dispersions

$$S_i^2 = \frac{1}{n_i - 1} \sum_{j=1}^{n_i} (F_{ij} - \overline{F}_i)^2$$

and the corresponding root-mean-square deviation $S_i = \sqrt{S_i^2}$. The values S_i can serve as estimates for δ_i^2, since $MS_i^2 = \delta_i^2$.

3) Root-mean-square deviations of the sample means $S_{\overline{F}_i} = S_i/(n_i)^{\frac{1}{2}}$. The mean experimental points (t_i, F_i) together with the intervals $\pm S_{\overline{F}_i}$ can be plotted (Figure 7a). These intervals determine the scope of deviations of the sample means $\overline{F}_i$ from the true averages $\mu(t_i)$.

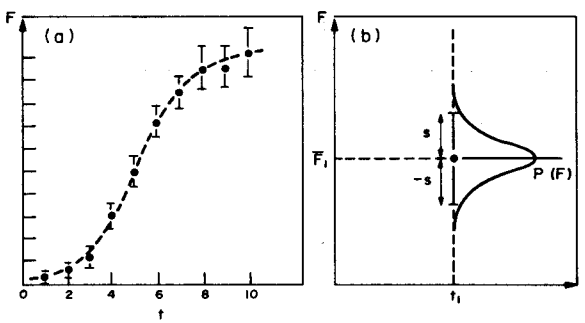

Fig. 7 Experimental kinetic curve (a) and the mean experimental point (b). S — mean quadratic equation; $P(F)$ — distribution density of experimental points.