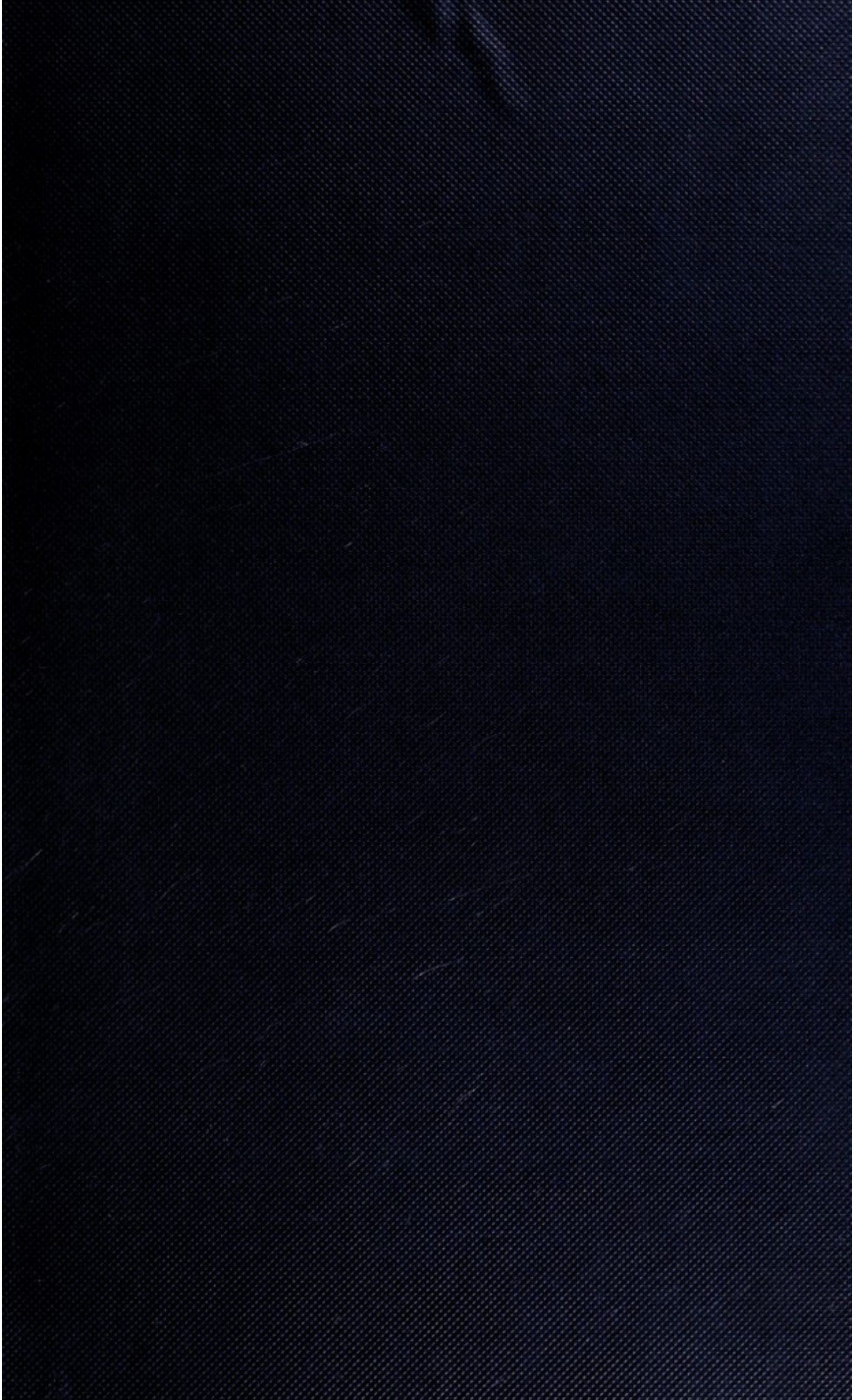
The mode of action of drugs on cells / by A.J. Clark.



H. Sandon

THE MODE OF ACTION OF DRUGS ON CELLS

Entia non sunt multiplicanda praeter necessitatem.

WILLIAM OF OCCAM.

THE MODE OF ACTION OF DRUGS ON CELLS

BY

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PREFACE

This book is based on three lectures delivered by the author in 1932 at the invitation of the University of London. He desires to record his thanks to the University both for this honour and also for a grant made from their Publication Fund.

The original lectures have been expanded considerably in order to present a moderately complete survey of the evidence available. A perusal of the volume shows that it deals almost entirely with the balancing of probabilities and that in scarcely any instance is it possible to produce clear-cut proof. The author has indeed felt serious doubts as to whether the evidence was sufficiently definite to be worth collecting, but the following two points seem to justify the collection of this data. In the first place the general problems dealt with involve many widely separated fields of knowledge, and there are many instances of workers advancing theories regarding the mode of action of drugs, which, although possible as regards their own subject, involve obvious impossibilities when applied to other subjects. In the second place the very vagueness of the biological evidence often produces a deceptive aspect of simplicity, and this results in the paradox of the most complex systems being interpreted by the simplest theories.

The probability of the truth of a theory regarding the action of drugs on cells can often be tested by considering the facts established regarding the simpler non-living systems dealt with by the colloidal chemist. In most cases the application of this method indicates that the action of drugs on cells must be far more complex than has been assumed.

The author apologizes for the fact that so much of the argument in the volume is destructive criticism of hypotheses that are attractive in their simplicity. There is however little hope for future advance unless the complexity of the factors regulating drug action is fully realized.

The author has much pleasure in acknowledging the help he has received in various forms. Drs. G. H. Percival, A. C. White, and C. M. Scott have kindly communicated to him unpublished data. The writer desires to thank the following authors for their permission to reproduce certain figures: Dr. J. Henderson Smith (Fig. 3), Miss Florence M. Durham and co-workers (Fig. 18), Dr. D. Wilkie (Fig. 35), Professor L. T. Hogben and co-workers (Fig. 47) and Professor J. Gray (Fig. 61). In addition the author has used the figures of many other writers in a modified form and in particular is indebted to Dr. Harriette Chick, Dr. J. W. Trevan, Dr. F. Tattersfield, and Professor S. Hecht and Professor C. Packard. Finally, the author desires to thank the editors of the *Journal of Physiology*, *Annals of Applied Biology*, *Journal of Cancer Research*, *Quarterly Journal of Experimental Physiology*, *Journal of Pharmacology and Experimental Therapeutics*, and *Journal of Experimental Biology*, fo their permission to reproduce figures.

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THE MODE OF ACTION OF DRUGS ON CELLS

CHAPTER I

INTRODUCTION

General Considerations

Pharmacology is one of the youngest of the biological sciences, and its youth was naturally occupied by the task of determining accurately the qualitative nature of the action of drugs. During the present century more and more attention has been paid to the quantitative aspect of pharmacology. Such studies have been stimulated in particular by the remarkable advances made in chemotherapy and also by the necessity for finding methods for estimating the activity of drugs by biological tests, and in consequence the general problems of quantitative pharmacology are beginning to receive more attention.

Various problems of quantitative pharmacology are dealt with in books such as that of Hoeber (1924) on the Physical Chemistry of Cells. Loewe (1928) has written an important article on the quantitative problems of pharmacology, and Zunz (1930) has reviewed this subject in his book *Eléments de Pharmacodynamie Générale.*

The purpose of the present volume is to try to discover what laws can be postulated regarding the combinations formed between drugs and cells, and the scope of this work is therefore narrower than that of the works already mentioned. The literature regarding those aspects of quantitative pharmacology dealt with in this volume is however very extensive, very scattered and of varied quality. The writer has not attempted to provide complete references to this literature, but has simply selected certain pieces of work which appear to throw light on problems of general interest.

The commonest defect noted in the literature studied has been the failure to recognize the extreme complexity of all systems that contain living organisms or cells. Quantitative pharmacology must seek explanations for the phenomena with which it deals from the laws of physical chemistry, but it must never be forgotten that the simplest living cell constitutes a system far more complex than any that would be considered worth investigating by a physical chemist. The requirements of physical chemistry are indicated by the fact that the relatively smooth surfaces of glass and metal are preferred to charcoal for the study of adsorption, because the latter is an inconveniently complex system. Such standards place living protoplasm far outside the limits of possible experimental material.

Quantitative investigations upon the living cell are indeed limited in every direction. The cell is a highly complex system and life is only maintained over certain narrow ranges of chemical and physical conditions. For these reasons quantitative pharmacology although it may use the laws of physical chemistry cannot really follow the methods of the physical chemist. The physical chemist can reasonably hope to simplify his conditions and to reduce the number of variables, until he obtains a system that provides formal proof of the laws which he enunciates, but the pharmacologist is interested in the action of drugs on the living cell and any attempt to simplify this material results in death. Hence he cannot hope to obtain formal proof for his theories and must be content with intelligent guesses. Even in the most favourable cases where quantitative relations have been established for the action of drugs on cells there probably remain dozens of unknown variables, and there is usually a considerable range of possible alternative explanations.

The aim of the present work is not to attempt to establish laws regarding the reactions that occur between drugs and cells in the sense that the term "establish" would be used in physical chemistry. The author has limited himself to the more modest aim of picking out cases in which the evidence available seems most complete and trying to find if the phenomena can be explained by the application of the laws of physical chemistry. There is no question of trying to

explain all the reactions that occur between drugs and cells ; the question is whether a certain proportion of these can be explained by the known laws of physical chemistry without making highly improbable assumptions.

On the other hand, the actions produced by drugs on living cells have certain features of a unique interest. Many specific drugs act at extraordinarily high dilutions, and the selectivity of the actions and antagonisms observed is without parallel in inorganic chemistry. In some cases it can be shown that living tissues detect differences that are not discerned by physico-chemical methods. For example, in the case of many synthetic organic drugs (e.g. the organic arsenicals), it has been found necessary to supplement the physical and chemical tests for purity by biological tests for therapeutic activity and toxicity. The physical and chemical tests are far more exact than are the biological tests, but unfortunately the former fail to provide information about the most important properties of the drug. Biological reactions therefore provide unique information regarding the action of drugs, but the information always tends to be vague and inexact.

Quantitative pharmacology is therefore something more than an inaccurate form of physical chemistry. It is true that the information provided by biological reactions must be regarded as vague and inexact when judged by the standards of physical chemistry, but these reactions often provide information that cannot be obtained by any other means.

Mathematical Treatment of Pharmacological Data

Quantitative data regarding the action of drugs on cells usually have the following unfortunate characteristics. The standard of accuracy is relatively low, because all living material varies and it is seldom possible to be certain that two samples are identical, and even in favourable cases, where an experiment can be repeated many times on the same population, the latter is undergoing continuous change. In practice it is seldom possible to get data accurate to within less than $\pm$ 5 per cent. Moreover, the range over which it is practicable to vary the conditions is usually narrow. Finally, there are always many unknown variables in the system under observation. In consequence of these facts nearly all pharmacological data would be summarily rejected by any physical chemist

as being far too inaccurate and uncertain for profitable mathematical analysis.

The writer made a few experiments with varying constants to see how many different formulæ could be used to interpret the same set of figures if a variation of ± 5 p.c. were allowed. The result is shown in Fig. 1. The three curves show various relations between concentration (x) and action (y) and do not deviate by more than ± 5 p.c. of the maximum value of y when y varies from 20 to 60 per cent. of its maximum value. Curve 1 is a hyperbola, curve 2 is an exponential curve and

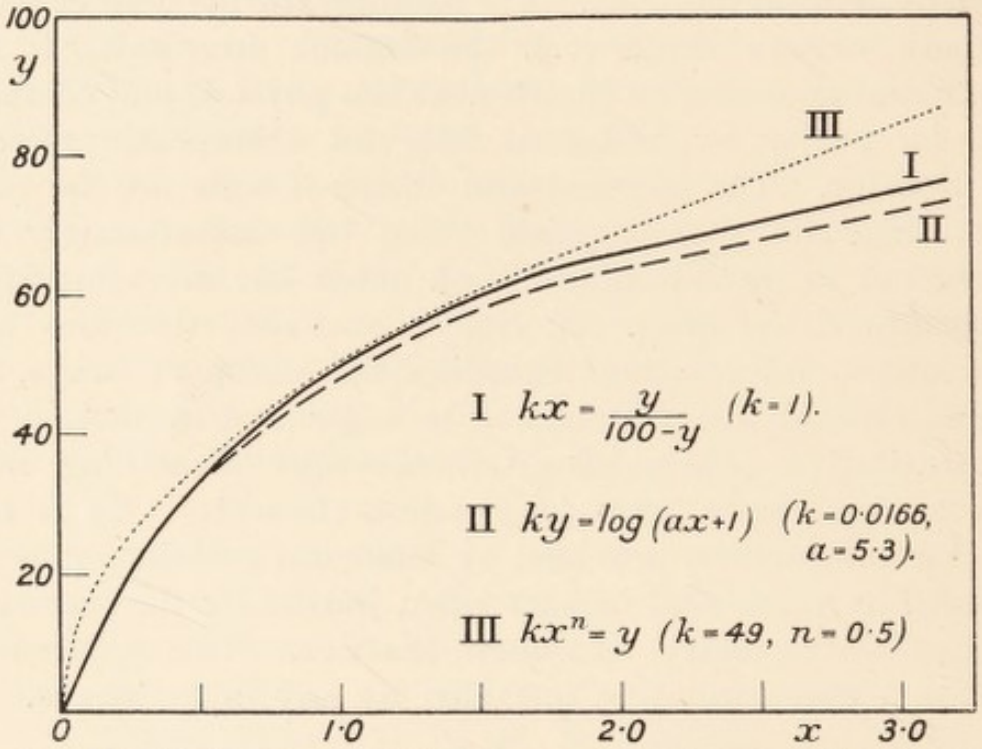

Fig. 1.—The similarity possible between the curves expressing three different formulæ of types used in biology.

curve 3 is a parabola. They are all drawn to formulæ which have been used to express pharmacological data. The first formula is the simplest of those used by Langmuir to express adsorption equilibrium. The second formula expresses the well-known Weber-Fechner law, and the third is the well-known adsorption formula popularized by Freundlich.

The majority of pharmacological data expressing equilibria between drugs and cells approximate to an exponential form. In most cases it is very difficult to get data accurate to within ± 5 p.c. of the maximum effect, and only in exceptionally favourable circumstances can the effect of a drug be measured

from 0 to 100 per cent. of its possible action. Consequently Fig. 1 expresses the nature of the evidence usually provided by quantitative pharmacological data, and, as the figure shows, the results can be interpreted almost equally readily in several different ways.

The writer must apologize for mentioning the following principles regarding the use of formulæ in interpretation of biological data. They appear to be self-evident, but they are very frequently disregarded. In the first place, there is no advantage in fitting curves by a formula unless this expresses some possible physico-chemical process, and it is undesirable to employ formulæ that imply impossibilities. For example, the simple kinetic formula—concentration $\times$ time $=$ constant, implies that an infinite dilution can produce an action in infinite time and that a sufficiently high concentration will produce an instantaneous action. Both these postulates are usually untrue, for there is always a threshold concentration below which a drug produces no action, and there is usually a lower limit to the time in which an action can be produced.

Pharmacological data usually have a low standard of accuracy and express relations over a narrow range of variation, but the accuracy of the data cannot be improved by mathematical treatment because the original quality of the material unfortunately is not affected by such treatment. On the other hand, mathematical treatment will often obscure experimental errors in a manner that is gratifying to the author, but not to the reader.

The fact that a particular set of data are fitted by a particular formula merely establishes a probability that the chief variable measured is some particular physico-chemical process. Formal proof of the occurrence of a particular process is rarely if ever possible because of the number of unknown variables that are nearly always present. Consequently regard must always be paid to general probabilities, and if the assumption of the occurrence of a particular physico-chemical process involves assumptions that appear absurd on general grounds, there is no necessity to violate reason in obedience to a formula. It must be remembered that a formula is merely a convenient form of shorthand and is an aid to and not a substitute for reason.

Material Suitable for Quantitative Study

There are obvious advantages in studying quantitatively the action of a drug over as wide a field as is possible. At the same time it must be recognized that only a limited number of systems are suitable for quantitative work. The action produced by a drug on a biological system not only must be measurable, but also it is very important that it should be measurable over the full range of possible action. There must be an upper limit to the action of any drug, but in many cases this cannot be determined because of secondary effects that appear when the concentration of drug is increased. For example, the depressant action of chloroform on most isolated tissues can be measured from 0 to 100 per cent. action, but in the case of a weak narcotic such as alcohol the effects produced by doses approaching the upper limit are of doubtful quantitative significance because the concentration of alcohol is so great as to produce an extensive change in the physical properties of the perfusion fluid, and the action measured is probably a mixed effect of narcotic action and a variety of other effects. Fig. 1 shows the great importance of the measurement of the top and bottom 10 p.c. of the possible response and systems in which these can be measured are preferable.

Another fundamental point is that the action produced should be as simple as possible. An example of a satisfactory system is the effect of a narcotic on the respiration of blood-corpuscles or sea-urchin eggs. In this case there is a reasonable probability that the changes observed are a direct result of the fixation of the drug by the cells. In other cases it is practically certain that the relation between dosage and action produced will be complex. For example, there is a quantitative relation between the dosage of a vitamin and the effect produced by its administration to an animal on a diet deficient in this vitamin. The easiest effect to measure is the increase in weight. The relation between rate of growth and increase in weight in a given period is however of a logarithmic character. Hence even if there happens to be a simple linear relation between vitamin dosage and the rate of growth this same relation will not obtain between the vitamin dosage and the gain in weight in a given period.

The pharmacologist must be moderate in his ambitions. It is not a question of finding laws which will interpret all actions of drugs on all systems. It is a question of finding a few systems so simple that it is possible to establish with reasonable probability the relation between quantity of drug and the action produced in these cases. The simplest systems present so many difficulties that it may be taken for granted that analysis is impossible in the case of complex systems. This need for caution is sometimes forgotten because highly complex systems may provide the simplest quantitative relations between dosage and action of drugs, but the most probable reason for any such apparent simplicity is that a large number of variables are present but mutually cancel each other.

Colloidal chemistry provides the best control for pharmacological theories. The living cell is more complex than any non-living colloidal system, and it is therefore absurd to expect the relations found in pharmacology to be simpler than those found in colloidal chemistry. Colloidal chemistry has made great advances in recent years, but the general trend of these advances has been to reveal an increasing complexity in the laws governing colloidal reactions.

It seems fair to assume as a general principle that if a pharmacological reaction appears simpler than an analogous reaction in non-living systems, the simplicity must be apparent rather than real. This line of argument will be used frequently in this book because it is often the only method of deciding whether the fact that a set of data are fitted by a formula has any theoretical significance. This attitude is moreover in general accordance with the experimental evidence, for an intensive study of any particular pharmacological action nearly always results in showing it to be more complex than was at first supposed.

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CHAPTER II

THE LIVING CELL CONSIDERED AS A PHYSICO-CHEMICAL SYSTEM

The Size of Cells and Molecules

Pharmacology is concerned with the effects produced by drugs on living organisms, and it is important to consider the general properties of the simplest systems available for quantitative study.

The simplest living system is a population of free-living cells, and a stationary population is preferable to one that is dividing freely because the former is more uniform. Hence spermatozoa, sea-urchin eggs, red blood-corpuscles, amœbæ or bacterial spores are preferable to freely growing bacteria. A suspension of even the simplest cells in a drug solution constitutes however a complex heterogeneous system, because the smallest cell is enormously larger than any molecule and the simplest living cell is composed of several phases.

Table I shows the sizes of certain molecules, whilst Table II shows the sizes of typical monocellular organisms and body cells. The range of size of monocellular organisms is very great and the manner in which their functions are related to their size has been discussed by Adolph (1931). The few examples given in Table II show that the lower limit of size for ultra-microscopic organisms is less than the size of the larger protein molecules, but that on the other hand a small bacteria is enormously larger than the molecule of an ordinary drug. The order of magnitude of the diameter of simple molecules is 10 Å, and of a protein molecule 100 Å, whilst on the other hand in the case of a small coccus the corresponding figure is 10,000 Å. This implies that the volume of a coccus is about 1,000,000 as great as that of a protein molecule.

A large number of drugs are known to exert their action upon the surface of cells, and therefore it is of interest to consider the number of drug molecules necessary to cover cells

with a monomolecular layer. These quantities can be calculated from the figures in Tables I and II.

In regard to the area covered by drugs Paneth and Radu (1924) found that 1 mgm. of methylene blue could cover a surface of 1 sq. metre with a monomolecular layer. The amount of this dye needed to cover a staphylococcus with a surface area of 2 sq. μ would therefore be 2×10^{-12} mgm., and this quantity would contain 4 million molecules. (In this and subsequent calculations the figure 1 gm.-mol. $= 6{\cdot}4 \times 10^{23}$ molecules has been used.) The molecule of phenol is smaller than that of methylene blue, and it may be assumed that about 10 million molecules of phenol would be needed to cover a coccus with a monomolecular layer. This number would have a total volume of about $200 \times 10^7 = 2 \times 10^9$ cu. Å, which is about 1 p.c. of the volume of the coccus. Similarly, a human erythrocyte would be covered by 240 million molecules of

TABLE I

The Dimensions of Molecules

Molecule.	Molecular weight.	Linear dimensions in Å.	Area covered by adsorbed molecule in square Å.	Volume in cubic Å.
Ethyl alcohol	46	$(3{\cdot}6)^3$ (1)	21·6 (2)	—
Chloroform .	119	$(4{\cdot}8)^3$ (1)	—	—
Palmitic acid.	256	$24 \times 4{\cdot}5 \times 4{\cdot}5$ (2)	20·5 (2)	495
Sodium oleate	304	$12{\cdot}3 \times 7{\cdot}6 \times 6{\cdot}6$ (3)	50 (3)	616
Methylene blue	320	—	50 (4)	—
Castor oil .	929	$17 \times 17 \times 5{\cdot}7$ (5)	280 (5)	1,600
Gelatine . .	—	$30 \times 22 \times 22$ (10)	450	13,500
Egg albumen.	36,000 (7)	$42 \times 30 \times 30$ (6)	905 (6)	39,000
Hæmoglobin .	34,000 (7)	—	—	—
	66,000 (8 & 9)	$53 \times 53 \times 7$ (10)	2,800	19,000
Muscle protein	—	$76 \times 76 \times 7{\cdot}5$ (10)	5,800	43,000
Phyko-erythrin	210,000 (11)	—	—	—

(1) Landolt-Börnstein (1923).
(2) Adam (1930).
(3) du Noüy (1926, p. 99).
(4) Paneth and Radu (1924).
(5) Langmuir (1917).
(6) du Noüy (1926, p. 115).
(7) Busk and Greenberg (1930).
(8) Svedberg and Fahraeus (1926).
(9) Adair (1925).
(10) Gorter and Grendell (1926A).
(11) Svedberg (1928).

TABLE II

The Dimensions of Cells

	Linear dimensions in micra ($1\mu = 10^4$ Å).	Surface area in square micra ($1\mu^2 = 10^8$ sq. Å).	Volume in cubic micra ($1\mu^3 = 10^{12}$ cu. Å).
Ultra-microscopic viruses . .	$(0{\cdot}01)^3$ to $(0{\cdot}1)^3$	0·000,3 to 0·03	0·000,000,5 to 0·000,5
B. pneumosintes	$(0{\cdot}2)^3$	0·12	0·004
Staphylococci and Streptococci (1)	$(0{\cdot}8)^3$	2	0·26
B. coli (1)	$3 \times 1{\cdot}2$	11·6	3·4
Botrytris spores (2) . . .	10×8	250	380
Yeast cells (3)	$(7)^3$	150	180
Amœba limax (4)	80×30	8,000	50,000
Eggs of *Psammechinus* (5) .	$(29)^3$	2,600	12,500
Human erythrocyte (6) . .	$8{\cdot}8 \times 2{\cdot}4$	119	120
Cell of heart ventricle:			
(*a*) Frog (7)	131×9	1,900	2,600
(*b*) Human (8)	50×20	3,100	15,000
Stomach-muscle of frog (8) .	60×2	180	60
Uterine muscle of virgin rat (9)	30×2	90	30

(1) Schmidt and Fischer (1930).
(2) Smith (1921).
(3) Pütter (1924).
(4) Pantin (1924).
(5) Gray (1931, p. 6).
(6) Ponder (1921).
(7) Skramlik (1921).
(8) McGill (1909).
(9) Gander (1930).

sodium oleate. Acetyl choline and adrenaline are two hormones of particular interest in quantitative pharmacology. Their molecular weight lies between 100 and 200, and one of their molecules probably covers an area between 20 and 50 sq. Å. If the latter figure be taken, then the number of molecules required to cover one heart-cell of the frog is of the order of 10^{10} molecules. Even in the case of proteins the number of molecules of egg albumen needed to cover a coccus is about 200,000, and 13 million would be needed to cover an erythrocyte.

These figures suffice to indicate the enormous disproportion between the sizes of molecules and of small cells. The ratio of size between a molecule of phenol and a coccus is similar to that between a milligram and a ton.

Although the number of molecules needed to cover a cell is large, yet the concentration resulting from the coating of a cell with a monomolecular layer of drug is not very great. These quantities can be calculated readily from the data given in the first three tables. Table III shows, for example, that the cells in 1 cu. mm. of frog's-heart tissue have a total surface of 6 sq. cm. The amount of acetyl choline needed to cover this area is 3×10^{-7} grammes or 2×10^{-9} gramme-molecules or 10^{15} molecules. The concentration of drug in the tissue when the cell surfaces are completely covered would be about one in 1000. Similarly when an erythrocyte is covered with

TABLE III

Number of cells in 1 cu. mm. and their total surface

Nature of cells.	Number in 1 cu. mm.	Total surface in sq. cm. of cells in 1 cu. mm.
B. coli	975×10^6 (2)	36 (1)
Staphylococci	$2{,}130 \times 10^6$ (2)	76 (1)
		175 (3)
Human erythrocyte	8×10^6 (1)	10 (1)
Muscle cells of frog's ventricle	$0{\cdot}33 \times 10^6$ (1)	6 (1)
Fibrils in rabbit's muscle :		
(*a*) Skeletal muscle	—	50 (4)
(*b*) Heart muscle	—	about 150 (4)

(1) Calculated from Table II.
(2) Schmidt and Fischer (1930).
(3) Rideal (1930).
(4) Gorter and Grendell (1926B).

a monomolecular layer of sodium oleate the total volume of the drug is about one-thousandth that of the cell. The surface per unit volume is greater in the case of bacteria than in the cases mentioned above, and the quantity of drug required to cover a bacteria with a monomolecular later is equal to about 1 per cent. of the volume of the bacteria.

The question as to the relative size of molecules and cells is important because the problem arises whether the fundamental laws of chemistry and physics can be expected to apply in a volume as small as that of a living cell. This question is discussed by Gray (1931, Chapter 2), who points out that the laws of mass action and the second law of thermo-

dynamics are both based on the conception of a large population of molecules moving at varying velocities. The validity of these laws depends on the assumption that the populations are large enough for the statistical laws of chance to apply, and therefore it is of interest to consider the probable number of molecules in a cell.

The Number of Molecules in Single Cells

Cameron (1929) calculated the number of certain molecules and ions present in a red blood-corpuscle and obtained results of the following order:

Phosphatides, cholesterol, glucose and urea	200–300 million
Adenosin and glutathione	50–100 ,,
Potassium and chlorine	2000–10,000 ,,
Hydrogen ions	220,000

The limit of direct visibility is usually considered to be a sphere $0{\cdot}2\mu$ in diameter. The volume of such an organism is only 1/30,000 that of a red blood-corpuscle, but even in this case each cell will contain thousands of all the molecules and ions mentioned except the hydrogen ion, and according to the calculation given above it will only contain about 7 hydrogen ions. Peters (1930) calculated that an organism 0·2 micron in diameter (volume $= 4 \times 10^9$ cu. Å), when its contents were neutral (pH 7·0), would only contain one hydrogen ion.

Errera (1903) was one of the first to calculate the probable lower limit of size compatible with life. He reckoned that the diameter of *Micrococcus progrediens* was $0{\cdot}15\mu$ and calculated the volume as 0·0018 cu. μ. He found that if the organism contained 14 p.c. of protein with a molecular weight of 10,000, it would contain 30,000 molecules of protein. The molecular weight of protein is now believed to be at least three times as great as he assumed, and therefore his figure for the protein molecules per cell must be divided by three. The surface area of the organism is about 0·07 sq. μ, and 10,000 molecules of protein would cover about 0·1 sq. μ. The protein content therefore is not much greater than the amount needed to form a monomolecular layer on the surface.

Errera also calculated that a spherical organism with a diameter of $0{\cdot}05\mu$ would contain 1000 molecules of protein, whilst one with a diameter of $0{\cdot}01\mu$ would only contain 12

molecules. These figures must be divided by three to accord with the more modern estimate of the molecular weight of protein. Errera concluded that ultra-microscopic viruses could not be very much smaller than the smallest visible organisms.

According to d'Herelle (1928–9) the bacteriophage and the majority of ultra-microscopic viruses have a diameter of about $0{\cdot}01\mu$, although in certain cases the diameter of the latter is as great as $0{\cdot}05\mu$.

Elford (1931) estimated the size of organisms by means of filtration through graded collodion filters and obtained the figures shown below.

Organism	*Diameter in micra*
B. prodigiosus	0·5 –1·0
Bovine pleuropneumonia	0·1 –0·5
Vaccinia virus	0·12 –0·17
Infectious ectomelia	0·1 –0·15
Bacteriophage (B. coli)	0·02 –0·03
Foot-and-mouth-disease virus	0·008–0.012
Oxyhæmoglobin	0·003–0·005

These results show a continuous gradation of size ranging downwards from organisms only slightly smaller than visible organisms to those of the size of protein molecules. The majority of authorities agree with these figures, although some authors have obtained smaller ones. For example, Hitler and Bronfenbrenner (1931) found that the average diameter of the particles carrying bacteriophage was $0{\cdot}004\mu$.

These calculations suggest that the number of molecules in a cell the size of a red blood-corpuscle is sufficiently large for the ordinary laws of chemistry and physics to apply. In the case of small bacteria it seems probable that the greater portion of the protein is orientated on the surface, and the number of hydrogen ions is too small for the ordinary laws of mass action to apply. As regards most of the molecules of which such an organism is composed, these will number thousands if not tens of thousands. The volume of such an organism is however about one million times greater than the volume of a molecule of a drug with a molecular weight of a few hundred. Hence even in the case of the smallest visible organisms there

is no reason to assume that they will behave as molecules and that one molecule of drug will suffice to kill an organism.

It seems probable that an ultra-microscopic virus contains a small number of molecules of protein. Our knowledge concerning these forms of life is very scanty but they would appear to present special problems in physical chemistry.

The Number of Molecules of Drugs that React with a Single Cell

The understanding of the possible modes of action of drugs on cells is facilitated by the estimation of the amounts of drug that react with cells. It is particularly interesting to consider the case of drugs that act in high dilutions, since these figures give an indication of the smallest quantities of drug that can affect a cell.

The following methods of measurement are available for estimating the dose of drug needed to produce an effect on a single cell.

(*a*) The micro-injection of drugs.

(*b*) The chemical estimation of the concentration of drugs in cells.

(*c*) The estimation of the quantity of drug fixed by cells by the measurement of the amount of drug disappearing from the surrounding solution.

The first of these methods appears to be the most reliable but has yielded somewhat unexpected results, for it has been found that many drugs when injected into cells produce actions that are qualitatively different from those produced when the cell is immersed in a solution of the drug. These results are interesting and important, but they do not indicate the quantity of drug needed to produce the normal drug action on cells.

The chemical estimation of the concentration of drugs in cells is possible in certain cases, but unfortunately it is not possible in the case of the most powerful and therefore the most interesting drugs because in these cases the quantities fixed are too small to be estimated by chemical methods.

In the case of phenol, estimations have been made of the concentration of drug in micro-organisms when the external concentration is just sufficient to produce death. Fig. 2 shows the results obtained by Herzog and Betzel (1911). This shows that yeast cells are killed when the concentration

of phenol in the cells is about 0·3 p.c. If the cell volume be taken as 200 cu. micra there will be about 3×10^9 molecules per cell.

In most cases the fixation of drugs by cells has been measured biologically. A certain volume of drug solution has been allowed to act on a certain quantity of cells, then the solution has been separated and the amount of free drug has been estimated by biological tests. An alternative method that is possible in some cases is to reduce the volume of drug solu-

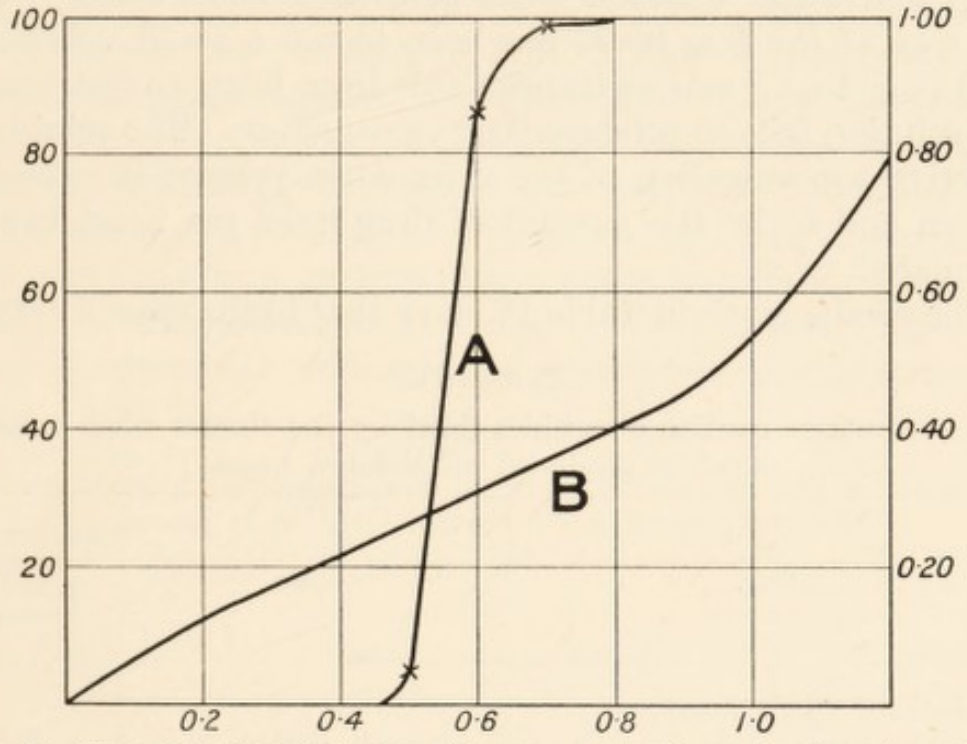

Fig. 2.—The relation between the concentration of phenol and its action upon and uptake by yeast.

Abscissa : per cent. concentration of phenol in solution. Left Ordinate : per cent. mortality. Right Ordinate : per cent. concentration of phenol in cells.

Curve A. Per cent. mortality.

Curve B. Adsorption of phenol by cells. (Herzog and Betzel. 1911.)

tion to a minimum and thus measure the smallest quantity of drug that can produce a given action on a known quantity of cells.

Ponder (1930) studied the hæmolytic action of saponin and of sodium oleate and concluded that hæmolysis was produced by the combination of 10^{-13} gm. lysin with a human erythrocyte. If the molecular weight be taken as 600, then the amount fixed corresponds to 10^8 molecules per cell. In the

case of sodium oleate Ponder calculated the corresponding figure as 2×10^8 molecules. He found that this quantity was just sufficient to cover the cell surface with a monomolecular layer. Christophers (1929) found that about 0·0002 gramme-molecules of acid or alkali were required to hæmolyse 1 gm. of erythrocytes. This works out at about 10^9 molecules per erythrocyte.

A number of authors have calculated the quantity of cardiac glucosides needed to produce arrest of the isolated hearts of frogs and other animals. The general method employed in the case of the frog heart has been to use a small volume of fluid (e.g. 1 c.c.) and to transfer this from heart to heart until the solution fails to produce the typical effect. The minimum effective concentration of the drug when present in excess is known and hence the amount of drug fixed per heart can be calculated.

The results given in Table IV show that in the case of certain

TABLE IV

The amounts of cardiac glucosides fixed by the tissues when a lethal effect is produced on isolated hearts

Drug.	Author.	Drug fixed by tissue (γ per g. moist weight).
(*a*) *Isolated frog's heart :*		
Ouabain.	Straub (1910)	2·2
	Weizsäcker (1917)	3
Strophanthin Kombé . .	Clark (1912)	3·2
Digitoxin	Fischer (1928)	20
Gitalin	Gander (1932)	50
Digitalin Kiliani . . .	Weizsäcker (1917)	25
Gitalin Kraft	Idem	80
Digitalinum verum . .	Idem	600
Scillaren	Oliaro and Rothlin (1928)	1·2
Antiarin.	Sluytermann (1911)	2
(*b*) *Heart-lung preparation of cat :*		
Ouabain.	Weese (1928)	2
Digitoxin	Idem	4·6
Scillaren	Idem	1·65

$\gamma = \frac{1}{1000}$ mgm.

drugs the heart cells can be poisoned by the fixation of about 2γ of glucoside per gramme of heart tissue. This result is of interest because this quantity of drug is much too small to cover the surface of the heart cells.

The molecular weight of ouabain is 760 and hence 2γ of drug contain about 2×10^{15} molecules. One gramme of frog's ventricle contains 3×10^8 cells and therefore each cell fixes about 10^7 molecules. A molecule of strophanthin will certainly cover less surface than 500 sq. Å and hence 10^7 molecules cannot cover more than 5×10^9 sq. Å. The frog's heart cell, however, has an area of 2×10^{11} sq. Å, and hence the strophanthin fixed cannot cover more than 3 p.c. of the surface of the cell. The author has estimated the amount of various other drugs fixed by the tissues upon which they act, and the results are shown in Table V. In most cases frog tissues were used, and the effect produced on a tissue immersed in a large volume of dilute solution of the drug was compared with the effect produced by adding small quantities of concentrated

TABLE V

The amounts of various potent drugs fixed by the tissues when an action of a given intensity has been produced

Drug.	Tissue.	Action.	Amount of drug fixed (γ per g. moist weight).	Author.
Acetyl choline HCl	Frog's heart	50 per cent. inhibition	0·02	Clark (1926A)
	Frog's rectus abdominis	50 per cent. contracture		Idem
Atropine sulphate	Frog's heart	Demonstrable antagonism of acetyl choline	0·5	Clark (1926B)
Potassium chloride	Frog's heart	50 per cent. inhibition	100	Clark (1926C)
Higher alcohols (Heptyl-dodecyl)	Frog's heart	50 per cent. inhibition	500	Clark (1930)
Caffeine citrate .	Frog's rectus abdominis	50 per cent. contraction	800	Clark (unpubl.)
Adrenaline HCl .	Frog's stomach	Marked inhibition	0·06	Idem
	Rat's uterus	Idem	0·01	Idem
Histamine HCl .	Rat's uterus	Idem	0·2	Idem

solution to the tissue when this was suspended in a moist chamber. In the case of rat's uterus the action of a large volume of solution (25 c.c.) was compared with the action of the drug when added to a bath containing about 0·5 c.c. of fluid. In the case of such drugs as acetyl choline and adrenaline the figures given are maximum ones, for the drug is destroyed rapidly by the tissues, and this must introduce a considerable error.

The results given in Table V show in the case of the alkaloids, which exert a powerful specific action on cells, that the amount of these drugs that must react with the cells lies between 0·01 and 0·1γ per gramme of cells, i.e. about one-hundredth of the corresponding amount of strophanthin.

Although these figures are only very approximate, yet they provide important information regarding the mode of action of these drugs, for their molecular weight lies between 100 and 300, and hence a quantity between 0·01 and 0·1γ, which is absorbed by 1 gm. of heart, contains about 10^{-10} gramme-molecules or about 10^{14} molecules. Even if each molecule covers 100 sq. Å this quantity would only cover 10^{16} sq. Å or 1 sq. cm. The surface area of the cells in 1 gm. of frog's heart is about 6,000 sq. cm., and hence the drug fixed can only cover about 1/6,000 of this surface. These figures therefore preclude completely any idea that these drugs can form a monomolecular layer over the heart cells and practically prove that the drugs must exert their action by uniting with certain specific receptors in or on the heart cells, and these receptors must form only an insignificant proportion of the total surface of the cells.

On the other hand, the amounts of the higher alcohols fixed by the heart are of the order necessary to form a monomolecular layer over the heart cells, for the amount fixed per unit of weight is about 10,000 times as great as in the case of the specific drugs.

In the case of potassium chloride another interesting point arises. The heart cells contain about 0·3 p.c. of KCl, but yet a powerful action is produced on the cell by the fixation of an amount of KCl equal to only 0·01 per cent. of the tissue weight.

Although the weight of drug fixed per cell is extremely

small, yet the number of molecules fixed per cell is large. The author has calculated that in the case of acetyl choline a demonstrable action is produced on the heart cells when 10,000 molecules per cell are fixed, but about twenty times this quantity is needed to produce a 50 per cent. inhibition.

In connection with these calculations it may be noted that it is difficult to express the quantitative reactions between drugs and tissues without the use of either cumbersome or else unfamiliar expressions. Quantities of drugs can be expressed fairly conveniently as micrograms (γ) per gramme or per kilo. Concentrations of drugs are however more difficult to express without the use of an inconveniently large number of noughts. In the case of the liminal concentrations of powerful drugs the millimolar terminology is but little more convenient than the molar. On the other hand, the number of molecules in a gramme-molecule is so great that this gives inconveniently large figures. For example, several drugs produce an action in a dilution of 10^{-10} molar, and this is equivalent to 6×10^{10} molecules per c.c.

Minimum Concentrations of Drugs effective on Cells

Drugs such as acetyl choline are well known to produce an action at extraordinary dilutions, and therefore it is of interest to consider the highest dilutions at which drugs can produce a demonstrable action, and whether there are a sufficient number of molecules per cell present to permit the ordinary laws of physical chemistry to operate.

The minimum concentrations of certain powerful drugs that have been proved with fair certainty to produce an effect are given in Table VI. This table shows that five drugs produce a recognizable action on isolated tissues in a concentration of 10^{-9} molar or less. In most cases the results quoted have been confirmed by several independent workers, and can therefore be regarded as firmly established.

Table VII shows the minimum doses of certain hormones that produce recognizable effects in intact animals, and the lowest figures in this table suggest that drugs can act at concentrations even lower than those given in Table VI. There are other cases in which drugs have been shown to produce a biological effect in comparable dilutions. For example, bioluminescence is an effect which permits the detection of

TABLE VI

Minimum concentrations of drugs that produce demonstrable actions on isolated organs

Drug.	Organ.	Action.	Concentration.	Author.
Acetyl choline HCl	Frog's heart	Inhibition	10^{-9} molar	Clark (1926A)
Histamine HCl	Rabbit's ear	Vaso-constriction	5×10^{-10} molar	Rothlin (1920)
Adrenaline HCl	Rabbit's intestine	Inhibition	5×10^{-9} molar	Trendelenburg (1924)
	Rabbit's ear	Vaso-constriction	5×10^{-10} molar	Rothlin (1920)
	Rat's uterus	Inhibition	5×10^{-10} molar	Knaus and Clark (1925)
Pituitary extract	Frog's skin	Melanophore dilatation	One part in 10^{12} of the active principle	Krogh (1926)
	Guinea-pig's uterus	Contraction	One part in 5×10^{10} of the active principle	Abel (1924)

minute traces of drugs, and Newton Harvey (1924) has given the following figures. The concentration of oxygen necessary for the occurrence of luminescence is 0·0053 mm. Hg. oxygen pressure which equals 1 part of oxygen in $3{\cdot}7 \times 10^9$ parts of sea water. Luminescence is produced by the addition of luciferase to luciferin when the former is in a concentration of 1 part in 8×10^9 parts of sea water.

In the case of smell extraordinary figures for dilution have been recorded. Passy (1892) found that tri-nitro-butyl-toluol could be detected at a concentration of $10^{-5}\gamma$ per litre, and Fischer and Pentzoldt (1887) gave a similar threshold value for mercaptan. These concentrations sound incredibly small, but none the less they are sufficient to provide about 10^8 molecules per c.c. of air.

These concentrations are extremely small, but comparable figures have been obtained in certain cases with non-living systems. For example, Bredig and v. Berneck (1899) found that platinum sol in a concentration of 3×10^{-9} gm. platinum per c.c. caused a measurable decomposition of hydrogen

TABLE VII

Minimum doses of hormones and vitamins that produce responses in intact animals

Drug.	Animal.	Effect measured.	Dose given (γ per kg.).	Author.
Acetyl choline HCl	Cat	Fall in B.P.	0·000,002	Hunt (1918)
Adrenaline HCl	Cat	Vaso-dilatation of denervated limb	0·000,005	Dale and Richards (1918)
	Cat	Inhibition of intestine	0·000,7	Lim and Chen (1925)
	Cat	Rise in B.P.	0·07	Wilkie (1928)
	Dog	Rise in B.P.	0·01	Molinelli (1926)
	Rat	Inhibition of uterus	0·5	Knaus and Clark (1925)
	Man	Rise in pulse rate, etc.	0·025 (per min.)	Cori and Buchwald 1930)
Active principle of posterior lobe of pituitary (partly purified)	Cat	Rise of B.P.	0·05	Abel (1930)
Œstrin, crystalline	Rat	Production of œstrus	1–5	D'Amour and Gustavsen (1930)
Insulin, crystalline	Rabbit	Fall of blood-sugar	10	Abel (1926)
Thyroxin . .	Mouse	Rise in metabolism	200 per diem	Gaddum and Hetherington (1932)
Vitamin A . .	Rat	Effect on growth	3 per diem	Moore (1930)
Vitamin D . .	Rat	Effect on bones	0·02 per diem	Coward (1928)

peroxide, and Paal and Amberger (1907) found the same with osmium sol at a concentration of 9×10^{-10} gm. per c.c.

It also is interesting to note that visible colour can be produced by concentrations even lower than those recorded in the case of smell. Newton Harvey (1923) stated that fluorescine could be detected at a concentration of one part in 10^{15}.

Minimum Quantities of Drugs producing effects on Isolated Cells and Tissues

Some of the smallest quantities of drugs that can be proved to produce a measurable action on isolated organs are as follows. Ehrmann (1905–1906) showed that 0·01γ adrenaline produced a measurable effect on the isolated frog's eye.

Führer (1918) found that 0·1γ physostigmine produced a demonstrable effect in potentiating the action of acetyl choline on the frog's heart. Fleischmann (1911) demonstrated the effect of 0·01γ atropine on a frog's sinus that had been arrested with muscarine.

The author used strips of frog's ventricle suspended in a moist chamber and applied drug solutions in the form of small drops. This method permitted the demonstration of 0·002γ acetyl choline (Clark, 1926A), of 0·06γ atropine (Clark, 1926B), and of 0.01γ strophanthin (unpublished results). The same technique with strips of frog's stomach gave a positive effect with 0·002γ adrenaline. Other experiments were made with tissues suspended in very small volumes of fluid and pieces of guinea-pig uterus responded to 0·001γ adrenaline, and to 0·02γ of dried pituitary and to 0·01γ histamine (unpublished results).·

Various authors have shown that demonstrable effects can be produced by very minute quantities of drugs applied locally to certain tissues. Metzner (1912) found that a dose of 0·01γ atropine instilled into the human eye produced a just demonstrable effect, and Joachimoglu (1915) found the same figure for the minimum dose of hyoscine effective on the cat's eye. Koppanyi and Lieberson (1930) found that 0·05γ atropine produced an effect when injected into the anterior chamber of the cat's eye.

Weiss and Hatcher (1923) applied drugs to the floor of the fourth ventricle and found that emesis was produced by 0·1γ aconitine and by 0·2γ morphine.

Minimum Quantities of Drugs producing effects on Intact Mammals

Table VII shows figures for the minimum quantities of drugs that produce a recognizable response when administered to a mammal. The smallest values are those given by Reid Hunt for acetyl choline and by Dale and Richards for adrenaline, and, as far as the writer is aware, these two values represent the smallest quantities of drug that have been proved with certainty to produce an action on living tissues.

Other workers have shown that these drugs produce effects when given in very small quantities, but the values obtained are considerably larger than those mentioned above. Clark

and White (1927) found that 0·004γ acetyl choline per kilo. just produced a fall of blood-pressure in the cat, but this figure, although small, is nearly 1000 times greater than that obtained by Reid Hunt. In the case of adrenaline, Dale and Richards showed that the amount needed to produce vaso-dilatation in the denervated limb was far smaller than that required to produce a demonstrable rise of the blood-pressure. Wilkie (1928) found that the latter effect was produced by 0·07γ per kilo. in the cat, and similar figures have been obtained by many other authors. The following are a few typical figures for the dose per kilo. of adrenaline needed to produce a measurable rise of blood-pressure. In the dog, 0·01γ (Molinelli, 1926), 0·08γ (Reid Hunt, 1901). In the cat, 1γ (Storm van Leeuwen, 1920), 0·25γ (Cannon and Lyman, 1913). In the rabbit, 0·5γ (Launoy and Menguy, 1920).

The effects considered above were immediate responses to injections, but very small doses of hormones and vitamins are capable of causing a sustained alteration in function. Crystalline oestrin and calciferol appear to be the two most potent of these substances isolated at the present time.

The figures for the minimum effective dose of oestrin are as follows: D'Amour and Gustavson (1930) found the rat unit was equal to 0·38γ when the oestrin was given in a single dose. Laqueur and de Jongh (1929) gave a fairly pure preparation of oestrin divided in 6 doses, over three days, and obtained with the purest specimens a value of 0·1γ oestrin for the mouse unit. Allan, Dickens and Dodds (1930) found that oestrin, when given divided in 6 doses, was about 50 times more active than when given in a single dose. It would appear probable therefore that oestrin acts on a rat when the total amount in the blood at any time is not more than 0·01γ, which is a concentration of about 1 part in 10^9.

Vitamin D or irradiated ergosterol has an activity comparable to that of oestrin. Coward (1928) found that a daily dose of 0·01γ of vitamin D just produced a demonstrable effect on a rat of 100 gm., and since then purer and more potent preparations have been prepared. Several hormones and vitamins therefore produce an action when the amount present in the body is of the order of 0·01 to 0·1γ per kilo. (i.e. 1 part in 10^{10} to 10^{11}).

In the case of several of the toxins the lethal dose is not very much greater than the figures given above. Osborne, Mendel and Harris (1905) found that the M.L.D. of ricin for a rabbit was 1γ per kilo. Koulikoff and Smirnoff (1927) found that the M.L.D. of diphtheria toxin for a guinea-pig was also about 1γ per kilogram. Marchmann (1931) found that the M.L.D. of a purified tetanus toxin for mice was about 3γ of dry substance per kilogram.

Certain Extreme Figures found for Active Drug Dilutions

The figures given for minimum doses and concentrations producing a measurable response are surprisingly small. The number of molecules in a gramme-molecule is however so enormous ($6{\cdot}4 \times 10^{23}$) that these minimum figures represent huge populations of molecules. For example, a dilution of 1 in 10^{15} corresponds to more than 100,000 molecules per c.c. These dilutions mentioned in this paper bear no relation to the homœopathic dilutions. Hahnemann, for example, claimed that drugs at the 30th potency produced reliable effects, and actions produced by similar dilutions are still occasionally described (e.g. König, 1927). A homœopathic potency means a dilution one hundredfold, and hence the 30th potency corresponds to a concentration of 1 part in 10^{60}. This works out at about one molecule in a sphere with a circumference equal to the orbit of Venus. Such results may be either believed or disbelieved, but their acceptance involves discarding the fundamental laws of chemistry and physics.

Other results have been published which are almost equally improbable. For example, botulinus toxin is undoubtedly a very powerful poison, and several observers agree that the M.L.D. of a potent solution for a guinea-pig is less than 0·000,1 c.c. per kilo. Bronfenbrenner and Schlesinger (1922), however, found that the M.L.D. was 10^{21} c.c. per guinea-pig, and this would scarcely suffice to provide a molecule of toxin per animal. This fact was pointed out by Newton Harvey (1924), who drew attention to the extraordinary difficulty in freeing measuring apparatus from traces of adsorbed drugs. It has been the writer's custom when working with high dilutions of drugs to wash out all pipettes with fuming nitric acid at frequent intervals. Unless some precaution such as this is taken the

results obtained with high dilutions are of little value. The amount of washing needed to cleanse a pipette can easily be seen by filling a pipette with saturated trypan blue and then measuring the number of washes needed to cleanse it.

There are other figures which are not absurd but must be considered doubtful. Ahlgren (1926) and Euler (1930) describe adrenaline as influencing the oxygen uptake of tissues at a concentration of 1 part in 10^{14}, and give similar figures for thyroxin and for insulin. Other workers have failed to confirm these dilutions ; for example, Kisch and Leibwitz (1930) found that the limit of demonstrable activity was 1 in 10^8. Similarly Krawkow (1923) stated that adrenaline at a dilution of 1 in 10^{16} produced constriction in the perfused rabbit's ear, but Kopp and Mancke (1930) found that the limit of demonstrable activity was 1 in 10^8. Schlossmann (1927) concluded that adrenaline at a dilution of 1 in 10^{18} could produce an effect on a frog's heart partly poisoned with aconitine.

The Number of Molecules present in Minimum Doses

The minimum effective concentrations and doses described above are surprisingly small even if doubtful figures be disregarded. In the case of the two lowest figures given in Table VII, namely, the doses of acetyl choline and adrenaline, the drugs were given intravenously and produced an immediate and transient action. These figures should therefore be calculated as the probable concentrations produced in the bloodstream, rather than as the dose per kilo. of body weight. If it be assumed that the drug is mixed with half the volume of the blood, then the minimum effective concentration in these two cases is of the order of 1 in 10^{12}. This is about one-hundredth of the minimum concentration known to produce an action on an isolated organ. This dilution of one part in 10^{12} contains, however, about 10^8 molecules per c.c. and the actual dose administered, namely about $10^{-5}\gamma$, contains about 10^{10} molecules. The fact that a dose of this magnitude produces a pharmacological response does not therefore involve any special physico-chemical difficulty.

In the case of vitamins it is possible that the active principle acts on most of the cells in the body, and therefore it is of interest to consider the number of molecules available per cell.

Parker (1930) estimated the number of cells in the body of

a man weighing 70 kilo. as 26×10^{12}. This corresponds to an average size of cell of 2700 cu. μ, or about 3×10^{11} cells per kilo. If the molecular weight of calciferol be taken as 400, then $0{\cdot}1\gamma$ contains 10^{14} molecules. Hence a daily dose of even $0{\cdot}1\gamma$ per kilo. will supply 100 molecules to every cell in the body.

Calculations of this nature can only provide very rough approximations, because on the one hand the active principles are probably selectively fixed by the tissues on which they act, and on the other hand they are fairly rapidly broken down by or excreted from the body.

The organs of the special senses can record very small physical or chemical changes, and hence it is of interest to consider the smallest number of molecules that can produce a sensation. The minimum concentrations of drugs that can be detected by smell have already been discussed, and it was shown that although these concentrations are extremely weak yet they contain a large number of molecules per cubic centimetre. In the case of vision it is believed that the primary effect produced by light in the retina is to cause a breakdown of some photosensitive substance (cf. Chapter XII), and the amount of photosensitive material activated by a minimal effective light stimulus acting on the human retina must be extremely small. Snyder (1931) considered this problem and concluded that the light energy entering the retina from the feeblest visible flash was $2{\cdot}3 \times 10^{-10}$ ergs, and that this would activate about 60 molecules. The smallest area that must be stimulated to give a light perception he took as two rods, and calculated their volume as 188 cu. μ. If one-tenth of this volume were occupied by the solution of photosensitive material, then the concentration produced by the 60 activated molecules would be about 5×10^{-14} molar. These results suggest that the product of light activation must be more potent than any known hormone.

This general consideration of the smallest quantities of drugs that can produce an effect on body tissues shows that the minimum effective concentrations and quantities are extraordinarily small. Indeed their expression involves the use of figures of an unfamiliar order of magnitude. If, however, these figures are expressed as the number of molecules

present, then the results show that there is no necessity to assume special laws of chemistry or physics in order to explain the effects observed.

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CHAPTER III

THE FIXATION OF DRUGS BY CELLS

Nature of Cell Structure

In most cases the site in the cell at which drugs act is unknown, but in a certain number of cases there is definite evidence that they act on the plasmatic membrane. This makes it necessary to discuss this membrane, and to adopt some hypothesis regarding the structure of the cell.

The numerous unsolved problems connected with this subject are discussed fully in works such as those by Höber (1924) and Gray (1931). There is, for example, no general agreement as to whether cell protoplasm is to be regarded as a solid or a fluid. Gray concludes (p. 97) :

> When we attempt to picture the facts of cytoplasmic differentiation in physical terms, the difficulties are overwhelming. . . . It seems more rational to regard living material as a state of matter where the constituent molecules are organized in a way quite unknown in the inanimate world, and which will only be elucidated by methods of analysis which have yet to be discovered.

These remarks can be applied with equal truth to the properties of the cell surface. For example, the muscle-cells of a vertebrate contain much more potassium than sodium, whilst the reverse is true of the fluids which surround them. The evidence obtained from the study of the electrical conductivity of cells indicates definitely that the ions in the cell are in true solution. This conclusion has been fully confirmed by Hill (1931), by studies of the osmotic pressure and the vapour pressure of muscles and other tissues. He concluded from his work on vapour pressure (p. 53) : " The fundamental point, therefore, brought out by these experiments is that nearly the whole of the water of muscle is ' free ' in the sense that it can dissolve in a normal manner substances added to it."

Micro-injection experiments also have shown that protoplasm

is freely miscible with water, for Chambers and Reznikoff (1926) have shown that when large volumes of water are injected into amœbæ the water immediately diffuses throughout the cell.

The evidence available indicates therefore that the cell interior contains a solution of salts in water, and that these salts are in true solution and are not adsorbed or combined, but are capable of conducting electricity and of exerting osmotic pressure. Since this salt solution inside the cell is totally different in composition from the salt solution in the tissue fluids, the cell membrane must form a barrier impermeable to the passage of many salts. On the other hand, if the tissues are perfused with solutions containing rubidium, the potassium in the cell is partly replaced by this element. The cell surface must therefore be to some extent permeable to these ions, and exchange of ions can occur fairly rapidly.

This example is typical of the complexity of the evidence regarding the selective permeability of the cell surface. Some of the phenomena can be imitated in systems composed of dead membranes, but our present knowledge of physical chemistry is inadequate to explain the general behaviour of the surface of a living cell. Hill (1931, p. 79) concluded : " Throughout we are involved, not with genuine equilibria, but with conditions maintained constant by delicate governors and by a continual expenditure of energy. How that energy is supplied, how it is utilized to maintain the structure and the organization, is, I think, the major problem of biophysics."

The following assumptions regarding cell structure are made in this volume.

The protoplasm in the interior of the cell is known to have a complex structure, it contains a nucleus and may contain a variety of granules, globules of fat, etc. These complications can however be ignored because nothing is known of the manner in which the internal structure of the cell influences the effect of drugs after these have entered the cell. The interior of the cell can therefore be treated as a homogeneous protein-water phase, although it is certain that it is in reality more complex.

There is a large variety of evidence that indicates that a drug may either act on the surface of the cell, or may enter the cell and act on the interior, and that the effects produced

differ in the two cases. We must therefore assume that the activity of the cell can be modified by the fixation of a drug on the cell surface. The simplest physico-chemical explanation for the possibility of such an occurrence is to assume some form of orientation of the cell protoplasm, although it is difficult to reconcile this view with the conception of the cell contents as a fluid and freely moving water-protein phase that has already been outlined. A theory recently put forward by Peters (1930) suggests that the cell surface is a more or less rigid surface film, and that the proteins in the interior of the cell are orientated on the surface so that they form a structure but yet are capable of movement.

It must be assumed that the surface membrane possesses selective permeability. As regards drugs this selective permeability shows very wide gradations. Some substances enter very rapidly, other substances enter slowly, whilst the membrane is completely impermeable to certain drugs.

Relation between Drug Fixation and Action

The diagrammatic representation of the cell outlined above is the simplest representation that will permit the explanation of the facts of drug action. The most important postulate is that a drug may either act on the surface of the cell or may enter the cell interior or may do both, and in the last case its action on the cell surface may be different from its action in the cell interior.

This conception is of fundamental importance in the quantitative study of drug action. The first step in quantitative pharmacology is to relate changes in drug concentration with effects produced on the cell. This, however, is only the first step, and the next problem is to relate changes in drug concentration with the amount of drug fixed by the cell. In some favourable cases both these relations can be obtained, and the question then arises whether we can then state that a certain quantity of drug fixed produces a certain action. Such a calculation will however only be significant if it is probable that all the drug fixed by the cell partakes in producing the action observed. The evidence available suggests that this is not the case. The cell is a complex structure and the effect produced by the drug depends upon the portion of the cell on which it is fixed. In many cases a drug appears to produce

its effect by reacting with the cell surface and any drug that enters the cell interior has little action. There is moreover no reason to suppose that the cell surface is uniform and probably the effect produced by a molecule depends on the nature of the receptor on the cell surface on which it happens to be adsorbed.

Drugs can be shown to produce different actions according to whether they act on the cell surface or enter the cells by the following experimental methods. (i) Micro-injection experiments; (ii) quantitative estimates of drugs fixed on cell surface and entering cells; (iii) measurements of the rate of action of drugs; (iv) measurements of the rate of washout of drugs; (v) experiments showing the existence of active patches.

Micro-injection Experiments

The technique of micro-injection of drugs into amœbæ as developed by Chambers has permitted the comparison between the action of drugs applied outside the cell and their action when injected into the cell interior. The results show that the actions frequently are different in the two cases. Reznikoff and Chambers (1924) studied the effects of salts on amœbæ. They found that NaCl and KCl were the more toxic on external application, whilst $CaCl_2$ and $MgCl_2$ were the more toxic on micro-injection. The effects produced were moreover different with external and internal application. The presence of calcium in the suspension fluid is essential for the reformation of the plasmatic membrane of the amœba (Chambers, 1926, and Chambers and Reznikoff, 1926). The injection of calcium into the cell causes, on the other hand, solidification of the surrounding protoplasm. Hiller (1927) showed that amœbæ were paralysed by immersion in narcotics but that injection of these narcotics into the amœbæ produced no narcotic effect but caused a local coagulation of the protoplasm. Brinley (1928, A and B) showed that immersion in hydrogen cyanide paralysed amœbæ but that the drug when injected into the amœba produced no more effect than did the injection of distilled water. He obtained (1928 C) similar results with solutions of sulphuretted hydrogen. Reznikoff (1926) found that soap solutions caused disappearance of the plasmalemma of amœbæ and that this result was produced more readily by immersing

the amœba in the solutions than by injecting the solution into the amœba.

The case of *Valonia* which contains a large internal vacuole is somewhat different. Jacques and Osterhout (1929) showed that manganese chloride was more toxic when injected into the vacuole than when applied externally. It is however scarcely legitimate to compare the injection into the vacuole with an injection into cell protoplasm, and it seems fairer to regard the cell protoplasm of *Valonia* as having two surfaces, one external and one internal.

These results indicate that drugs such as ions, narcotics, cyanides, etc., exert their specific actions on the surface of cells such as amœbæ, and not upon the interior of the cells, for only thus can we explain the fact that the drugs do not produce their typical effects when injected.

Action of Drugs on Cell Surface and Interior

The action of potassium salts on the isolated frog heart provides a particularly clear example of the fact that a drug may produce completely different effects according to whether it is outside or inside a cell.

Frogs' muscles, both cardiac and skeletal, appear to contain about 0·3 p.c. KCl, and function normally in a perfusion fluid containing 0·015 p.c. KCl. The investigations of Hill (1931) make it certain that the salt inside the cells is in true solution.

The frog's heart is in equilibrium when bathed in a fluid containing 0·015 p.c. KCl. Increase of this external concentration to 0·09 p.c. causes profound depression of the heart in half a minute (De, 1928). On the other hand, decrease of the potassium content to about 0·004 p.c. KCl produces a typical change in the heart's action in a few minutes (Clark, 1926).

The excess of potassium inside the heart cannot therefore compensate for any sudden deficiency on the outside. The potassium in the cell contents can, however, leak out slowly, and does so when the heart is perfused with potassium free fluid. For example, a heart loses some 30 p.c. of its potassium content when perfused with potassium-free Ringer fluid for a few hours (Clark, 1922). If a heart is depressed by perfusion with potassium-free fluid and is then allowed to remain in contact with a small volume of fluid, it gradually recovers,

because the potassium leaks out of the cells and raises the external concentration. Moreover, a heart depressed by perfusion with potassium-free fluid is restored in a few seconds by perfusion with normal Ringer's fluid. This effect is too quick to permit of the entrance of potassium into the cells, and the probable explanation is that the function of the heart is dependent on the concentration of potassium in the fluid surrounding the cells and is to a certain extent independent of the amount of potassium within the cells.

All these effects can be easily explained if it be assumed that the cell membrane prevents any rapid passage of potassium and that the potassium in the perfusion fluid is in equilibrium with some easily dissociable compound that is located on the outside of the impermeable cell membrane.

Chase and Glaser (1930) studied the action of acids on paramœcia and concluded that these produced a double effect ; firstly a rapid surface action which caused excitation, and secondly a slowly developed inhibition that was produced by the acid penetrating the cell.

Experiments by Cook (1926) on the action of methylene blue on the frog's heart show very clearly that this dye acts on the cell surface and also enters the cell, but that one of its pharmacological actions is due entirely to the action of the drug on the cell surface. Methylene blue has a powerful atropine-like action on the frog's heart, and a heart perfused with methylene blue is dyed deeply. No measurable quantity of dye can be removed from the heart by washing out with Ringer's solution, but such washing out immediately abolishes the atropine-like action. Hence the pharmacological action of the dye can be abolished when the heart is deeply stained with the dye. On the other hand, the atropine-like action is produced rapidly whilst the dyeing of the heart proceeds slowly, and hence the atropine-like action can be demonstrated before there is any visible dyeing of the heart.

These experiments show clearly that two processes occur when a frog's heart is exposed to methylene blue. Firstly a pharmacological action which is probably a surface action, and secondly an entrance of the dye into the heart cells. The two processes appear to be completely independent of each other.

In considering this experiment it must be remembered that dyes when they enter a cell frequently do not mix with the protoplasm but accumulate into discrete granules; this happens particularly in the case of basic dyes (Chambers, 1928). These granules really represent foreign bodies, for in the case of indicators their colour does not measure the *p*H of the surrounding protoplasm.

The possibility that a dye or drug after entering a cell may be encysted as a granule supports however the general argument that the amount of action produced by a drug on a tissue is not necessarily proportional to the amount of drug fixed by the tissue.

The experiments of Cook show that even when time is allowed for equilibrium there is no necessary direct relation between the amount of drug entering the cell and the amount of cell response. This divergence between drug entry and cell response is much greater in the case of any actions in which time for equilibrium is not allowed, for it will be shown later that the rate of entrance of drug and the rate of cell response are two independent variables that often differ widely.

The Rate of Action of Drugs

The frog ventricle has a sponge-like structure and hence drugs normally take a considerable time to diffuse through its interstices. If, however, a strip of ventricle be taken and the drug solution applied as a fairly powerful jet, this lag due to diffusion is abolished, and since the network of the sponge-like trabeculæ of the ventricle is very fine, this tissue is an exceptionally favourable one on which to estimate the rate of drug action.

The following figures have been obtained with this method in the author's laboratory for the time of half action of various cardiac depressants.

Lack of calcium	2–3 seconds	
Excess of potassium	10 seconds	
Acids (*p*H 5–6)	2–3 minutes	(De, 1928)
Ethyl alcohol	1·5–5 seconds	(Pickford, 1927)
Butyl chloral hydrate	2 minutes	
Acetyl choline	1·5–4 seconds	(Clark, 1927)

The results fall into two groups: the drugs that act in a few seconds and those that act in a few minutes. It seems very

improbable that the rapid-acting drugs can penetrate the cell membrane in a few seconds. In the case of potassium cell penetration certainly does not occur, Similarly, lack of calcium does not cause any rapid depletion of the cell calcium, for the heart recovers immediately after being exposed to calcium lack for hours, and therefore the effect in this case is probably exerted on the surface and not on the interior of the cell. In the case of alcohols and narcotics there is evidence that equilibrium between the drug in the blood stream and the drug in the tissues is attained slowly. For example, Tissot (1906) showed that equilibrium was not fully established in a dog after it had been exposed to a constant concentration of chloroform for 8 hours.

Delay in the appearance of the cell response to a drug may be due to an indefinite number of causes. For example, it has been shown (Clark, Gaddie and Stewart, 1932) that the time required for lack of oxygen to cause arrest of the frog's heart depends on two independent variables, namely the reaction of the perfusion fluid and the amount of carbohydrate available. The heart can only obtain energy by the breakdown of carbohydrate to lactic acid. The acid poisons the heart unless it can be excreted, and the excretion depends on the reaction of the perfusion fluid, for it can occur with alkaline but not with neutral solutions. Hence the time taken for lack of oxygen (and probably also for cyanide) to produce arrest may vary from a few minutes to many hours.

In a few cases it is possible to determine the cause of delay in drug action. For example, in the case of the cardiac glucosides it is known that these are rapidly fixed by the cells and the delay occurs between drug fixation and cell response.

The Rate of Washout of Drugs

In the case of drugs that produce a reversible action on cells, this action disappears when the cells are changed to a drug-free solution. In the case of any one drug acting on a particular cell population the rate of recovery presumably depends on the time taken for the drug to diffuse out from the cells. If the volume of fluid around the cells is large, the concentration of drug washed out is negligible and the only variable is the amount of drug in the cells at the moment

that the washout commences. The times taken for recovery ought therefore to indicate the relative amounts of drug in the cells.

The author (1930) studied the rate of washout of alcohols from the frog's heart, and found that when equal effects were produced by a short exposure to a high concentration, or by a long exposure to a low concentration, the rate of recovery was quicker in the former case.

For example, a 95 p.c. inhibition of the ventricle was produced by both 0·5 min. exposure to a 0·5 m. molar octyl alcohol and by 10 min. exposure to a 0·25 m. molar solution. On washing out half-recovery occurred in the first case in 13 sec. and in the second case in 120 sec.

It has been already shown that the rate of commencement of the action of alcohols is so great that it is probable that their action is exerted on the cell surface. The simplest explanation of these washout experiments is that alcohols rapidly act on the cell surface and also enter the cell more slowly, and that the cell-surface receptors can be filled by the drug either from solutions surrounding the cell or by drug contained within the cell. In the case of high concentrations of alcohol the time of action was too short to permit drug entry, and hence recovery on washout was rapid. In the case of weak concentrations there was time for the alcohol to enter the cells, and hence recovery was a much slower process and was not complete until the cells had been cleared of the alcohol.

The Existence of Active Patches on the Cell Surface

Warburg (1929) concluded from experiments on birds' erythrocytes and sea-urchin eggs that oxygen consumption was dependent on the presence of a minute quantity of iron compound in the cells. His general conception of the process of oxygen uptake was that it occurred at certain molecules present in the stroma or on the surface of the cells, and that cyanides and narcotics inhibited respiration by putting these molecules out of action.

Warburg's views regarding the importance of iron have been criticized by Dixon (1929) and by Keilin (1929), and it is probable that other receptors are at least as important as iron. This problem is discussed in Chapter XI. There is,

however, general agreement with the conception that oxygen is fixed by certain specific molecules in the cell, that there are several different varieties of receptors, and that these constitute a very small proportion of the total cell substance. Hence respiration can be inhibited by very small quantities of a drug such as cyanide, which paralyses these receptors.

Quastel (1930) has shown that the oxidations carried out by bacteria occur on the cell surfaces and that they are carried out on specific centres on the cell surface. His evidence suggests that at least twelve varieties of specific centres occur on the surface of B. coli.

This theory also accords with the results obtained with powerful drugs, for in many cases the amount of drug required to produce a strong action on a cell is much too small to form a monomolecular layer over the whole cell surface.

The cell surface cannot therefore be pictured as a uniform structure but must be regarded as a mosaic. This introduces another difficulty into quantitative pharmacology, for even in the case of drugs that act wholly on the cell surface it cannot be assumed that the whole of the drug fixed produces equal actions.

The Nature of the Reaction between Drugs and Cells

The evidence presented in Chapter II showed that even in the case of the most powerful drugs the minimal effective dose was large enough to provide a considerable number of molecules for every cell upon which the drug acts. In the case of a number of powerful drugs with specific actions it can be shown that the drug probably acts on the surface of the cell, but that the number of molecules fixed by the cell is too small to cover more than a fraction of the surface.

In the case of acetyl choline acting on a heart-cell, the relative sizes of the cell and the molecule are as 10^{12} is to one. This is about the relation between a large whale and a small midge. The problem is to form some picture of cell structure that will explain the fact that a few thousand of these molecules when they unite with the cell suffice to modify its functions.

We are practically obliged to postulate active patches on the cell surface on which such functions as oxygen uptake and contraction depend. There is a considerable body of

evidence in favour of the hypothesis that oxygen uptake is dependent on a variety of active patches that only occupy a small proportion of the cell surface. As regards contraction it may be pointed out that most of the well-known depressant agents produce their action on the mechanical response far more rapidly than does oxygen lack. Therefore these drugs must depress the contraction process directly and not secondarily by interference with the oxygen uptake. This suggests that the functions of the cell other than those of division are controlled by the cell surface.

Peters (1930) has advanced a theory of cell structure that agrees fairly well with the pharmacological evidence. He pictures the cell as composed of a three-dimensional protein mosaic, with the molecules in the interior of the cell orientated on the surface film. Since the interior of many cells is known to be fluid the structure must be regarded as an orientation rather than as an anatomical skeleton, and this orientation is assumed to regulate the chemical processes that occur within the cell.

This conception of cell structure makes it possible to imagine how a drug by reacting with a few molecules on the cell surface can alter the activities of the cell by changing the surface pattern and thus affecting the orientation of the interior of the cell.

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CHAPTER IV

PHYSICO-CHEMICAL LAWS APPLICABLE TO CELL-DRUG REACTIONS

It has been shown that a suspension of even the smallest living cells constitutes a highly complex heterogeneous system. The ordinary laws of mass action are based on the chances of collision between molecules moving at a particular velocity, and it is obvious from theoretical considerations that these laws cannot be expected to apply to heterogeneous systems. This fact was pointed out long ago by Nernst (1916, p. 622), in the following terms :

" Since, according to this, in chemical reactions which occur merely at the boundary between two phases, the phenomenon is essentially one of diffusion, it is useless to try to determine the order of the reaction from the rate at which they proceed, as has often been attempted in recent years ; this method of argument is only applicable, according to kinetic considerations, to the probability of collisions in homogeneous systems, and loses its meaning when applied to heterogeneous systems."

This fact requires emphasis because Arrhenius (1915) lent his great authority to the idea that reactions between cells and drugs could be interpreted by the simple laws of mass action. Arrhenius performed a great service to biology by drawing general attention to the need for quantitative analysis of data, but his suggestion that reactions in complex heterogeneous systems could be interpreted by the simplest laws applicable to homogeneous systems had an unfortunate effect on the future development of the subject.

Knowledge regarding the physical chemistry of heterogeneous systems has advanced greatly since 1915, when Arrhenius put forward his views, and to-day it is scarcely necessary to argue the point that reactions between drugs and cells must be interpreted by the laws that are known to be applicable to

heterogeneous systems, and that a living cell is unlikely to behave in a simpler manner than does, for example, a solution of gelatine.

Formulæ expressing Adsorption Equilibria

The best-known adsorption formula is that popularized by Freundlich, which has the form $KC^{\frac{1}{n}} = \frac{x}{m}$ (C = concentration of solute; x = amount fixed and m = mass of adsorbent; K and n are constants; n is always greater than 1 and usually is less than 3).

According to Brownlee (1924) this formula has an honourable history of highly varied utility. It was introduced in 1867 by Harcourt and Esson to express the rate of chemical reaction between hydrogen peroxide and hydriodic acid. In 1875 Dr. Farr showed that it represented closely the association of the death-rate with the density of the population. In 1897 Pareto recommended it as a gradation formula in the statistics of the distribution of wealth. Unfortunately no theoretical basis has been supplied for the application of the formula to adsorption.

It will be seen that the formula can be transposed to the form $\log K + \frac{1}{n}(\log C) = \log \frac{x}{m}$, and hence if log C is plotted against $\log \frac{x}{m}$ a linear relation is obtained.

In the true adsorption formula $\frac{1}{n}$ is less than unity and very frequently has a value near 0·5, but there is a very similar formula that may be written $Kx^{n'} = y$, which expresses the differential solubility of a substance between two solvents a and b, when the substance is in molecular dispersion in solvent a, but forms aggregates of n' molecules in substance b. In this case x = concentration in a, and y = concentration in b. Similarly this formula would express a polymolecular reaction in which n' molecules of one substance united with one molecule of another substance.

Adsorption and differential solubility can therefore account for three types of relations between drug concentration and drug uptake by cells.

1. *True adsorption.*

The quantity of drug entering the cell varies as some power of the concentration less than unity.

2. *Simple differential solubility.*

The quantity of drug entering the cell varies as the concentration.

3. *Differential solubility and polymerization.*

The quantity of drug entering the cell varies as some power of the concentration greater than unity.

Langmuir's Theory of Adsorption.

The chief objection to Freundlich's empirical formula is that it implies that there is no limit to the amount of drug that can be fixed. This is undoubtedly incorrect and in practice it is usually admitted that the formula does not hold for high concentrations of drugs. Langmuir put forward a series of formulæ to interpret certain cases of adsorption, which account more exactly for many of the phenomena observed in pharmacology and are based on a few simple principles. These formulæ apply only to freely reversible processes. Although drugs often produce irreversible effects on cells, yet in many such cases there is evidence that the uptake of drug by the cells is a reversible action and that the irreversible effect is due to secondary actions, moreover the majority of the most interesting drug actions are reversible effects. Hence these formulæ can be applied legitimately to a large number of cases.

The simplest case is when a substance is present in large excess so that the amount fixed does not produce a significant change during the reaction and when one molecule of drug is fixed by one receptor on the surface. In such a case the rate of fixation of the drug is directly proportional to its concentration and to the number of unoccupied receptors on the surface. The rate of dissociation is directly proportional to the amount of drug fixed or to the number of receptors on the surface that are occupied.

If $x =$ concentration of drug, $A =$ total number of receptors and $y =$ receptors occupied.

Then the rate of combination $= K_1x(A - y)$ and the rate of dissociation $= K_2.y$.

Equilibrium occurs when combination and dissociation are equal and hence

$$K_1x(A - y) = K_2y \text{ or } Kx = \frac{y}{A - y}.$$

The relation between concentration and fixation is more complex when one molecule of drug splits into two portions which occupy two receptors, and the two portions must combine before dissociation can occur. Then the rate of combination $= K_1x(A - y)^2$ and the rate of dissociation $= K_2y^2$.

Hence equilibrium occurs when

$$Kx = \frac{y^2}{(A - y)^2}, \text{ or } Kx^{\frac{1}{2}} = \frac{y}{A - y}.$$

The chief disadvantage of Langmuir's formulæ is that even the simplest of them provides a curve that can be fitted for considerable stretches by an embarrassingly large number of other formulæ. In the case of the formula $Kx = \frac{y}{100 - y}$, the relation between x and y is nearly linear when y varies from 0 to nearly 20 p.c. of its maximum value. Secondly, there is a nearly linear relation between $\log x$ and y between 20 and 80 per cent. of the maximum value of y. Finally if $\log x$ be plotted against $\log y$, a nearly linear relation is obtained between 5 and 70 p.c. of the maximum value of y. It is therefore practically impossible to prove that the relation between concentration and fixation of drug follows Langmuir's formulæ unless it is possible to study the whole range of relations from 0 to 100 p.c. of the possible values of y.

If only a portion of the curve is obtained this may appear to follow either a simple linear relation (Henry's law), a logarithmic relation (Weber-Fechner law) or Freundlich's adsorption formulæ. Fig. 1 shows how closely the hyperbola obtained from Langmuir's formula approximates to the last mentioned two formulæ.

The relation between drug concentration and drug action is likely to be influenced by the ratio between the concentration that produces maximum action and the concentration that produces maximum drug fixation. There is no reason why these two figures should be identical, and in many cases it can be shown that a maximum physiological action is produced long before the cell is saturated with the drug.

Sumner and Myrbäck (1930) concluded that the ferment urease could fix ten times as much silver as was necessary to inactivate it, and they suggested that the enzyme was a large molecule but that its activity depended on a few receptors. This theory closely resembles the " active patch " hypothesis developed in the case of solid catalysts for very similar reasons. Ponder (1923) found that hæmolytics continued to be fixed by the debris of erythrocytes long after lysis had been completed.

It is possible therefore that in two cases in which there is a similar chemical reaction between drugs and cells the relation between drug concentration and physiological response may appear to be different. In one case the end-point of the physiological response may occur long before the chemical reaction approaches completion, and in the other case the two end-points may coincide. Some of the differences observed in the concentration-action curves obtained with narcotics appear to be due to differences of this nature. (*Cf.* curve B, Fig. 31, and curve A, Fig. 32.)

Finally, it must be remembered that " no single equation, other than purely thermodynamic ones, should be expected to cover all cases of adsorption, any more than a single equation should represent equilibrium pressures for all chemical reactions " (Garner, 1926). These remarks were made in reference to adsorption in simple inorganic systems and apply with still greater force to adsorption by living cells.

Selective Adsorption

Although the remarkable selective actions produced by many drugs and by all toxins cannot be paralleled in inorganic chemistry, yet selective adsorption is often obtained with quite simple systems. The following examples quoted from McBain (1932) show the variations that may occur in the behaviour of a substance such as charcoal, which represents a system infinitely simpler than the simplest living cell. Barker (1930) showed that activation of charcoal might increase the sorption of carbon tetrachloride one hundredfold. Ruff and Roesner (1927) found that activation increased the sorption of gases such as CO_2 and NH_3 to fivefold, but it increased the sorption of phenol as much as fiftyfold. Briggs (1921) studied the sorption of nitrogen and hydrogen by various

forms of charcoal, and his figures show that in the case of two charcoals from different sources one may adsorb hydrogen better and nitrogen worse than the other.

These examples show that the adsorptive activity of a charcoal surface is altered profoundly by activation by heating. Not only is the general adsorptive power increased but qualitative differences are produced so that the adsorption of one substance may be increased far more than is the adsorption of another substance. This provides an interesting inorganic model for the selective adsorption of drugs.

Active Patches

Measurements of the quantities of drugs that suffice to produce an action on cells, prove that in the case of powerful drugs the amount fixed is only sufficient to cover a small fraction of the cell surface. The speed with which drugs act and the slowness with which drugs penetrate cells indicates that many drugs probably act on cell surfaces.

The simplest probable conception of drug action is that potent drugs occupy certain specific receptors on the cell surfaces, and that these specific receptors only comprise a small fraction of the total cell surface.

The study of contact catalysis has in recent years provided evidence that even in simple inorganic systems chemical reactions may be due to certain active patches on a surface and that these active patches only constitute a very small proportion of the total surface. Armstrong and Hilditch (1922) showed that in some cases the quantity of poison that sufficed to arrest the activity of a catalyst was much less than the amount needed to cover the whole surface of the catalyst with a monomolecular layer. A good example of this fact is provided by the results of Pease and Stewart (1925). They found that a certain copper surface could adsorb strongly 5 c.c. of carbon monoxide, but that the adsorption of 0·05 c.c. of carbon monoxide reduced the catalytic activity of the surface by 90 p.c. Therefore 90 p.c. of the catalytic activity of the copper surface was due to certain active patches which constituted less than 1 p.c. of the total surface.

The conception of active patches, which was introduced to general recognition by Taylor (1925), is now fairly firmly established. It postulates that the surface of a contact

catalyst is not uniform but contains certain active molecules and that the catalytic action is due to these active molecules. The proportion of the total surface occupied by active patches varies in different systems from 1/100 to 1/100,000. For example, Fromherz and Menschick (1929) found that fluorescent substances were adsorbed selectively by imperfections on crystal surfaces which represent only 1/10,000 to 1/100,000 of the total surface.

There is further a large volume of evidence that shows that several different varieties of active patches may be present on the surface of some simple inorganic catalysts. Rideal (1926A, p. 141) stated: "In catalytic investigations a still more marked differentiation in the adsorptive capacities of various parts of the surface must be presumed. Thus in the combination of ethylene and hydrogen on a nickel surface we find that the fraction of the surface area which is catalytically active is of the order of 10^{-4}. There are thus areas of highly reactive localized patches which can adsorb and cause reactions in these gases, there are other less active areas which can adsorb both gases but fail to bring them into reaction, and there exist still less active areas which can adsorb only ethylene but not hydrogen."

Rideal and Wright (1926) studied the oxidation of oxalic acid by blood charcoal. Two types of active centres, Fe-C and C-C, were found. The former, which was 57 times as active as the latter, was selectively poisoned by HCN. Poisoning experiment indicated that the Fe-C complexes occupied 0·2 p.c. of the charcoal surface. Balandin (1926) put forward the conception that different points adsorbed different groups of the adsorbed molecule and hence the molecule might get pulled in half, and he suggested that catalysis depended on this effect.

It appears to the writer that the phenomena observed in regard to active patches in contact catalysis resemble those observed in the case of specific drug actions far more closely than do any other phenomena in inorganic chemistry.

The following are certain points established as regards the action of contact catalysts that suggest analogies to the action of drugs on cells.

1. The activity of the surface depends on the activity of

the patches which constitute a minute fraction of the whole surface.

2. On a single surface there may be patches showing several different types of activity. One type of patch selectivity adsorbs one chemical group and another type of patch adsorbs a different group.

3. The activity of a surface can be changed or abolished by minute amounts of poisons inhibiting the activity of some or all of the active patches.

4. The activity of a surface can be increased enormously by promoters. These are substances which are not themselves good catalysts but which increase the catalytic activity of surfaces.

The evidence quoted regarding the existence of active patches in charcoal is very strong, but in other cases the whole of the charcoal surface appears to adsorb in a fairly uniform manner. Ochrent studied the adsorption of organic acids by powdered charcoal and concluded (1932B) from measurements of the quantities adsorbed that about 1/25 of the total number of carbon atoms present in the charcoal were concerned in the adsorption. This result implies that not only is the total surface of the powdered charcoal enormous in proportion to its bulk, but also that adsorption occurs on the whole of the surface. Ochrent (1932A) also concluded that the adsorption of the different organic acids occurred on the same surface. This work indicates that in some cases the whole of a charcoal surface adsorbs in a fairly uniform manner. The conception of the adsorptive activity of charcoal depending on " active patches " appears therefore to be only true for certain forms of adsorption.

A contact catalyst such as charcoal may be regarded therefore in respect of its reactions to drugs as a greatly simplified model of the living cell. It appears certain that the reactions that occur in the living cell must be far more complex than those that occur on a charcoal surface, and if the former appear to be the simpler the simplicity must be apparent rather than real, and due to faulty methods of measurement and to ignorance. The general rule about heterogeneous reactions appears to be that their known complexity depends almost entirely upon the care and accuracy with which they have been

studied. Colloidal chemists have usually chosen the simplest possible systems for investigation, hence the more complex systems have been studied the least and therefore are interpreted by the simplest laws.

In general, it may be assumed that the living cell is such a complex system that only in the most favourable cases are we likely to succeed in establishing any certain laws regarding the mode of action of drugs.

The Fixation of Chemicals by Cells and Cell Constituents

The Forms of Equilibria observed

Most quantitative pharmacological experiments record the relation between the concentration of a drug and the action it produces. There is relatively little direct evidence relating the concentration of drug with the amount fixed by the cells, and hence such evidence must be supplemented by evidence regarding the uptake of drugs by such cell constituents as proteins, etc.

Herzog and Betzel (1911) studied the uptake of various drugs by yeast-cells and showed that the distribution of drug between solution and phase suggested three main types of processes.

1. Irreversible chemical reaction.
2. Adsorption.
3. Differential solubility.

The first process which was observed in the case of formaldehyde requires little comment. It is the usual result obtained in a chemical titration. The whole of the drug added is fixed until the substrate is exhausted and then any further excess of drug remains free in solution.

In some cases it can be shown that no reaction occurs until a certain critical concentration is reached, and then the reaction is completed at this concentration. For example, Walker and Appleyard (1896) studied the uptake of picric acid by solid diphenylamine. No picrate was formed until the concentration of the acid reached about 0·06 molar, but the reaction was completed at this concentration, and hence addition of picric acid did not raise the concentration in the solution until the diphenylamine present was saturated.

Adsorption. In many cases the uptake of drugs by cells or proteins has been found to follow a course approximating to Freundlich's adsorption formula $Kc^{\frac{1}{n}} = \frac{x}{m}$, which implies a linear relation between the logarithms of the concentration without and within the cells. The value of $\frac{1}{n}$ usually lies between 0·3 and 0·5. Relations of this type have been shown to occur in the case of heavy metals.

It is now recognized that many if not most cases of adsorption follow the formulæ put forward by Langmuir, the simplest of which is $kc = \frac{y}{100 - y}$. (y = drug fixed expressed as per cent. of the maximum.) This formula is based on the hypothesis that the adsorbed drug forms an easily reversible combination with a limited number of receptors on the surface of the adsorbent. McBain has shown however that in many cases the initial process of true adsorption is followed by slower processes and complex chain effects of this character are unlikely to follow Langmuir's formulæ accurately. Moreover, these formulæ assume that all the receptors on a surface have an equal affinity for the adsorbed drug. This is very unlikely to be true for cell surfaces.

The chances therefore are against any simple formula expressing accurately the relation between drug concentration and drug fixation by cells. The empirical formula of Freundlich tends to obscure minor errors and hence it has been very popular amongst biologists.

The Combination between Hæmoglobin and Gases. The uptake of oxygen and of carbon monoxide by red blood-corpuscles may be taken as an exceptionally simple example of the fixation of a drug by a cell.

This reaction has been studied with great accuracy by a large number of workers, and it is interesting to find that the relation between oxygen concentration and uptake is complex and varies under different conditions.

The uptake of oxygen by hæmoglobin, when the latter is dissolved in water in the absence of salts, follows accurately a right-angled hyperbola. This is the expected theoretical result. The oxygen is present in excess and the combination

formed is freely reversible. Hence the rate of fixation varies as the oxygen concentration and the amount of reduced hæmoglobin, whilst the dissociation varies as the amount of oxyhæmoglobin. Hence the reaction is expressed by the formula $Kx = \frac{y}{100 - y}$ where x = concentration of oxygen and y = p.c. of total hæmoglobin converted into oxyhæmoglobin. This formula also expresses the uptake of carbon monoxide by hæmoglobin.

When, however, the hæmoglobin is dissolved in a salt solution the uptake of oxygen follows the formula $Kx^n = \frac{y}{100 - y}$ and the value of n is usually about 2·5. Hill (1910) explained this on the assumption that in the presence of salts the hæmoglobin molecules are aggregated and that the value n indicates the average number of molecules per aggregate. The fact of chief importance for the present discussion is that the characteristic curve of this extremely simple system can be altered by a very slight change in the condition of the hæmoglobin.

Differential Solubility. If the uptake of a drug is determined by differential solubility then, according to Henry's law, the cell/water distribution coefficient should remain constant, unless the drug forms aggregates of molecules in one of the solvents. If in the latter case the molecular weight of the drug in the cell (concentration $= x$) is n times greater than that of the drug in the solution (concentration $= c$), then the distribution will follow the formula $Kc^n = x$.

If, therefore, the drug uptake varies as some power of the concentration less than unity the process is probably adsorption, if the drug uptake varies directly as concentration we have a simple example of Henry's law, and if the drug uptake varies as some power of the concentration greater than unity, an aggregation of molecules is probably occurring.

In actual practice it is often very difficult to decide the factors regulating drug uptake. This is well shown by the example of the narcotics.

The Uptake of Aliphatic Narcotics. The alcohols and aliphatic narcotics constitute a group of chemically inert substances which are more soluble in lipoid solvents than in

water. The well-known Overton-Meyer theory of narcotic action was based on the fact that as a general rule the narcotics with the strongest action had the highest oil/water distribution coefficient ; this theory assumed that the uptake of narcotics by the cells depended on differential solubility.

Traube pointed out that the activity of narcotics varied as did their power of lowering the air/water surface tension, and this suggested that the activity of the group was dependent on the degree to which they were adsorbed in the cells. Warburg has produced a large mass of evidence in support of the theory that narcotics are adsorbed on surfaces in the cells.

This dispute has led to the careful investigation of the distribution of narcotics between solutions and cells. This evidence has been summarized by Winterstein (1926, pp. 248–53), who concludes that the evidence is doubtful whether the relation between concentration outside and inside cells is linear (i.e. follows Henry's law) or is an adsorption isotherm. In most cases the curves obtained have not deviated greatly from the linear, but have nevertheless shown a distinct curvature.

It is interesting to note that Bubanovic (1913) found that the benzole/water distribution coefficient for alcohol and chloral hydrate was not constant but fell from 11 to 8 and from 13 to 7 respectively as the concentration was increased. He explained this by assuming that these substances formed complex molecules in benzole.

The adsorption of alcohols by charcoal has been studied carefully and it is interesting to note the variety of results that have been obtained with this simple system.

Garner, McKie and Knight (1926) found that there was a linear relation between the concentration of amyl alcohol and the amount adsorbed by charcoal, but that at a certain concentration there was an abrupt change. Rideal and Wright (1926) found the same relation between the concentration of amyl alcohol and the extent of the inhibition it produced on the power of charcoal to oxidize organic acids. There was a linear relation up to 80 per cent. inhibition, and at this point there was an abrupt change in the relation between concentration and action. Garner and Kingman (1929) in a later work with improved methods found, however, that the rela-

tion between concentration and adsorption was not discontinuous nor linear, but followed the formula $KC^{\frac{1}{n}} + \frac{x}{m}$. This is the usual adsorption formula, but in this case $\frac{1}{n}$ equals 2, which is a value quite atypical of ordinary adsorption and suggests that the drug is polymerized on the surface.

Warburg (1921) measured the power of various urethanes to inhibit the oxidation of cystin by blood-charcoal, and his concentration-action curves have an exponential form, although these same drugs show a linear relation between concentration and the inhibition of oxygen consumption by birds' erythrocytes.

These examples show how difficult it is to establish with certainty the relation between concentration of narcotic and its adsorption by such a relatively simple adsorbent as charcoal, and therefore it is not surprising that conflicting results have been obtained as regards the uptake of narcotics by cells.

The Overton-Meyer theory that narcotic uptake was determined by the differential solubility of the drug in water and in lipoids explained so many pharmacological facts that for a long time it received general acceptance. This theory is however definitely disproved by certain experiments.

Dorner (1910) found that the cell/water distribution coefficient of octyl alcohol was about 60 in the case of laked red blood-corpuscles of birds, but that after the stromata had been defatted the distribution coefficient was still 26. In the latter case the alcohol fixation could scarcely have been due to differential solubility and was probably due to adsorption.

Usui (1912) studied the uptake of thymol by intact birds' erythrocytes, by the stromata after laking, and by the defatted stromata. He concluded that the fixation of thymol was due to three factors, namely, differential solubility between the watery cell phase and the external solution, fixation by the soluble cell constituents and fixation by the insoluble non-lipoid cell constituents. These results indicate the complexity of the factors regulating the uptake of a narcotic by such a simple system as erythrocytes.

Moose and Roaf (1904 and 1906) studied the uptake of chloroform by serum and by tissue emulsions. They found

that the serum/vapour distribution coefficient was constant at low concentrations, but that at a certain concentration opalescence and precipitation of proteins occurred and the distribution coefficient rose.

Herzog and Betzel (1911) obtained a similar result with yeast, for their figures show that the amount of chloroform fixed by the cells varies as $(\text{conc.})^{1.15}$.

The uptake of chloroform by serum and by yeast-cells does not follow Henry's law, nor is it a case of simple adsorption. The distribution appears to depend on differential solubility until a certain concentration is attained, but above this point the distribution coefficient rises, and this change appears to be associated with the fact that chloroform begins to cause an alteration in the colloidal condition of the serum or the cells.

Loewe and Moljawko-Wyssotzki (1926) studied the uptake of narcotics by muscle- and nerve-pulp. They noted that the narcotics caused a swelling of the tissues and that it was necessary to correct this source of error. Their results indicate an adsorption process in the case of chloral hydrate, and the figures agree with the adsorption formula with a value for $\frac{1}{n}$ of about 0·5. In the case of bromal hydrate their figures approximate to a simple differential solubility.

These examples show that the factors regulating the uptake of narcotics by cells or cell constituents are evidently complex.

The Uptake of Phenol by Cells. The relation between concentration and uptake of phenol has been measured by several workers. Herzog and Betzel (1911) found that the distribution coefficient of phenol between solution and cells was approximately constant up to a certain concentration and then rose rapidly (cf. Fig. 2). This conclusion was confirmed by Cooper and his co-workers (1912, 1923, 1927). They studied the uptake of phenol by gelatine, egg albumen and casein, and found (1927) that the gelatine/water distribution coefficient of phenol had a constant value of about 3 for low concentrations but rose to 12 at higher concentrations.

Reichel (1909) found that the bacteria/saline distribution coefficient of phenol was about 10. Cooper (1912) concluded that the uptake of phenol by proteins was determined by

differential solubility until the concentration of phenol in the protein phase reached a certain critical figure when the phenol reacted with and precipitated the protein, and a sharp rise in the differential distribution occurred.

The figures of Herzog and Betzel and of Cooper show an approximately linear relation between log x and log y (x = concentration in solution and y = concentration in protein or cells). They are fitted approximately by the formula $Kx^3 = y$, and might be explained on the assumption that the phenol molecules were aggregated in the protein phase. The explanation offered by Cooper appears however to be more reasonable.

Cooper and Mason (1928) measured the protein/water distribution coefficient of cresol and *o*- and *p*-chlor-phenol and found that in these cases it increased as the external concentration was increased.

The cases of narcotics and of the coal tar disinfectants are of interest because the distribution of these drugs has been investigated with particular care, but nevertheless the laws determining the fixation of these drugs by proteins or by cells are not known with any certainty.

There does not appear to be much evidence for the assumption that the distribution of drugs of either of these classes is regulated simply by differential solubility, but it would appear that the distribution is affected by differential solubility, by adsorption and also by chemical reactions that only commence when a certain concentration has been attained in the cells.

The Uptake of Hæmolytics by Erythrocytes. The action of hæmolytics has been studied extensively. In many cases authors have assumed that the extent of hæmolysis indicated the amount of drug fixed, but this assumption appears to be unjustifiable.

Eisenberg (1913) showed that erythrocytes could combine with 400 times as much potassium permanganate as was required for their hæmolysis. Klinke, Knauer and Kramer (1926) concluded that the amount of hæmolysis of sheep's corpuscles produced by pig's serum did not give a direct measure of the amount of lysin fixed; Ponder (1932) showed that in saponin hæmolysis a portion of the saponin was fixed by the laked corpuscles.

A certain number of authors have made more or less direct estimates of the amount of hæmolytics disappearing from solutions, and in most cases they have found that the relation between concentration and fixation follows the usual adsorption formula $\left(kc^{\frac{1}{n}} = \frac{x}{m}\right)$, and have obtained normal values for n (i.e. $n = 2$ to 3). Morawitz (1910) found this relation in the case of mercuric chloride acting on washed corpuscles. Stadler and Kleemann (1911) measured the uptake of ammonia and of acetic acid and found that it followed the adsorption formula, with a value for n of 2·5.

Eisenberg (1913) studied the fixation of mercuric chloride, potassium thiosulphate and potassium permanganate by erythrocytes. He found that when the concentration was increased the fraction of drug fixed decreased (i.e. an adsorption effect). Ponder measured the fixation of sodium taurocholate (1923A) and saponin (1925) by serum and found that these processes followed the adsorption formula and that the value of n was respectively 2·66 and 1·9. On the other hand, Piettre and Chrétien (1927) concluded that the fixation of various hæmolytic agents by the stromata of corpuscles was an ordinary chemical reaction and was not an adsorption process.

The evidence obtained in this field is therefore somewhat indecisive. This is not surprising because erythrocytes are obviously an unsatisfactory material upon which to study drug fixation, because the occurrence of hæmolysis alters the physical condition of the adsorbent so profoundly.

Summary

Our knowledge regarding the fixation of drugs by proteins and by cells has not advanced greatly beyond the original conclusions of Herzog and Betzel, who described the three chief types of relation between concentration and drug fixation, namely a steep sigmoid curve, a simple linear relation and a linear relation between the logarithms of the quantities of free and fixed drug.

The complexity of the results obtained with simpler systems such as charcoal, suggests that the factors determining drug fixation by cells are extremely complex and that any relations found are likely to be only first approximations.

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CHAPTER V

KINETICS OF CELL-DRUG REACTIONS

General Theory

The chief fundamental difficulty of quantitative pharmacology which has already been pointed out in Chapter III is that although the concentration of a drug applied to cells and the consequent biological response can both be measured accurately, yet there is no means of determining the quantity of drug that has produced this response. There is indeed very little direct evidence that the biological response is produced by a chemical reaction between the drug and the cell constituents. This assumption is chiefly justified by the facts that it is supported by much indirect evidence, and that there is no alternative hypothesis which has stronger evidence in its support. If we assume that there is some simple relation between the amount of drug entering into combination and the extent of the biological response, then the following consideration must apply.

If the fundamental chemical reaction consists in fixation of the drug by some particular constituent of the cell, then this must result in a reduction both of the free drug and of the free receptors in the cell. The relative amounts of drug solution and cells can however be varied at will, and hence the concentration of the drug can always be kept constant by providing a sufficient excess of drug solution ; indeed, in many cases the quantity of drug fixed is so minute that it is difficult to reduce the drug solution to a volume small enough to show changes of concentration.

There are indeed four possibilities regarding the variations in the amount of free drug and in the number of free cell receptors.

Firstly, both drug receptors may be reduced by a significant quantity ; secondly, the drug may be in such excess that it

undergoes no significant change ; thirdly, a full action may be produced when only a small fraction of the available cell receptors has been occupied, and in this last case there are two alternatives, because the free drug may or may not be reduced by a significant quantity.

If no significant change of concentration occurs in either drug or receptors, then the rate of reaction will be constant ; if only one component changes by a significant amount, then the rate of reaction will vary as the concentration of this component (monomolecular reaction) ; if both free drug and cell receptors change significantly, then the rate of reaction will be expressed by the bimolecular formula. The formula expressing the rate of progress of a monomolecular reaction is : $Kt = \log \frac{a}{a-x}$; where t = time, a = original concentration and x = amount combined. The corresponding formula for a bimolecular reaction is : $Kt = \frac{x}{a(a-x)}$. It has been pointed out previously that these fundamental laws of mass action express the course of reactions which occur in homogeneous solutions, and in the case of drugs and cells the rates of reaction are very unlikely to be expressed by the simple formulæ mentioned above. Even in simple heterogeneous systems there is a tendency for the rate of reactions to be regulated by the rate of diffusion, and the probability of this is greatly increased in the case of complex heterogeneous systems such as living cells.

Another point is that the equations mentioned apply to irreversible processes, whereas the majority of the more important and interesting actions produced by drugs are freely reversible. The possible errors due to other causes are, however, so great that it appears to the writer that kinetic studies are only likely on rare occasions to express the progress of a single chemical reaction and hence it is unnecessary to argue regarding the exact form of reaction they may be expected to express.

Kinetic studies of drug action have been a very popular type of experiment because it is easy to measure the time interval between the addition of a drug and the appearance of some particular response, and the results can usually be fitted by some fairly simple formula. Difficulties arise, how-

ever, when the results are analysed in the light of the knowledge gained regarding simpler heterogeneous systems.

The addition of a drug to a population of cells causes the following events : (i) fixation of drug by cells, (ii) secondary chemical reactions between drug and cell constituents, and (iii) biological response to injury.

The rate of fixation of drugs by cells can only be measured by chemical estimations. The quantity of drug fixed is usually small and hence such estimations are difficult, and it is only in exceptional cases that this evidence is available.

Fixation of the drug is followed by the production of changes in the cell. In many cases these occur slowly and appear to indicate alterations in colloidal aggregation. The time taken for some biological response to appear is usually the easiest of all time measurements and therefore the commonest. For example, in the case of disinfection the progress of such changes is estimated by removing samples of the cell population and determining whether they have or have not suffered irreversible injury.

The time measured in kinetic studies is very frequently of a very different nature in different systems. In the case of kinetic studies on disinfectants " time " is assumed to measure the time taken for some irreversible and lethal change to be produced in the cells. In the case of hæmolytic studies the time measured is the time required for some chemical change to be produced plus the time required for this change to cause rupture of the cell. The time taken by different drugs to produce their action varies over a remarkably wide range, as is shown by the following examples. In the case of specific drugs such as acetyl choline acting on a sensitive tissue such as the frog's heart, equilibrium is attained in a few seconds and this period covers the time taken for the drug to be fixed by the cell and to cause a biological response. In the case of cardiac glucosides acting on the frog's heart the full effect may only be produced after more than an hour, although the drug is fixed in a few minutes.

It is often assumed that measurements of the time of occurrence of a biological response measure the rate of progress of a chemical reaction. This assumption is unjustifiable, and in every case it is necessary to determine the probable nature

of the " time " measured. In most cases it can be shown that the time measured is not the measure of any simple chemical reaction, and only in a minority of cases is it possible that this may be the case.

Kinetic studies unfortunately are profoundly affected by experimental conditions, and therefore it is necessary to consider certain gross experimental errors.

Gross Errors due to Diffusion

No reaction can occur between a drug and a cell before the drug has reached the cell, and hence efficiency of mixing is of prime importance in kinetic studies. In the case of free cells it is possible to mix the cells and the drug rapidly so that delay in the drug reaching the cell is not a serious error. In some cases, however, the cells are covered with a thick protective coating, e.g. anthrax spores, and such a coating will hinder and may even prevent a drug from acting on the cell. In such cases the rate of action of drugs must measure the rate of diffusion through the capsule rather than the rate of any chemical reaction.

The most favourable systems on which to study drug kinetics are therefore free cells devoid of any protective covering (e.g. red blood-corpuscles, spermatozoa, sea-urchin eggs, etc.). In the case of isolated tissues there is the possibility of an indefinite delay due to the time required by the drug to diffuse through the tissue to reach the cells. An isolated medullated nerve represents an extremely unfavourable system, for it consists of closely packed nerve-fibres each of which is surrounded by a protective layer of fat. Isolated skeletal muscles vary greatly in the ease with which diffusion occurs, but the extent of the delay that can be caused by diffusion in this case is illustrated by experiments by Ing and Wright (1932). The following figures show the times taken for 2 m. molar octyl trimethyl ammonium iodide to produce 50 p.c. reduction in the response of frogs' muscles.

	Time in Minutes before Paralysis	
	Immersed	*Perfused*
Gastrocnemius	13	0·25
Sartorius	1·6	1·6

The figure for the perfused gastrocnemius shows that the drug at the concentration employed can combine with the cell

receptors on which it acts in less than 15 seconds. When the gastrocnemius is immersed in solution without perfusion the drug acts fifty times more slowly, and this delay must be due to diffusion. The figures for the sartorius considered alone suggest that they are not influenced by diffusion, but the fact that the perfused sartorius reacts to the drug six times more slowly than does the perfused gastrocnemius raises doubts as to the exact significance of the time measurements.

The frog's heart is an exceptionally favourable muscle on which to study the rate of action of drugs, because it has a sponge-like structure which facilitates the exposure of all its cells to drugs, but even in this case the writer found that the rate of action of drugs depended very largely on the mechanical conditions of the experiment. For example, the time taken by acetyl choline to produce half action was about 30 seconds in the case of a ventricular strip immersed in a bath that was stirred vigorously, but when the drug solution was applied as a strong jet of fluid playing on the ventricular slip this time was reduced to less than 5 seconds.

These examples show that in many cases the time taken by drugs to produce an action on cells expresses the time taken by the drug to diffuse up to the cells or through the protective covering that surrounds free cells. Time measurements of drug action can only be assumed to express the progress of a chemical reaction when gross errors due to delay caused by diffusion have been excluded.

The evidence obtained from inorganic heterogeneous systems proves however that, even when gross errors due to delay in mixing are excluded, the rate of reactions proceeding in such systems is more likely to be governed by diffusion than by the ordinary laws of mass action.

Noyes and Whitney (1897) studied the rate of solution of small cylinders of solids rotated rapidly in fluids. They concluded that the solid was always surrounded by a thin layer of saturated solution and that the rate at which the substance dissolved was determined by the rate of diffusion away from this layer. Brunner (1904) concluded that when solids were stirred about 150 times a minute the Noyes-Whitney layer was between 20 and 50 micra in thickness.

In these cases the delay in the solution of the solids was

due to a layer of saturated solution. In the case of drugs acting on cells a similar delay may be expected if the cells carry with them a layer of water, for the fixation of the drug by the cells will deplete the layer of solution immediately surrounding them and the depleted layer will have to be refilled by diffusion. Brunner's experiments indicate that such a layer is likely to occur even when free cells are stirred vigorously in a solution.

Lag Inherent in Biological Response

The difficulty of obtaining mechanical records free from instrumental lag is very well known, but far greater errors may be imposed on kinetic measurements by the simple fact that the tissue studied is incapable of a rapid response.

The contracture of plain muscle in general, or of the rectus abdominis of the frog, is a simple example of this fact. Acetyl choline produces its full action on the frog's heart in less than half a minute, but the contracture this drug produces in the rectus abdominis may take more than half an hour to be completed. Most of this delay is due, however, to the sluggish response of the tissue, for the muscle may take 20 minutes or more to attain equilibrium when subjected to some simple instantaneous change such as an alteration of its load.

Hill (1909) found that the rate of contraction of a rectus abdominis produced by nicotine followed a logarithmic curve and was fitted by the formula $h = \mathrm{K}(1 - \varepsilon^{-at})$ where h is height of contraction and K and a are constants, but this curve cannot be assumed to measure the rate of the reaction between nicotine and the muscle because an instantaneous stimulus, such as changing the load on the muscle, produces a similar curve.

The following general rule may be formulated. The measurement of the interval between application of drug and biological response measures the time taken by at least two separate processes, namely a chemical reaction and the biological response to this stimulus. Unless it can be proved that the interval between the chemical reaction and the biological response is much shorter than the total time measured, the latter cannot be assumed to be a measure of the time occupied by the chemical reaction.

The influence of lag in biological response on the rate of

action of drugs frequently can be demonstrated by measuring the rate of action of a single drug on a number of different tissues.

The rate of action of ergotamine has been studied on a number of tissues and the results appear to indicate that the drug initiates changes that proceed at a very different rate in different tissues. The drug renders isolated tissues insensitive to adrenaline. Braun (1925) found that in the case of the rabbit's uterus the effect of ergotamine was produced very slowly and he concluded that the amount of effect was directly proportional to the exposure. Gaddum (1926) obtained results consistent with this hypothesis, but Mendez (1928) found that the reaction probably was completed after one or two hours' exposure. Braun believed that the action produced was irreversible, but Mendez found that a slight reversal was obtained after prolonged washout. Issekutz and Leinzinger (1928), Rothlin (1929) and Nanda (1931) all found that ergotamine produced its full action on the isolated rabbit's intestine in a few minutes and that the action could be rapidly reversed by washing out.

These differences in time relations cannot be explained by delays due to diffusion, for the uterus is not thicker than the intestine, and the vas deferens of the guinea-pig, which behaves like the rabbit's uterus, is much thinner than the intestine. The uterus is more sensitive to the action of ergotamine than is the intestine, and the action is much more difficult to reverse in the former case. Both these differences would, in the case of an ordinary chemical reaction, cause the combination to proceed to completion quicker in the uterus than in the intestine, whereas the reverse is what happens.

The results suggest that ergotamine causes some slow change in the cells on which it acts and that the times of action required for action measures the rate at which these biological changes occur rather than the rate at which the drug combines with the tissue.

Measurements of the rate of action of lethal agents on bacteria or larger organisms avoid errors due to lag in the biological response, since the action of the drug is stopped at a particular time and the cells are tested to determine whether or no they are still capable of life or of reproduction.

On the other hand, this source of error is important in the case of most experiments with isolated tissues including the hæmolysis of red blood-corpuscles.

Kinetics of Heterogeneous Systems

The cell-drug system is a complex heterogeneous system and therefore it is necessary to consider the facts known about kinetics of simple heterogeneous systems, for it is irrational to expect kinetic laws applicable to homogeneous systems to apply. A study of non-living heterogeneous systems shows that even when the gross errors already considered have been eliminated there are a large number of factors that may influence the rate at which a reaction occurs.

(i) **Sorption.** The term sorption was introduced by McBain in 1909 to express the composite processes that occur in heterogeneous systems. In a simple case such as the fixation of gases by charcoal there is, for example, a rapid reversible process (true adsorption), but this is followed by slow irreversible chemical changes which may still be incomplete after several years. McBain (1932) does not claim that all cases of sorption are of composite type, but considers that until any particular case has received adequate investigation it should be referred to as sorption and should not be given the more definite names such as adsorption or chemical reaction.

In the case of drugs acting on living material, evidence regarding the nature of the process of drug fixation is at the best scanty and usually is non-existent. In a few cases the effects produced are so rapid that it is probable that the fixation of a drug on a cell surface produces an immediate change in the biological condition of the cell, e.g. alcohol, acetyl choline, etc. In the case of drugs producing lethal effects there is in many cases evidence of two processes, namely a rapid fixation of the drug which is followed by much slower irreversible chemical changes.

True adsorption appears usually to be a rapid process. It can, for example, be studied in the case of substances that reduce the air/water surface tension. Freundlich (1926, p. 51) states that the adsorption of amyl alcohol on an air/water surface is completed in a few hundredths of a second. On

the other hand, composite sorption effects are usually much slower and may be extremely slow. For example, Seth (1923) found that the sorption of heavy metals by caseinogen was not completed after 3 hours, and Lockemann and Picher (1928) found that the adsorption of mercuric chloride and silver nitrate by wool was only completed after 24 hours.

The complexity of the sorption processes in such a simple case as the sorption of oxygen by charcoal is indicated by the fact that Garner (1926) found that at 1000° C. the adsorption of oxygen by charcoal was practically instantaneous but that the resultant heat was liberated over periods varying from 1 to 2 minutes. Hence the rate of heat liberation in this case does not measure the rate of fixation of the gas by the solid. The fact that difficulties of this type arise in such a simple system as charcoal and oxygen is a good indication of the degree of inevitable uncertainty regarding the significance of time measurements made on living systems.

Liepatoff (1924, 1925 and 1926) found that the rate of adsorption could be expressed by the formula $kt = ln.\frac{a}{a - \gamma x}$ (a = original amount of drug, x = amount fixed and γ = constant). He found that this formula expressed the rate of both adsorption and imbibition and concluded that the reason for this was that the rate of both these processes was determined by diffusion. These results confirm the general conclusion of Nernst that the rate of all reactions in heterogeneous systems is regulated by diffusion, and hence the kinetics of such reactions always resemble those of a monomolecular reaction.

(ii) **Depletion.** Drugs that act in high dilutions are of particular interest in pharmacology, and in such cases true adsorption may be delayed by the depletion of the layer of solution adjoining the surface. Freundlich (1926, pp. 169–70) states: "The simpler the conditions chosen and the smaller the difficultly accessible inner surface, the quicker is the adsorption. We again have a strong depletion in a layer directly adjoining the surface layer, and a diffusion into it from more distant parts of the solution. In the case of well-powdered adsorbents the equilibrium is mostly reached in seconds or minutes."

The rate of surface adsorption of substances of a high molecular weight which act in high dilutions may however be very slow. Johlin (1925A) found that a solution of 0·1 per cent. gelatine took 13 min. to produce its full effect on surface tension. He also found (1925B) that the rate of fall of surface tension produced by various proteins followed the formula $\sigma = \frac{a}{t^n}$ (σ = surface tension, t = time and a and n are constants). This formula gives a linear relation between $\log \sigma$ and $\log t$, a relation which is very frequently found when the rate of action of drugs on cells is measured.

Bigelow and Washburn (1928) found that with dilute solutions of sodium oleate equilibrium of surface tension was only attained after 300 minutes. Du Noüy (1926 and 1930) showed delays of from 30 minutes upwards in the attainment of equilibrium of surface tension with serum and saponin. Gaddum (1931) found that saponins even in strong solutions took more than 10 minutes to produce their full effect on surface tension.

One probable reason for the delay in these cases is depletion of the area adjoining the surface. In the case of cell-drug solution systems in which vigorous stirring is possible such delay ought to be reduced. It is however necessary to remember that particles above a certain size carry with them a layer of water several micra thick, even when they are stirred thoroughly. Hence even in cases where such stirring is possible the delay due to depletion cannot be ignored. In all cases where stirring is not possible the delay due to depletion is likely to occupy a considerable time, when the action of drugs in dilute solution is studied.

Table VIII shows the rates of action of various alcohols as measured by the writer. The drug solution was applied as a jet to strips of frog's ventricle, and the delay due to diffusion was thus reduced to a minimum. It seems probable that the alcohols produce their action by forming a monomolecular layer over the surface of the heart-cells, but the alcohols that act in dilute solution take much longer to produce their action than do those that act in concentrated solution.

Ponder (1926) discussed this question of depletion and concluded (1927) that the volume of the zone of action was proportional to the resistance of the red blood-corpuscles to

TABLE VIII

The rate of action of alcohols on the isolated frog's heart (A. J. Clark, unpublished results)

Alcohol.	Molar concentration which produces 50 per cent. inhibition.	Time in seconds required for aequi-active solutions to produce half action.
Ethyl	0·65	1–3
Octyl	0·000,2	8
Decyl	0·000,03	30
Dodecyl	0·000,006	500

the lysin. In the case of the resistant corpuscles of sheep he calculated that the zone of action of saponin had a radius of 9·5μ, whereas with the sensitive corpuscles of the rabbit the radius was about half of this.

Ponder (1930) found that a red blood-corpuscle was hæmolysed when about one million molecules of sodium oleate were fixed by each square micron of surface, and this corresponds to about 10^8 molecules per corpuscle. A solution of 1 in 100,000 sodium oleate contains 10,000 molecules per cubic micron, and hence 10^8 molecules are contained in a sphere with a diameter of 25 micra. The fixation of the amount of sodium oleate necessary for hæmolysis must therefore involve depletion to a distance of more than 10 micra from the cell surface. It is interesting to note that when the concentration of the solution is doubled, the depth of depletion is reduced and the rate of diffusion into the depleted area is increased, and hence the delay due to depletion will vary inversely as $(\text{conc.})^n$, and n will be greater than unity.

Some of the figures for the relation between time and concentration in saponin hæmolysis given by Ponder and Yeager (1931) approximate to the relation $C^2t = \text{constant}$. The delay due to depletion appears to be a possible and even probable reason for a relation of this type between concentration and time of action.

(iii) **Diffusion.** A process such as hæmolysis may possibly be of the same order of complexity as the adsorption of drugs by powdered charcoal. A process such as the destruction of

bacteria by disinfectants must however be more complex, for in this case it is probable that the drug must penetrate the cell interior before it produces its action.

For the sake of simplicity the cell may be regarded as a protein gel surrounded by a semi-permeable layer. Stella (1928) calculated that the amount of drug (x) that diffused from a solution into a solid of unlimited depth was $x = 2C\sqrt{\frac{Kt}{\pi}}$. The relation between variations of concentration (C) and time (t) that cause the entry of a certain fixed amount of drug is therefore C varies as $\frac{1}{\sqrt{t}}$ or C^2 varies as $\frac{1}{t}$.

Bigwood (1930) found that the penetration of ions into gelatine was expressed by the following formula. Distance of penetration $= k_1\sqrt{t}.c^{k_2}$ (k_1 and k_2 = constants). The time required to produce equal penetration, and hence an equal biological effect, will therefore probably vary as some power of the concentration. Diffusion can therefore account for the relation $c^n.t$ = constant, when the times required for the production of an equal biological effect by various concentrations are measured.

Calculations of this type do not however make allowance for the changes in the condition of a gel that may be caused by diffusion into it of a substance. Jordan Lloyd (1930) concluded that the simple process of absorption of water by gelatine was regulated by three separate factors, namely imbibition or restoration of water to a preformed structure, osmotic pressure due to an unequal solvent pressure in the solution and in the jelly, and swelling of hydration.

Diffusion into a living cell of substances such as disinfectants that produce extensive changes in its colloidal structure, is likely to be affected by a very large number of variables. Irwin (1925) found that the rate of diffusion of brilliant cresyl green into *Nitella* was expressed approximately by the formula $\frac{dx}{dt} = K(a - x)$. This process cannot however have been one of simple diffusion, because when equilibrium occurred the concentration of the dye in the water of the central vacuole of *Nitella* was about ten times as great as the outside concentration.

The evidence regarding the uptake of drugs by cells indicates

that in most cases it is a very complex process and cannot be interpreted as a simple diffusion from a solution into a gel. The example of *Nitella* shows however that the general rate of a chain of processes of unknown complexity may resemble a very simple diffusion process.

Time-Action and Time-Concentration Measurements

The rate of action of drugs can be measured in two ways, firstly by measuring the rate at which a particular concentration produces a series of actions varying from 0 to 100 per cent. of the maximum effect which it can produce (time-action curves), and secondly by measuring the times taken by a series of concentrations to produce some particular selected effect (time-concentration curves).

Time-Action Curves. A curve of this type measures the rate at which a particular concentration of drug produces an effect in a population of cells. It has already been shown that the time measured does not necessarily indicate the time taken by a chemical reaction, and indeed it frequently can be proved to measure a rate of diffusion, or some lag in a biological response. The nature of the action measured in curves of this type is also uncertain. The usual conception of the mode of action of drugs on cells is that thousands of molecules react with a single cell and their combination with the cell produces a cumulative effect until finally some particular action such as death is produced. There is a mass of evidence in support of this view, e.g. relative size of cells and molecules, number of molecules shown to react with cells, etc. If a drug in a concentration sufficient to produce death acted in this way on a population of cells that were absolutely uniform, then all the cells would die simultaneously. This result is never obtained, but time-action curves vary from sigmoid to logarithmic or die-away curves. The writer believes that these curves are chiefly a measure of the individual variation of the population observed.

Arrhenius however supported the hypothesis that drugs produced death of cells by a single event or at most by a few events, and hence that the reaction between drugs and cells resembled a monomolecular chemical reaction. This hypothesis involves a number of assumptions that appear obviously

absurd to pharmacologists, but controversy regarding its truth has continued since the commencement of this century, and since this controversy involves the question of individual variation its discussion is deferred to Chapter VIII. The existence of this unsettled controversy indicates however the difficulty of interpreting the nature of time-action curves and suggests that they are unlikely to provide a satisfactory foundation for any theory of drug action.

Time-Concentration Curves. In this case the time taken to produce some definite action is measured with a series of concentrations. Errors due to cell variation are thus avoided provided that the samples of cell population taken are sufficiently large and are uniform.

Henderson Smith (1923) has pointed out one avoidable source of error in the construction of those curves. The obvious method is to measure the time at which equal actions are produced. Sometimes, however, comparisons are made by comparing the amounts of action produced at a given time. The latter method is erroneous unless there happens to be a linear relation between action and time, a relation which is extremely rare, because time-action curves usually are either sigmoid or logarithmic in shape. If the rate of action is logarithmic and action A proceeds at twice the rate of action B, then the ratio between the amount of action A and the amount of action B will vary with every time taken; at first it will be nearly 2, but as time proceeds it will approach nearer and nearer to unity. This error appears obvious, but it is encountered fairly frequently.

Another difficulty regarding the construction of time-concentration curves is that the ratio between the times taken by different concentrations to produce a given effect, differs according to the effect measured. Henderson Smith (1921) measured time-action curves for the killing of botrytis spores by phenol (Fig. 3). A comparison of the times taken by 0·7 and 0·4 p.c. phenol to kill varying proportions of the populations gives the following figures. The ratio of times for 25 p.c. action is 1/35, for 50 p.c. action 1/25 and for 75 p.c. action 1/19. In the majority of cases the rate of action is greatest round about 50 p.c. action, and since most populations show the greatest uniformity in their rate of

response at about 50 p.c. action this is the most favourable end-point for measurement of rates of action.

The very existence of this variation in the ratio indicates, however, the inherent uncertainty regarding measurements of the times taken for actions to be produced.

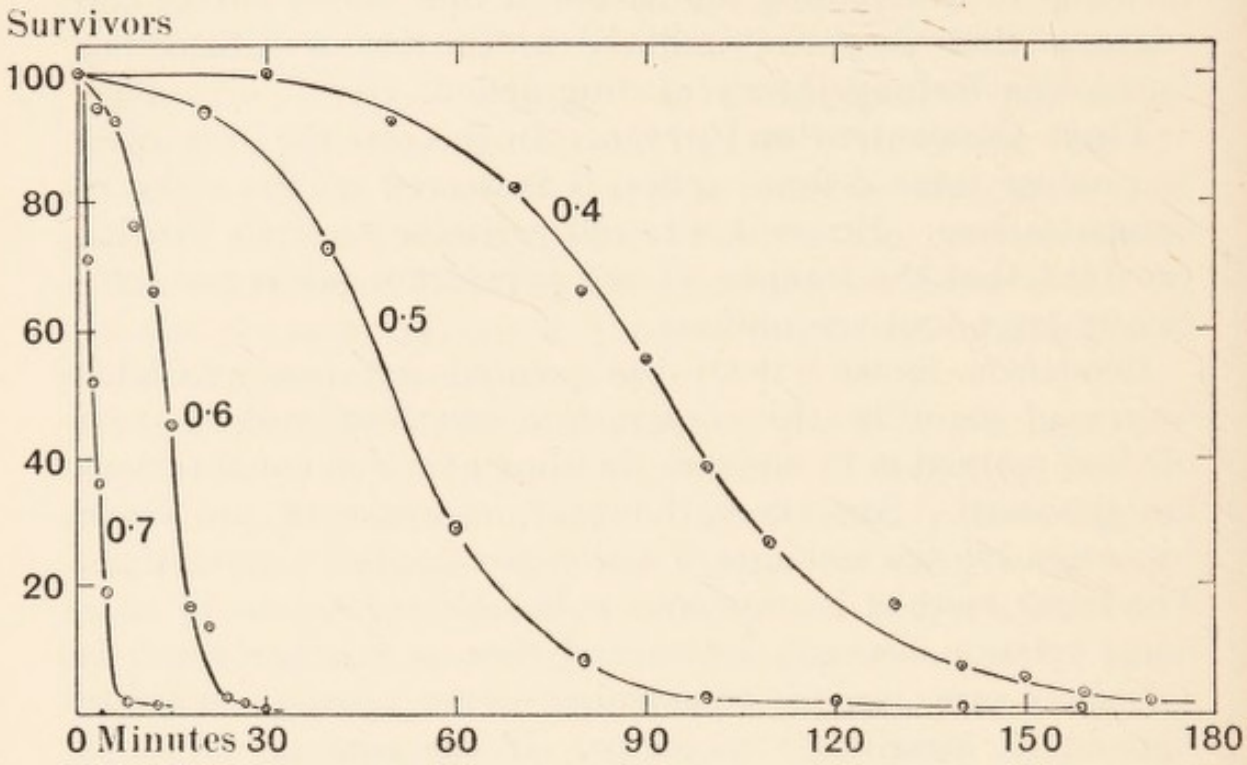

Fig. 3.—The rate of destruction of Botrytis spores by concentrations of phenol of 0·7, 0·6, 0·5, and 0·4 per cent.

Abscissa: time of exposure to drug in minutes. Ordinate: per cent. mortality. (Henderson Smith. 1921.)
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The General Form of Time-Concentration Curves

The literature regarding time-concentration relations is extensive and varied, and perusal of it shows that authors have used a very wide variety of formulæ, but that in most cases no attempt has been made to explain the significance of the formulæ employed.

In a number of cases the relation is expressed by the formula $Ct = \text{constant}$, and most curves can be interpreted by one of the following formulæ: $(C - C_m)t = \text{constant}$ (where C_m is the concentration that just fails to produce an action in infinite time); or $C^n t = \text{constant}$ (where n is a constant that may vary from 0·3 to 7·0); or finally $(C - C_m)^n t$

= constant. The formulæ are of a type that can be adjusted to fit a wide variety of results, and hence the fact that they fit experimental results proves very little. In many cases a set of results can be fitted by two formulæ, and hence it is necessary to consider what type of formula is most likely to provide information regarding the nature of the drug action that is being measured. In the first place it must be remembered that the action of any drug on cells probably depends on a chain of events and that the rate of action will be determined by the slowest process. When one event proceeds much more slowly than the rest a simple relation between concentration and time of action may result, but in most cases the relation is likely to be too complex to interpret.

Several authors have tried to determine the nature of drug actions by study of time-concentration curves ; their theories will be considered later, but in the writer's opinion all these interpretations are very doubtful, and hence it seems most profitable to consider first the types of time-concentration curves obtained in a few simple cases.

No drug produces an effect at infinite dilution, for the living cell possesses powers of resistance and repair, and hence there is always a certain threshold concentration necessary for the production of any given effect. All time-concentration curves will therefore start from a certain threshold concentration at which the effect measured is only produced after a delay so long that time may be regarded as equal to infinity. At the other extreme conditions may vary, for in some cases a sufficiently high concentration of drug may produce instantaneous action, whereas in other cases a certain minimum time is needed for the production of an action, however high be the concentration.

Some figures obtained by Jenkins (1926) for the action of alkalies on the protozoa *Spirostomum ambiguum* provide a simple example of the general nature of time-concentration curves. Her figures for the duration of life of the protozoa in tap water buffered with carbonate are as follows :

*p*H . .	7·35	7·65	7·95	8·37	8·57	8·78	8·98	9·13	9·29	9·39
$C_{OH} \times 10^7$.	2·2	4·5	8·9	23	37	60	96	135	195	246
Duration of life in hours (t)	∞	20	13	6	4	$2\frac{1}{4}$	$1\frac{3}{4}$	1	$\frac{1}{3}$	$\frac{1}{4}$
$(C_{OH} \times 10^7)t$	∞	90	116	138	148	135	167	135	65	62

With regard to these figures the purpose of changing the *p*H nomenclature to values of C_{OH} was to make the results more readily comparable with those obtained with drugs. The figures cover a very wide range of concentration, moreover the concentration measured is that of the hydroxyl ion, which is the active lethal agent. The figures are therefore exceptionally favourable. In the first place they cover a wide range and in the second place the concentration of the active

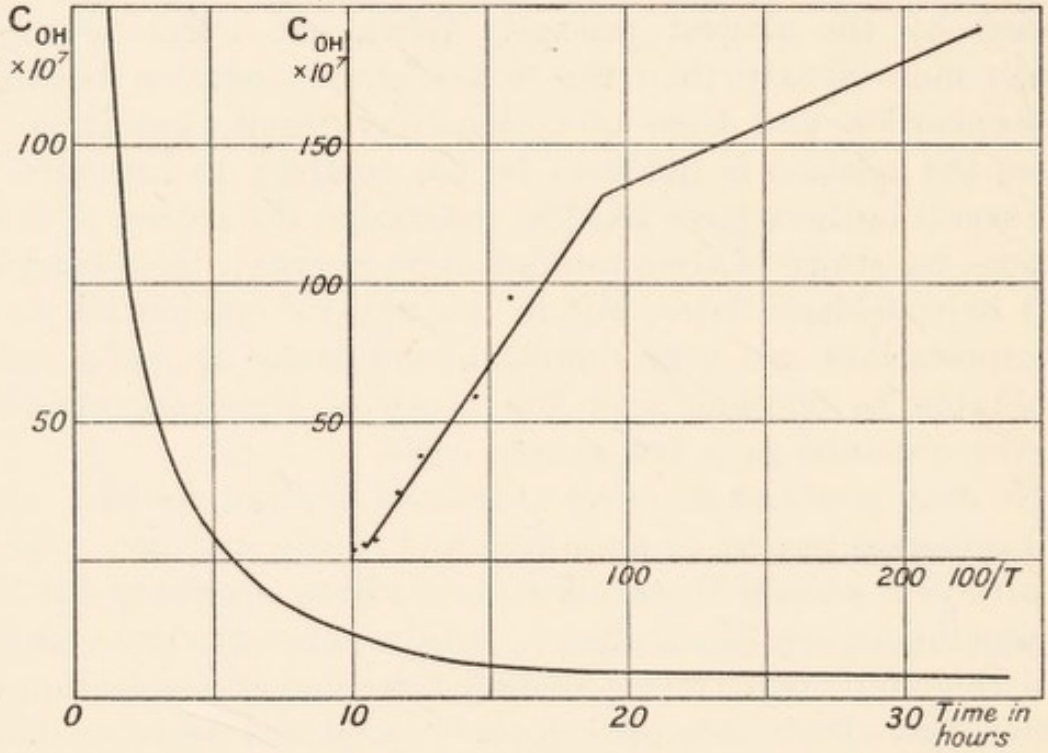

Fig. 4.—The relation between the concentration of hydroxyl ions and the duration of life of *Spirostomum ambiguum*.

Abscissa : time in hours. Ordinate : $C_{OH} \times 10^7$.
Inset. The relation between C_{OH} and the reciprocal of the time.
(Penelope M. Jenkins. 1926.)

ion is measured, whereas in most cases only the total concentration of a drug can be measured.

The figures in the last line of the table show that the product *c.t* is constant over a range of value of $C_{OH} \times 10^7$ from 23 to 135, but that it is not constant outside these limits. These results when plotted in various ways furnish attractive curves. When time is plotted against concentration a nearly symmetrical hyperbola is obtained (Fig. 4). When the reciprocal of the time is plotted against concentration a linear relation is obtained over a considerable stretch (Fig. 4 inset). When

the logarithm of the time is plotted against the logarithm of the concentration (Fig. 5 A) a sigmoid curve is obtained, which is linear over a considerable range.

Unfortunately these curves do not establish any results of theoretical significance, beyond the fact, which was shown in the original figures, that $c.t$ is constant over a considerable range.

The formula $c.t =$ constant, cannot however be modified readily to cover the extremes of concentration. Time becomes

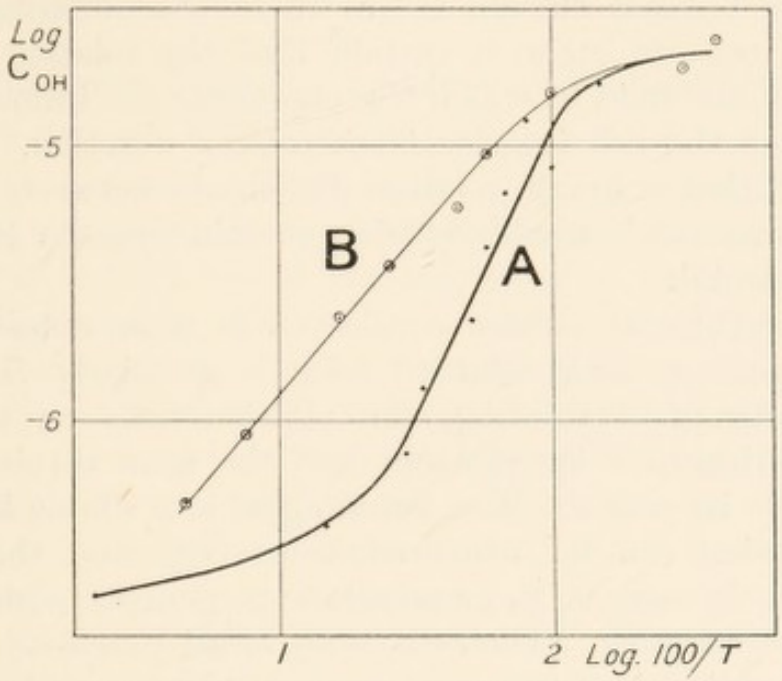

Fig. 5.—The relation between the logarithm of C_{OH} and the logarithm of the reciprocal of the duration of life in hours of *Spirostomum ambiguum*.

Curve A. Organism in tap water containing CO_2.
Curve B. Organism in borax-boric acid solution. (Penelope M. Jenkins. 1926.)

infinite when $C_{OH} = 2{\cdot}2 \times 10^{-7}$, but if this figure be taken as the threshold concentration and be subtracted from the other values, then the divergence of the product $c.t$ in the case of values of pH less than 8 is increased considerably. These latter figures could be fitted accurately if the threshold concentration were taken as pH 6·5, but there is no justification for this assumption, and it would not correct the divergence in the product $c.t$ observed when the pH is greater than 9.

The linear portion of curve A in Fig. 5 can be fitted by the formula $c_{0\cdot5}.t =$ constant, but this does not express the

extremities of the curve. Moreover, a curve with a different slope was obtained when similar experiments were made in fluids buffered with borax and boric acid (Fig. 5 curve B).

Attempts at the interpretation of the time-concentration curves obtained in this case show that a variety of approximate relations can be obtained, but that no formula expresses more than a portion of the results. Moreover, the study of these curves gives extremely little information regarding the mode of action of hydroxyl ions on protozoa. This is really not surprising because enough is known of the buffering powers of living cells to make it certain that the relation between the *p*H of the fluid in which the organisms are immersed and the *p*H of the cell interior is extremely complex. If it so happened that a simple relation did obtain between C_{OH} and time of survival it would be fairly certain that the simplicity was accidental.

These results have been considered in some detail because they appear at first sight to offer a peculiarly favourable opportunity of establishing the relation between time and concentration of a lethal agent and the time required for it to produce its action. The lethal agent is a simple ion whose concentration can be measured accurately, and the system on which it acts is a monocellular organism without any protective covering. The cells were killed practically instantaneously when put in a solution at *p*H 9·4 and therefore there was no unavoidable lag due to delay in biological response in this case.

In most cases the data relating the concentration of drugs and the times required for their action cover a much smaller range of concentration and the number of variables is much greater than in the case just considered.

As a general rule, the narrower the range of the data and the greater the number of the possible variables, the easier it is to obtain a satisfactory fit with some simple formula. The example studied above suggests that the theoretical significance of such agreements is likely to be extremely small.

The most profitable method of treating time-concentration curves appears to be to plot the logarithm of the concentration against the logarithm of the reciprocal of the time and to estimate the general slope of the curve obtained.

The results just considered show that it is perfectly useless to consider cases in which there is not a reasonably wide range of time and concentration, but even in such cases the results can often be fitted by two or three different formulæ and often they are fitted best by formulæ that appear devoid of any significance. Finally, it is desirable to choose examples in which there is evidence regarding the nature of the time measured, i.e. whether it expresses a rate of adsorption, a rate of colloidal change or the interval between chemical change and biological response.

Various Time-Concentration Relations

(i) **Disinfectant Action of Phenol.** Measurements in the case of disinfectants do not measure the time of biological response (i.e. time of death of the bacteria) but measure the time at which the bacteria has taken up so much drug that it will subsequently die. Cooper (1912) showed, however, that the toxic action of phenol on bacteria was a complex process. He studied the action of phenol on proteins and found that the drug was fixed rapidly by the proteins, and that this fixation was followed by a slower process of protein precipitation. Cooper and Haines (1928) found that at 20° C. the precipitation of egg albumen by 1 per cent. phenol was only half completed in 50 minutes, and that at least 5 hours were required for the completion of the process. Cooper concluded that the absorption of phenols by bacteria was only the initial stage in the process of disinfection and that the germicidal action was due to the phenol altering the colloidal condition of the cell proteins. This conclusion is confirmed by the results of Küster and Rothaub (1913), who found that when phenol acted on anthrax spores and on yeast the fixation of the drug by the cells was nearly completed in 24 hours, but that the cells were not killed until 5 days and 12 days respectively.

Chick (1908) has given figures for the time of destruction of *B. paratyphosus* at 20° C. by phenol (loc. cit., Table 16) and Disinfectant A (loc. cit., Table 18). She interpreted her results by the empirical formula $Ct = K^c$. The figures show, however, a fairly exact linear relation when concentration is plotted against the logarithm of the time, as is shown in Fig. 6,

and the same is true of the figures given by Henderson Smith (1923) for the destruction of *Botrytis* spores by phenol. These figures also give an approximately linear relation when the logarithm of the concentration is plotted against the logarithm of the time, as is shown in Fig. 7. The reason why both concentration and the logarithm of the concentration give an approximately linear relation when plotted against the logarithm of the time is that the figures cover only a small range of concentrations, and small changes

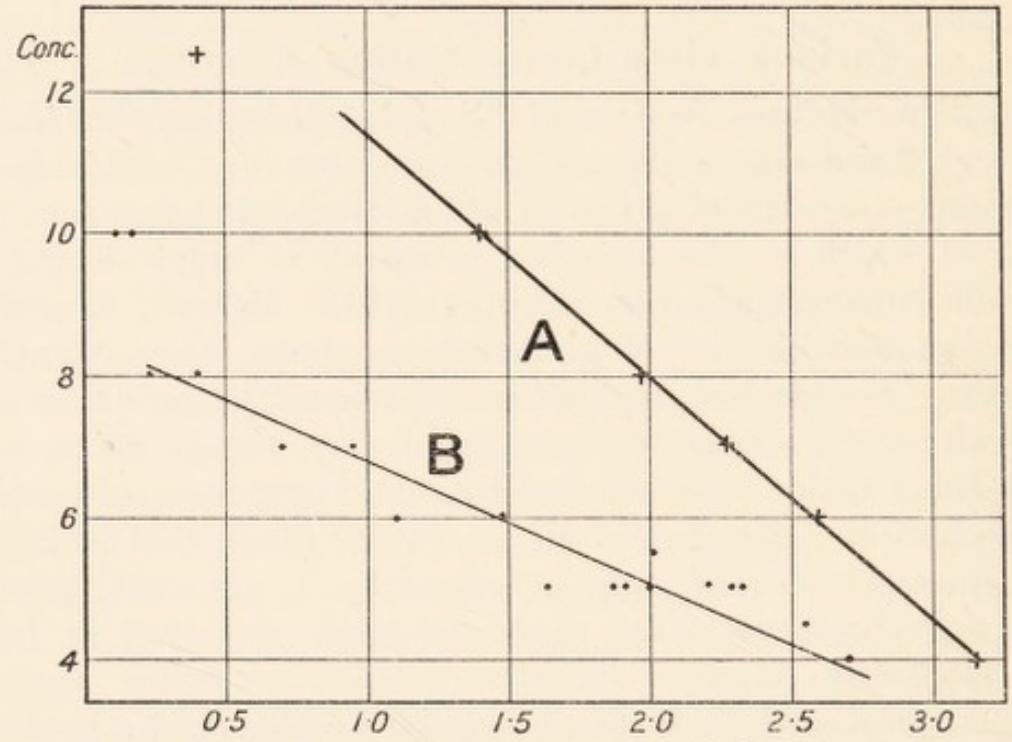

Fig. 6.—Time-concentration curves of disinfectants.

Abscissa : logarithm of time in minutes. Ordinate : concentration.
Curve A. Phenol (parts per 1000) and *Staph. pyog. aureus*. (Harriette Chick. 1908. Table XVII.)
Curve B. Coal-tar disinfectant (parts per 10,000) and *B. paratyphosus*. (Harriette Chick. 1908. Table XVIII.)

in concentration cause enormous differences in the time of action. The figures follow the relation $C^{6\cdot1}t$ = constant. This is a very surprising relation, but the same method of calculation gives similar values from the figures obtained by other workers who have studied the disinfectant action of phenol, e.g. Henderson Smith (1923), using *Botrytis* spores, found $C^{5\cdot26}t$ = constant ; Reichel (1909a), using *B. paratyphosus*, found $(C - C_m)^4 t$ = constant. As far as the writer can determine it is not possible to obtain any simple relation-

ship between C and t by subtraction of constants, and the evidence definitely shows that the time taken for phenol to produce disinfection varies inversely as the fifth power or more of the concentration.

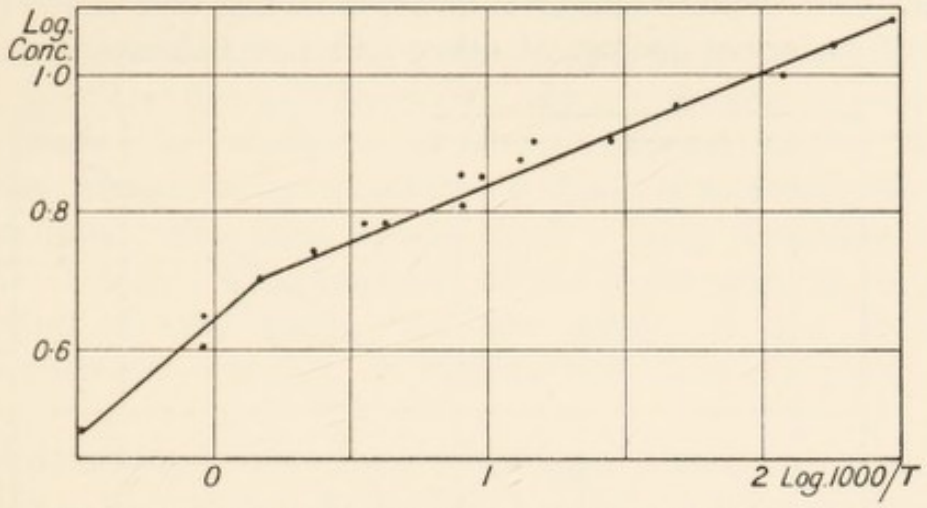

Fig. 7.—Time-concentration curve of phenol acting on *B. paratyphosus*.

Abscissa : logarithm of the reciprocal of the time in minutes. Ordinate : logarithm of concentration in parts per 1000. (Harriette Chick. 1908. Table XVI.)

Shackell (1922) studied the action of phenol on *Limnoria lignorum*. His figures for the rate of paralysis approximate to the formula $c^{0{\cdot}3}t = \text{constant}$. He also studied the rate of recovery by removing the animals to pure water as soon as they were paralysed, and measuring the time before movement was restored. His results are shown in Table IX. Animals paralysed by strong solutions took much longer to

Table IX

The rate of paralysis and recovery of *Limnoria* exposed to phenol (Shackell. 1922)

Concentration of phenol (per cent.).	Time in minutes.	
	Paralysis.	Recovery after paralysis.
0·125	10	7·42
0·25	8·34	15
0·375	7·54	26·55
0·5	6·82	31·79

recover than those paralysed by weak solutions. The only reasonable explanation for this difference is that at the moment of paralysis the animals exposed to strong solutions had taken up more phenol than had those exposed to weak solutions. In this case therefore there is direct evidence that the production of an equal biological effect did not indicate an equal

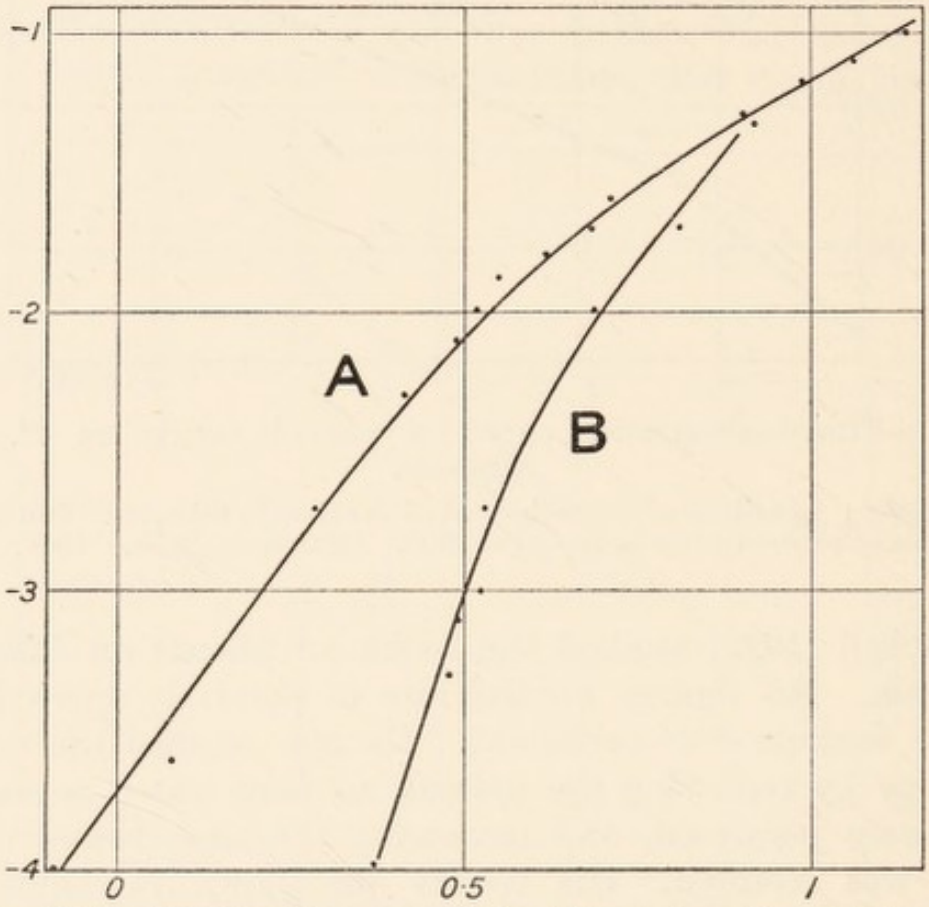

Fig. 8.—Time-concentration curves for the destruction of *Leuciscus phoxinus* by lead nitrate (curve A), and by mercuric chloride (curve B).

Abscissa: logarithm of the reciprocal of the time in minutes. (Curve A = log. 1000/T; curve B = log. 100/T.) Ordinate: logarithm of molar concentration. (Kathleen E. Carpenter. 1927.)

uptake of drug. This is a striking example of the uncertainty of the significance of measurements of the rate of action of drugs.

(ii) **Disinfectant Action of Heavy Metals.** The action of heavy metals forms an interesting contrast to that of phenol, for in the former case the time of action usually varies inversely as some power of the concentration between 0·3 and 0·5. The results obtained by Carpenter (1927) on a small fish cover a wide range of concentration and are shown in Fig. 8.

The relation between the logarithm of the concentration and log. $\frac{1}{t}$ is not linear, but the tangent of the central portion of the curves in the case of lead nitrate corresponds to $C^{0\cdot4}t =$ constant and in the case of mercuric chloride to $C^{0\cdot2}t =$ constant. The metals probably act by precipitating mucus and covering the gills of the fish and hence the times represent in part the time needed for asphyxia to occur. Several authors have, however, measured the disinfectant action of mercuric chloride and in most cases their figures show a fairly accurate linear relation between the logarithm of the concentration and the logarithm of the time, and are fitted by the formula $C^n t =$ constant when n is either less than or equal to unity.

The following values for n have been found with mercuric chloride: Krönig and Paul (1897) anthrax spores on garnets $n = 0{\cdot}8$. Chick (1908) suspension of *B. paratyphosus* $n = 0{\cdot}9$. Gregerson (1915) staphylococci and bacilli on garnets $n = 1{\cdot}0$. Gegenbauer (1921) staphylococci in suspension, $n = 0{\cdot}5$. Hartmann (1918) gives figures for the rate of destruction of plankton by mercuric chloride which approximate to $C^{0\cdot5}t =$ constant. Cook (1926B) measured the time taken by copper chloride to produce an equal effect in reducing the respiration of *Nitella* and found a linear relation between the logarithm of the concentration and the logarithm of the time; n had a value of between 0·22 and 0·35.

There is therefore a very general agreement that the rate of action of heavy metals on organisms varies as some power of the concentration less than unity. There is also direct evidence that the rate of action of mercuric chloride on the cells does not measure the rate at which the drug is fixed by the cells. Chick (1908) noted that mercuric chloride was rapidly adsorbed by bacteria but that it took some minutes to penetrate the cell wall. Cook (1926A) concluded that copper chloride entered the cells of *Nitella* almost immediately, but that there was a latent period of some fifteen minutes before a measurable depression of respiration was produced. Jowett and Brooks (1928) concluded that the diffusion of mercuric chloride into tissues was rapid as compared with the rate at which the drug acted on cellular enzymes.

(iii) **Various Delayed Actions.** In the case of some drugs it can be shown experimentally that they combine rapidly with the tissues but that there is a considerable delay before they produce any pharmacological action. This is true of the action of strophanthin and digitoxin on the vertebrate heart (Straub, 1931). The relations between the concentration and time of action of these substances have been measured by

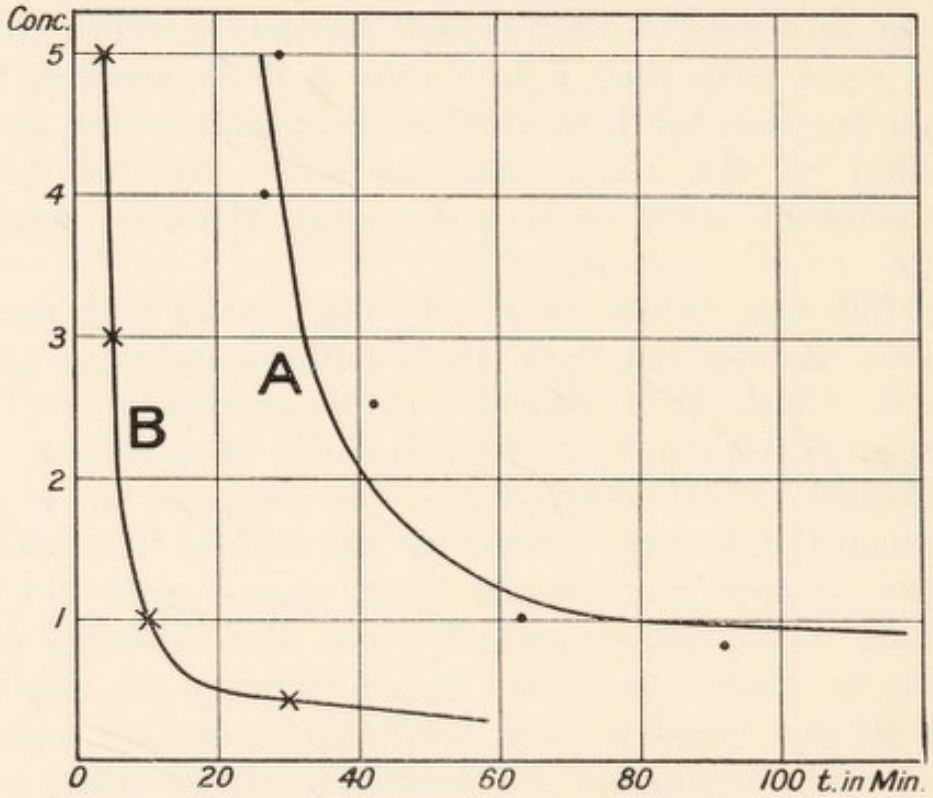

Fig. 9.—Time-concentration curves for the arrest of the frog's isolated heart by cardiac glucosides.

Abscissa: time in minutes until systolic arrest. Ordinate: concentration.

Curve A. Gratus strophanthin. Conc. = parts per 10,000,000. Curve drawn to formula $(C - 0{\cdot}5)(t - 20) = 30$. (Holste. 1912.)

Curve B. Digitoxin. Conc. = parts per 100,000. Curve drawn to formula $(C - 0{\cdot}2)(t - 3{\cdot}3) = 5{\cdot}2$. (Oppenheimer. 1913.)

several workers and two of the more complete sets of figures are shown in Fig. 9. These figures only cover a narrow range of concentration and they can be fitted by several different formulæ.

It is an undoubted fact that there is a minimum period within which an action cannot be produced and that there is a minimum concentration below which an action is not produced in infinite time. There is therefore a practical

justification for the use of the formula $(C - C_m)\ (t - t_m) =$ constant. The results obtained in this class of experiment can be fitted fairly well by such formulæ as is shown in Fig. 9. The results shown in this figure can, on the other hand, be fitted almost equally well by the formula $(C - C_m)^n t =$ constant. In the case of Oppenheimer's figures a good fit is obtained by the formula $C^{0\cdot8}t =$ constant and in the case of Holste's figures by the formula $(C - 0\cdot3)^{0\cdot6}t =$ constant. On the other hand, figures given by Sollmann, Mendenhall

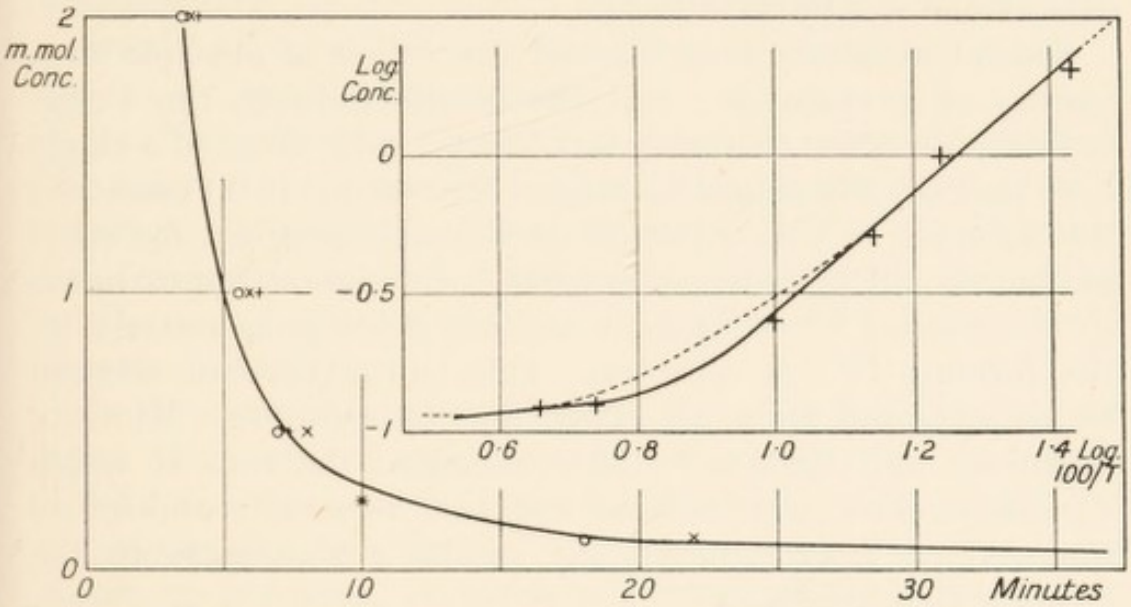

Fig. 10.—Time-concentration curves for the action of quaternary ammonium iodides upon nerve-muscle preparations of the frog.

Abscissa: time in minutes. Ordinate: millimolar concentration. Points: o = amyl trimethyl ammonium iodide; + = hexyl t.m.a.i.; × = octyl t.m.a.i. Curve drawn to the formula $c(t - 2\cdot5) = 2\cdot2$.

Inset. Abscissa: logarithm of 100/T. Ordinate: logarithm of m.molar concentration. Continuous line and crosses = results observed. Dotted line drawn to the formula $k(c - 0\cdot1)^{0\cdot4} = 100/T$. (Ing and Wright. 1932.)

and Stingel (1914) for the relation between concentration of ouabain and time of arrest of the isolated frog heart approximate to the formula $C^{1\cdot3}t =$ constant.

These curves can be compared with the time-concentration curves obtained by Ing and Wright (1931) for the rate of paralysis of isolated nerve-muscle preparations of the frog's sartorius by quaternary ammonium iodides. In this case also the drug was proved to be fixed rapidly by the tissues, and this rapid fixation was followed by some slower secondary process. Fig. 10 shows that the times observed can be fitted

fairly accurately by the formula $C(t - 2{\cdot}5) = \text{constant}$, whilst the inset shows an equally good fit with the alternative formula $(C - 0{\cdot}1)^{0{\cdot}416}t = \text{constant}$.

(iv) **Alcohol Paralysis.** The action of alcohols on cells is fairly certainly of a physical rather than of a chemical nature. Moreover, they probably act on the surface of cells and their rate of action on exposed cells such as the frog's heart is very rapid. Hence it may be assumed that the narcotics act quickly once they reach the cell surface, and that delay in action in this case is due chiefly to diffusion.

Several observers have studied the action of alcohols on a variety of preparations and the results indicate the extraordinary diversity that may be obtained with drugs of a single type that act in a simple manner. Hartmann (1918) measured the influence of concentration on the time required for ethyl alcohol to kill plankton organisms. His figures approximate to the relation $Ct = \text{constant}$ and are fitted more exactly by the formula $C^{1{\cdot}2}t = \text{constant}$. This is the type of relation to be expected from an effect due to diffusion. Kuuisto, Suominen and Renqvist (1294) measured the rate at which various alcohols paralysed the frog's gastrocnemius and for all the alcohols (methyl-octyl) the results approximate to the formula $Ct = \text{constant}$.

Blume (1925) measured the rate of paralysis of both the nerve-endings and muscles of the frog. In the case of heptyl alcohol he obtained graded effects over a range of concentration from 0·006 to 0·04 per cent. and the figures approximate to the formula $C^{0{\cdot}8}t = \text{constant}$, which is in fair agreement with the results already quoted. In the case of ethyl alcohol he obtained the following peculiar results in the case of both muscle and nerve-endings : 5 p.c. (by wt.) paralysed in 2 hours, 4·5 p.c. in 4 hours, 3·5 p.c. in 12 hours, whilst less than 3 p.c. did not produce paralysis. These figures would correspond to a formula $C^5t = \text{constant}$. An alternative method is to subtract the threshold concentration of 3 p.c. and then $(C - 3)t$ gives an approximately constant figure.

Similarly Cole and Allison (1930) studied the stimulant action of alcohols on organisms. Their figures for the time required by alcohols (methyl-amyl) to stimulate planaria are the fullest. These can be fitted approximately by the formula

C^3t = constant or alternatively by $(C - C_m)t$ = constant, where C_m is the threshold concentration that produces an action. It will be seen from these examples that the interpretation of the time-concentration curves of alcohols depends entirely on the mode of treatment of the figures. The majority of the results appear to approximate to the formula Ct = constant.

The examples discussed above illustrate certain striking variations in the relations between concentration and time. The literature of this subject is too extensive to permit any general review in this volume, but a few other examples of special interest deserve mention.

(v) **Hæmolysis.** Ponder has made an extensive study of the kinetics of saponin hæmolysis. His arguments are mostly based on the assumption that the rate of production of hæmolysis measures the rate of some form of chemical combination and he has advanced evidence in support of this belief as regards saponin hæmolysis.

In the case of many hæmolytics it is certain that the measurement of the rate of appearance of hæmolysis measures not the rate of combination of drug with the red blood-corpuscle, but the time taken by the red blood-corpuscle to swell after it has taken up the drug. Christophers (1929) showed this was the case with hæmolysis produced by acid and alkalies, for these substances in weak concentrations reacted with the corpuscles in less than 5 minutes, but hæmolysis did not commence for an hour. Fig. 11 gives an example of the results obtained by Ponder and Yeager (1931). The figures show the relation between saponin concentration and the time at which 50 p.c. hæmolysis occurs. The figures approximate fairly closely to the relation C^2t = constant, as is shown in Fig. 11, but Ponder interprets them by a much more complex formula. He found (1932) that the time-concentration curve of hæmolysis could be interpreted by the formula $\frac{dx}{dt} = K\,(C - y - x)^n$. ($C$ = original conc. of saponin, x = saponin fixed by erythrocytes and y = saponin inactivated by the hæmoglobin released into the solution.) A correction also was necessary for delays due to the initial mixing process. Hæmolysis appears at first sight to be a process particularly suitable for the study of drug

kinetics, but Ponder's results show that the system is much more complex than it appears and that the interpretation of the results is extremely difficult.

The relation between concentration and time of action of saponins is necessarily complex because a large proportion of the saponin present in the solution is fixed by the cells or by the laked hæmoglobin. In the case of such drugs as ammonia, no significant change in drug concentration is likely

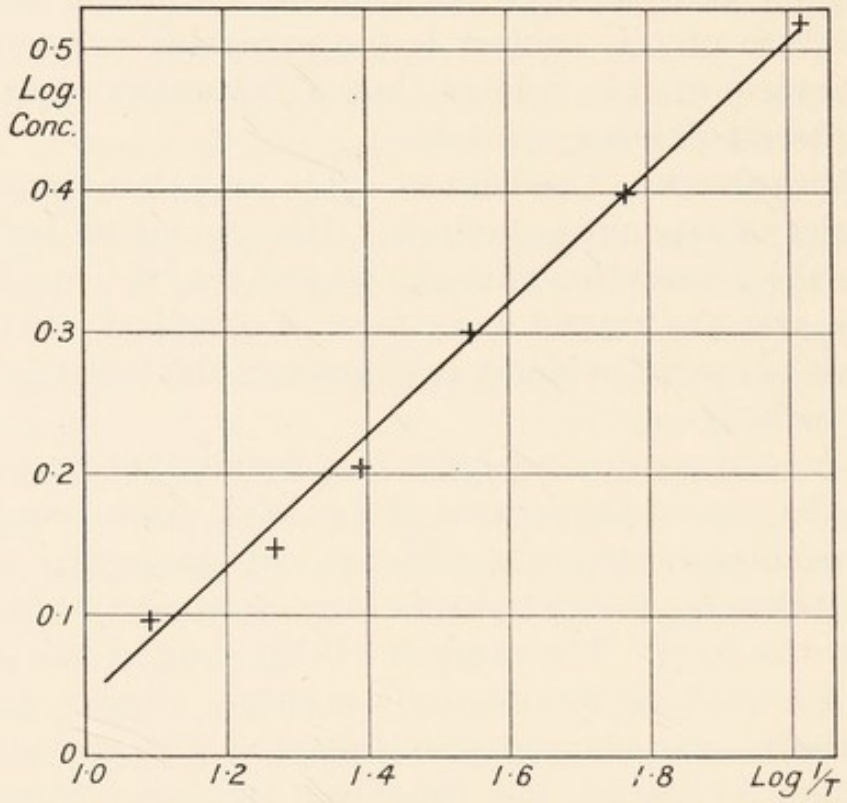

Fig. 11.—Time-concentration curve for the hæmolytic action of saponin.

Abscissa : logarithm of the reciprocal of the time in minutes. Ordinate : logarithm of micrograms of saponin added. (Ponder and Yeager. 1931.)

to occur during the action. In such cases most observers have found an approximation to the formula $Ct = \text{constant}$. If this formula be written $C^n t = \text{constant}$, then the values of n found by various observers for various simple hæmolytics are as follows :

Ammonia : $n = 0{\cdot}71$ (Gros, 1910) ; $n = 1{\cdot}077$ (Stadler and Kleeman, 1911) ; $n = 1$ (Arrhenius, 1915, p. 63).

Sodium hydrate : $n = 1{\cdot}3$ (Gros, 1910) ; $n = 1$ (Arrhenius, 1915).

Sodium carbonate : $n = 0{\cdot}68$ (Gros, 1910).

(vi) **Various Disinfectants.** The results obtained with phenol and with heavy metals have already been considered. Chick (1930) has collected and tabulated the evidence available on this subject and has worked out the following values of n in the formula $C^n t$ = constant from the figures given by various authors.

As already mentioned $n = 0{\cdot}5$ to $1{\cdot}0$ in the case of mercuric chloride, and the same is true of silver nitrate. With hydrogen peroxide $n = 0{\cdot}5$ (Reichel, 1908, 1909B). In the case of iodine and formaldehyde $n = 1{\cdot}0$ (Gregerson, 1915). Values of n from $0{\cdot}5$ to $2{\cdot}0$ have been found with acids. HCl $n = 0{\cdot}5$ (Paul, Birstein and Reuss, 1910); $n = 1{\cdot}0$ (Gregerson, 1915); $n = 1{\cdot}5$ (Gegenbauer and Reichel, 1913); acetic acid $n = 1{\cdot}0$ and n-butyric acid $n = 2$ (Paul, Birstein and Reuss, 1910). High values of n have been obtained for phenol ($n = 4{\cdot}0$–$6{\cdot}25$) as already mentioned; for coal-tar disinfectants ($n = 8{\cdot}3$ (Chick, 1908)); thymol and chloral hydrate ($n = 4$ (Gregerson, 1915)).

(vii) **Lethal Agents on Small Organisms.** The relation between concentration and time of death of small organisms has been measured for a large number of drugs. The author has estimated the general slope of the curves obtained when log. concentration is plotted against log. time. The results are shown in Table X. These results differ from those obtained with disinfectants in that the time measured is the time at which the organisms are paralysed and hence is the sum of

TABLE X

Time-concentration relations obtained with small organisms. Values of n in the formula $C^n t$ = constant

Drug.	Range of molar concentration.	Organisms.	Value of n.	Observer.
Acids.				
HCl . . .	0·1–0·01	Cladocera (13° C.)	1·2	Hartmann (1918)
HNO_3 . . .	,,	,,	1·3	,,
H_2SO_4 . . .	,,	,,	1·04	,,
Formic . . .	,,	,,	1·12	,,
Acetic . . .	,,	,,	1·2	,,
Alkali.	0·1–0·01	,,	1·1	,,
NaOH . . .	*p*H7·5–9·2	Spirostomum ambiguum	0·5 and 0·83	Jenkins (1926)

TABLE X—*continued*

Drug.	Range of molar concentration.	Organisms.	Value of n.	Observer.
Heavy Metals.				
$FeCl_3$ . . .	0·1–0·01	Cladocera	1·0	Hartmann (1918)
$CuSO_4$ . . .	,,	,,	0·7	,,
$HgCl_2$ . . .	,,	,,	0·55	,,
	0·0001–0·01	Leuciscus phoxinus	0·16	Carpenter (1927)
$Pb(NO_3)_2$ . .	,,	,,	0·32	,,
$CuCl_2$ . . .	0·0001–0·1	Nitella	0·22–0·34	Cook (1926B)
Alkaloids.				
Strychnine. .		Fundulus	0·35	Sollmann (1906)
Phenol and Derivatives.				
Phenol . .	0·125–0·5 per cent.	Limnoria	0·3	Shackell (1922)
Cresols . . .	0·125–0·25 per cent.	,,	0·25	,,
Resorcinol .	0·25–0·5 per cent.	,,	1·0	,,
Pyrocatechin .	,,	,,	1·0	,,
Narcotics.				
Chloral hyd. .	1·0–0·1 molar	Cladocera	0·75	Hartmann (1918)
Ethyl alcohol	3·0–0·3 ,,	,,	1·2	,,
Various organic agents.				
Glycerine . .	3·0–0·3 M.	Cladocera	1·3	Hartmann (1918)
Saline changes.				
Sea water . .		Gammarus	3·1	Ostwald (1907)
		Daphnia	5·0	Dernoscheck (1911)
		Limnoria	4·0	White (1929)
Neutral salts.				
KNO_3, KBr, $CuCl_2$, NaCl		Cladocera	0·7–1·1	Hartmann (1918)
		Gammarus	0·83	Ostwald (1907)
		,,	1·4	Pawlow (1925)

the delays due to chemical reactions, diffusion and lag in the biological response. In most cases the figures appear to

approximate to Ct = constant, but the heavy metals show a value for n lower than unity in nearly all cases.

(viii) **Inhaled Gases.** The relation between concentration and rate of uptake of narcotics has been studied by Henderson and Haggard (1927). In this case the rate of action depends on the rate of excretion as well as on the rate of uptake of the drug, and hence the conditions are very complex.

The poisoning of vertebrates by hydrocyanic acid is a somewhat simpler process because most of the drug inhaled is fixed

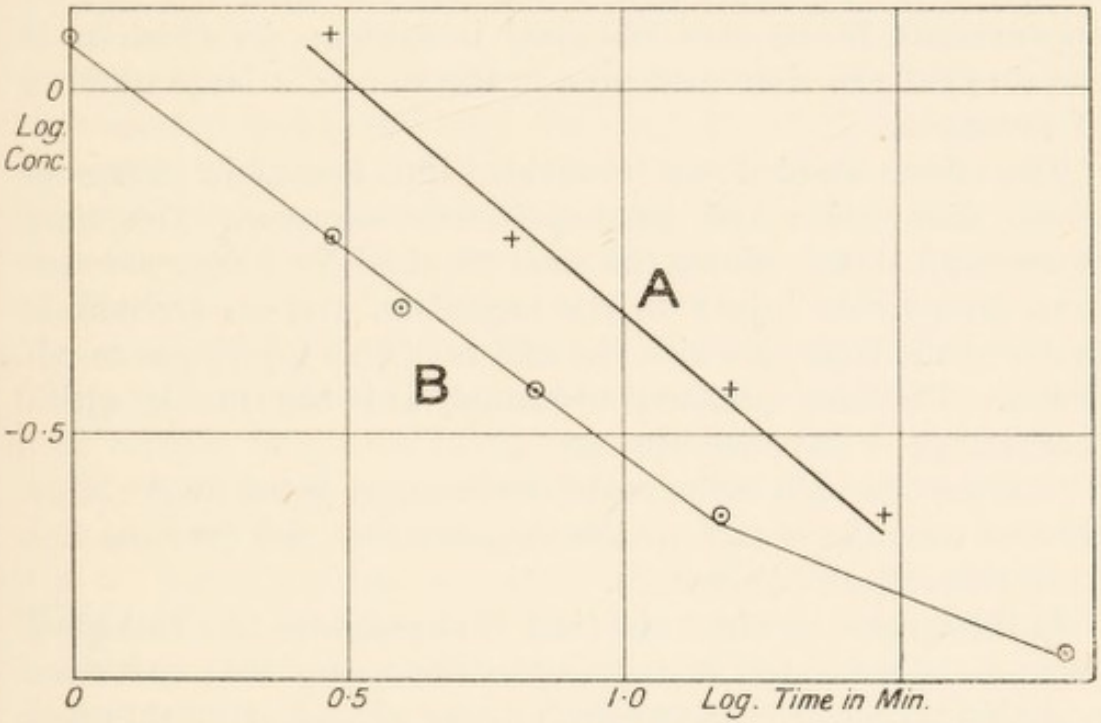

Fig. 12.—Time-concentration curves for the action of hydrocyanic acid vapour upon goats (curve A) and upon rabbits (curve B).

Abscissa: logarithm of time in minutes. Ordinate: logarithm of the concentration of HCN in mg. per litre of air. (Barcroft. 1932.)

by the tissues. This has been studied by Barcroft (1931), who has shown that there are several variables. For example, the cat is killed more rapidly than the rabbit, and this is due chiefly to the fact that hydrocyanic acid acts as a stimulant to the respiratory centre of the cat but not to the rabbit. The figures for goats and rabbits are the fullest, and Fig. 12 shows that in these cases relation between log. concentration and log. time is nearly linear, and the rate of poisoning varies as $C^{1\cdot7}$ in the case of rabbits, and as $C^{1\cdot4}$ in the case of goats.

The approximate agreement in this case is interesting because

it is obvious that the time measured must depend on a number of independent variables such as rate of ventilation, minute volume of blood, etc., and cannot possibly express the rate of any chemical reaction.

Discussion

The evidence presented in this chapter shows that the relation between concentration and time of action of drugs can be expressed by the formula $C^n t = \text{constant}$. This formula usually gives an approximate but not an exact fit, but an approximate fit is often obtained in systems in which it is certain that the time measured is the sum of a large number of processes.

The effects studied can be divided into two main groups of which disinfection and hæmolysis are examples. The time measured in disinfection is the time required for a drug to cause some irreparable injury to the organism, and no account is taken of the time at which the effects of this injury are manifested. The time measured in hæmolysis is the time at which the biological response appears.

Curiously enough both groups of effects are fitted by the same general formula, a fact which suggests that the formula has little theoretical significance.

In those cases in which the time of appearance of a biological response is measured it appears to the writer that the most probable processes that are likely to be expressed by the time are, firstly, time of diffusion and, secondly, time of diffusion plus time spent in alteration of the colloids inside the cells.

Ostwald (1907) suggested that since the fixation of drugs by organisms was an adsorption effect, and since adsorption equilibrium was expressed by the formula $KC^n = \frac{x}{m}$ ($C = $ conc. of solution, $x =$ amount adsorbed by mass m), therefore the formula $KC^n = \frac{1}{t}$ ought to express the time relations of fixation.

The theoretical justification for this argument is not clear, but there is a more direct objection, namely that adsorption itself is a relatively rapid process, and that delays in the uptake of drugs from solution in sorption processes are believed to

be due either to diffusion, consequent on depletion, or to slow secondary processes that follow the initial fixation.

Watson (1908) interpreted the formula $C^n t = K$ as a proof of the occurrence of polymolecular reactions. This theory is subject to Nernst's objection that the probability of the simultaneous collision of several molecules is extremely small, and hence the velocity of polymolecular reactions can only be appreciable under quite exceptional conditions.

The fact that the value of n can vary from 0·3 to 8 appears surprising, but such variations can be paralleled in non-living colloidal systems. Gann (1916) studied the rate of precipitation of an aluminium hydrate sol by various inorganic salts and found that the rate of precipitation varied as a very high power of the concentration of the coagulator; in some cases as the seventh power. Freundlich and Gann in a discussion of these results pointed out their remarkable similarity to certain biological phenomena. On the other hand, Moravek (1929) found that the rate of diffusion of lead nitrate into gelatine containing chromate was highly complex and that over certain ranges of concentration the rate of penetration might decrease as the concentration rose. It seems possible that the very low values of n found with heavy metals may be due to these altering the permeability of the cell surfaces. Stiles (1920) gives figures for the rate of penetration of gelatine by sodium chloride, and the times needed to penetrate an equal depth indicate that the rate of penetration varies as the square root of the concentration, or even as some lower power.

The relations between concentration and rate of action that are obtained with living cells can therefore be paralleled by results obtained with fairly simple colloidal systems. The information regarding the kinetics of colloidal changes is somewhat meagre because colloidal systems have been found inconveniently complex for such studies, and this fact is the best indication of the difficulties attending kinetic studies on living organisms.

The outstanding facts about the relations between concentration and time taken for a biological response appear to the writer to be as follows. The rate of action usually varies approximately as some power of the concentration and this power may vary from 0·2 to 8. Usually the time-concentration

relations of a drug are similar when it is tested on different organisms, but when the active concentration is much lower in one organism than another, then the time-concentration relations may be totally different. It seems doubtful if the time taken by an organism to show some response to a drug is ever a simple measure of the rate at which a single chemical reaction is proceeding.

Porodko (1926) found that the relation between the intensity (x) of a stimulus and the time (y) necessary to provoke reactions in plants could be expressed by the formula $y = k/x^m$. In this case the time must be a measure of the lag in the biological response.

Since the relation between the strength of stimulus and the duration of the latent period is the same as that between the concentration of drug and the duration of the latent period it appears most probable that the delay in both cases is of a similar nature and expresses the time taken for the occurrence in the cells of secondary processes which are initiated by the stimulus or by the combination of the drug with the cell.

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CHAPTER VI

INDIVIDUAL VARIATION IN POPULATIONS

The Measurement of Individual Variation

A simple example of the individual variation of the response of animals to drugs is shown in Fig. 13. The frequency polygon expresses the results obtained with 573 cats, on each of which

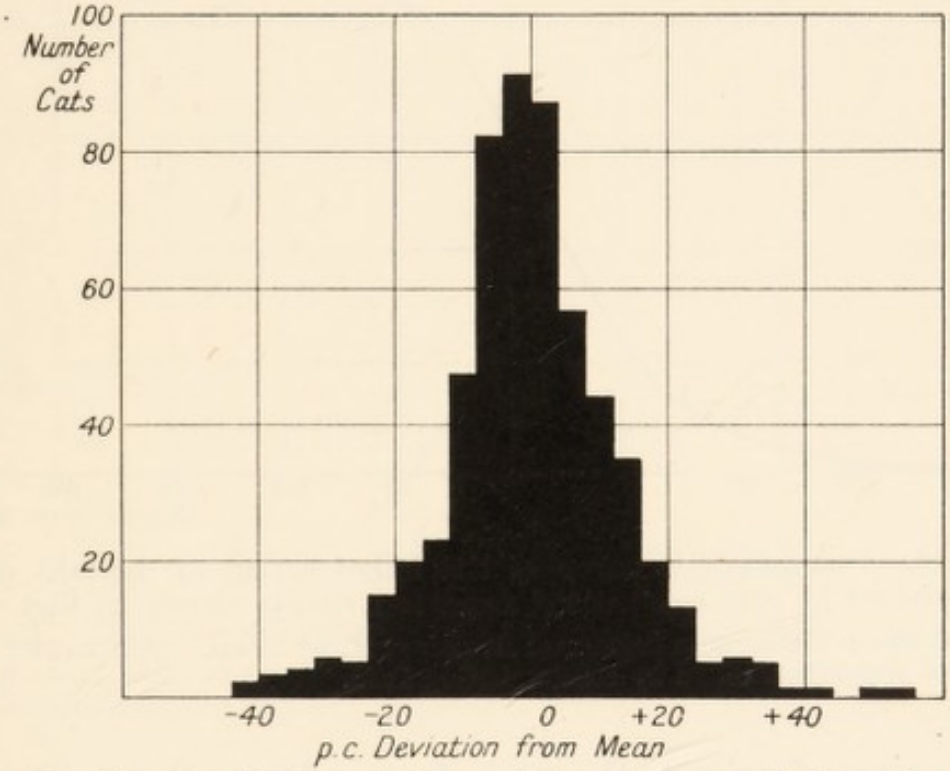

Fig. 13.—The distribution of the variation in susceptibility of cats to digitalis.

Abscissa: Deviation of lethal dose of tincture of digitalis from mean lethal dose, expressed as per cent. of the mean. Ordinate: number of cats (total number 573). (Lind van Wijngaarden. 1924.)

the minimal lethal dose of a standard preparation of digitalis was measured. The drug was given as an intravenous injection to anæsthetized animals and was run in slowly so that the exact dose needed to arrest the heart could be measured in each animal. (Lind van Wijngaarden, 1924.) The percentage

of a population of cats that will be killed by any particular dose of digitalis can be calculated by integrating these figures, and the curve relating dosage and percentage of deaths is shown in Fig. 14. This type of curve is termed a "characteristic curve." In the particular example chosen it was possible to measure the individual lethal dose in a large population and thus obtain directly a frequency polygon from which a characteristic curve could be constructed.

In most cases the measurement of the lethal dose for each individual is impossible and it is necessary to give each member

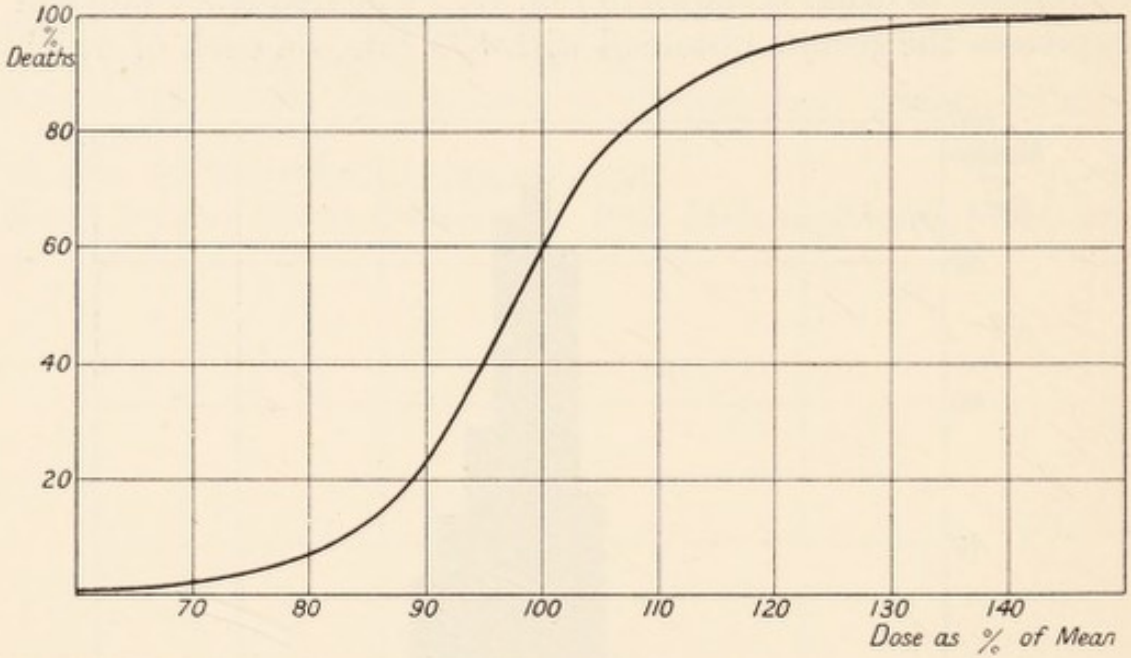

Fig. 14.—Characteristic curve of the lethal action of digitalis on a population of cats constructed from the results shown in Fig. 13.

ABSCISSA: lethal dose expressed as per cent. of mean. ORDINATE: per cent. of population killed by corresponding dose.

of a group of animals the same dose and note the number of survivors. The repetition of this procedure with a range of doses finally provides a characteristic curve. For example, Fig. 15 shows the results obtained by injecting 8 groups, each comprising about 200 frogs, with 8 different doses of ouabain (Chapman and Morrell, 1931).

A characteristic curve can be analysed, and from the analysis a frequency polygon can be constructed. From the frequency polygon it is possible to calculate the standard deviation of the population. This is the most scientific method of describing

variation and this technique has been described in detail by Trevan (1927). This method is unnecessarily laborious for the purposes of the present discussion. It is however desirable to have some method for describing the shape and spread of characteristic curves. This can be done most simply by measuring the median (50 p.c. action) and the lower and upper quartiles (25 and 75 p.c. action). The semi-interquartile range expressed as the percentage of the median provides a simple measure of the spread of the curve. This measurement also

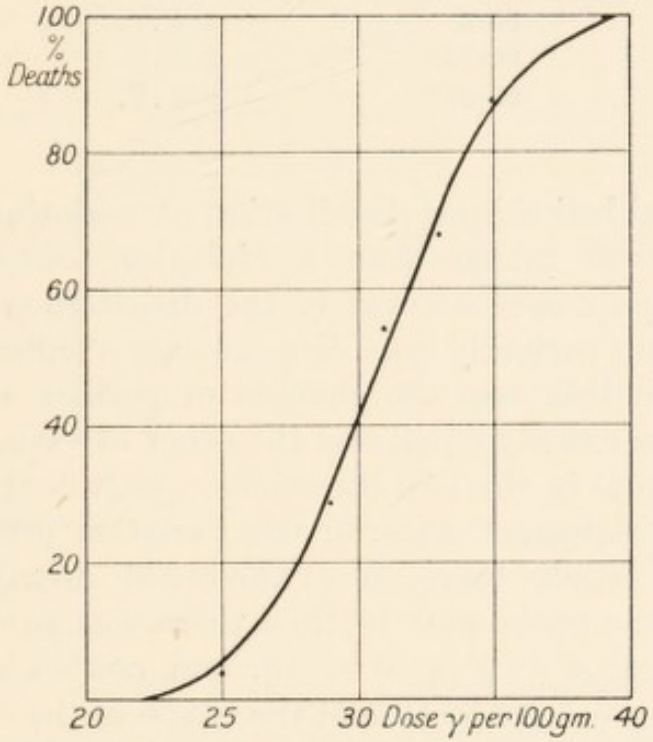

Fig. 15.—Characteristic curve of the lethal action of ouabain on frogs. ABSCISSA : dose in γ per 100 gm. ORDINATE : per cent. of deaths. (Chapman and Morrell. 1931.)

gives the probable variation because there is an exactly even chance of any individual falling within or without the interquartile range. The point of steepest rise of the curve indicates the mode. In cases of symmetrical variation the median and mode are identical, but in skew curves they are not identical and the difference between the median and the mode provides a standard of comparison regarding the degree of skew in curves expressing biological variation.

In the case of a population which shows a " normal " bell-shaped distribution of variation, the proportion which is

included within any given multiple of the standard deviation or of the probable variation can be calculated exactly, and some of these figures are given in Table XI.

TABLE XI

Limit of variation. (σ = standard deviation.)	Per cent. of population included within limit.	Limit of variation. (P.V. = probable variation.)	Per cent. of population included within limit.
$\pm 1\sigma$	68·5	$\pm$ 1 P.V.	50
$\pm 2\sigma$	95·5	$\pm$ 2 P.V.	83·4
$\pm 3\sigma$	99·73	$\pm$ 3 P.V.	95·6
$\pm 4\sigma$	99·992	$\pm$ 4 P.V.	99·3

The normal bell-shaped distribution of variation is however a mathematical rather than a biological conception. For example, it expresses accurately the distribution of numbers obtained when perfectly true dice are cast a sufficient number of times. In this case the chances of positive and negative variations are exactly equal and the range of variation possible is exactly equal in the two directions. Galton (1879) pointed out that in biological material the variation often showed a geometrical rather than an arithmetical distribution. For example, if the mean were 10, then there was an equal chance of a variation of -5 or of $+10$, and not an equal chance of -5 and $+5$. Inspection of the shape of the characteristic curves obtained with drugs shows that this type of distribution of variation occurs frequently.

Another important point is that if one characteristic A has a symmetrical distribution of variation, then a second characteristic B can only have a symmetrical distribution of variation if B varies directly as A. For example, if the variation of length of an organism is distributed in a symmetrical manner, then its area or volume are likely to be distributed asymmetrically. It is rare in pharmacology to find simple linear relations between the amount of drug and the amount of action produced, and it is much commoner to find logarithmic relations. It is indeed somewhat surprising that symmetrical distributions of variation in response to drugs should occur as frequently as they do.

Types of Characteristic Curves obtained

Curves relating the dosage of drugs and the incidence of some effect are usually termed characteristic curves. Figs. 14 and 15 are examples of steep and nearly symmetrical characteristic curves. The probable variations are about 8 p.c. of the mean, which is an unusually low figure to obtain with drug responses. Usually the probable variation is more than 10 p.c. of the median and frequently is more than 20 p.c.

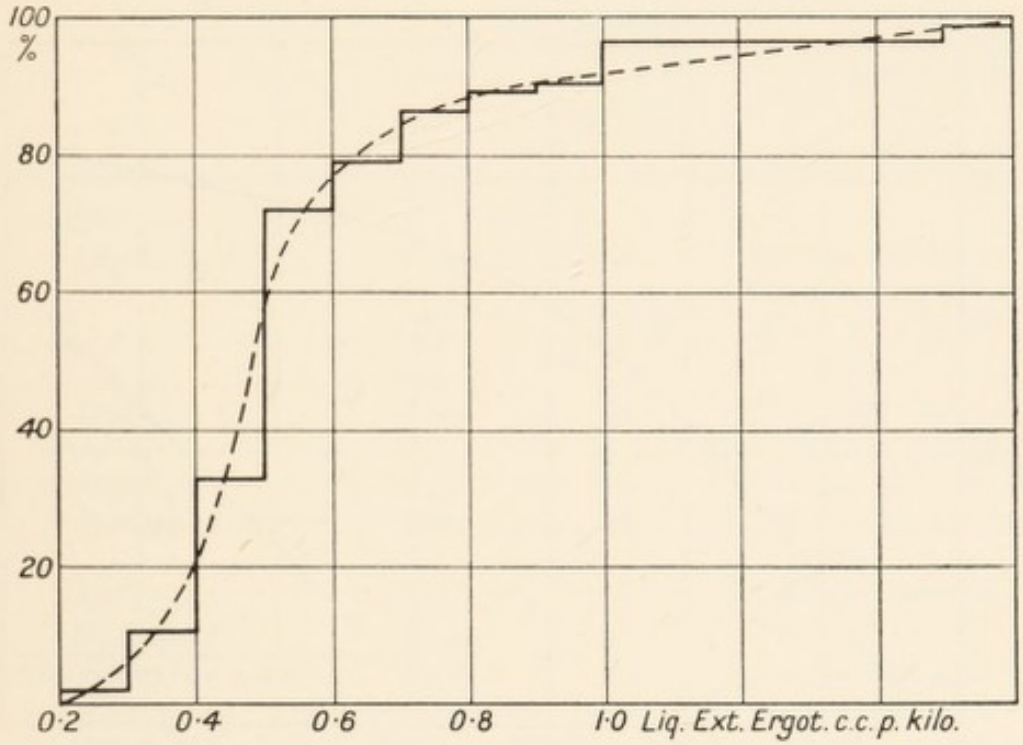

Fig. 16.—Characteristic curve of the action of liquid extract of ergot in producing cyanosis in cocks' combs.

Abscissa: dose in c.c. per kilo. Ordinate: per cent. of population showing response. (Gittinger and Munch. 1927.)

For example, the British Pharmacopœia 1932 gives figures for action of digitalis in killing frogs, and these show a probable variation of 17 p.c. of the median. This is equivalent to a standard deviation of about 25 p.c. of the mean.

The probable variation can be reduced to a minimum by working with as uniform a population as possible, under strictly uniform conditions and by choosing as sharp an end-point as is possible. Very frequently the characteristic curves show a considerable amount of skew and Fig. 16 shows a shape of curve that is frequently obtained. This curve is asym-

metrical with a long tail running out to the right and the probable variation is 18 p.c. of the median.

Slightly skewed curves of this type are met with frequently in biological data and are of interest because a nearly linear relation is obtained if the dosage is plotted against the logarithm of the "surviving" population (i.e. the proportion that fails to respond). This fact was pointed out by Peters (1920) and it has an important bearing on the theoretical significance of the logarithmic relationships that are frequently observed in

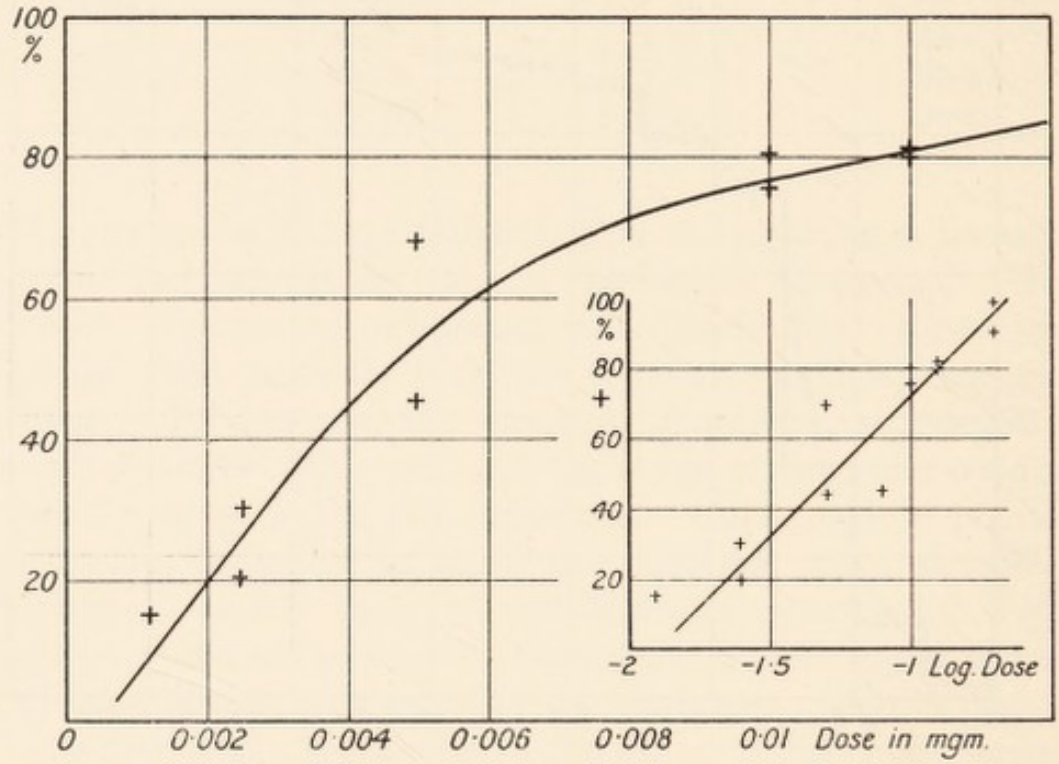

Fig. 17.—Characteristic curve of the lethal action of dysentery toxin on mice.

Abscissa: dose of toxin in mg. per 20 gm. Ordinate: per cent. mortality.

Inset. Logarithm of the dose plotted against the per cent. mortality. (Trevan. 1929.)

the case of time-action curves. Curves of the type shown in Fig. 16 express approximately the geometric distribution of variation described by Galton.

In the cases considered hitherto the organisms were large enough to permit the measurement of the dose administered to each individual. In such cases the characteristic curves are usually either symmetrical or else slightly skewed. Occasionally very skew curves are obtained. For example, Fig. 17 shows the characteristic curve of the destruction of

mice with dysentery toxin (Trevan, 1929). The curve has a general exponential form and the inset in Fig. 17 shows that there is a nearly linear relation between action and log. dose.

Table XII shows the probable variations of the characteristic curves of a number of drugs. These values were in most cases estimated by the writer from the data given by the various authors.

TABLE XII

Drug.	Animal.	Probable variation expressed as per cent. of median.	Author.
(i) *Lethal doses :*			
Digitalis . .	Frogs	13	Trevan (1926)
,, . .	Frogs	12·5	Behrens (1929)
,, . .	Cats	7·25	van Wijngaarden (1926)
	Frogs		
Ouabain . .	Rats	8·3	Chapman and Morrell (1931)
Squills . . .	Rats	19	Winton (1927)
Aconitine . .	Rats	21	Munch and Gittinger (1929)
Cocaine. . .	Mice	16	Trevan (1927)
Echitamine .	Mice	25	Trevan (1927)
Neosalvarsan .	Mice	12	Durham et al (1929)
Thallium sulphate	Rats	17	Munch (1931)
Adrenaline. .	Mice	71	Schultz (1909)
Diphtheria toxin	Guinea-pigs	9	Sudmersen and Glenny (1910)
Dysentery toxin	Mice	27	Sudmersen, Runge and O'Brien (1924)
,, ,,	Mice	88	Trevan (1929)
(ii) *Various effects :*			
Insulin convulsions	Mice	46	Hemmingsen and Krogh (1926)
Insulin convulsions	Mice	29	Trevan and Boock (1926)
	Rabbits	45	Marks (1926)
Ergot cyanosis	Cockerels	12	Gittinger and Munch (1927)
Salicylism . .	Male patients	24	Hanzlik (1913)

Factors causing Individual Variation in Response to Drugs

Variation may be regarded as one of the fundamental characteristics of living matter, and it is always found when the individuals of any population are measured in any way. The occurrence of variation in the response to drugs is therefore not a matter for surprise, moreover certain variables are known to occur which are likely to cause variation in response to drugs.

In the first place there is an extensive individual variation in the ratios between the weights of organs and the body weight. Brown, Pearce and van Allen (1926) measured the organ and body weights of 645 rabbits and found that the coefficient of variation (i.e. standard deviation expressed as the percentage of the mean) varied from 12 in the case of the heart ratio to 61 in the case of the thyroid ratio. The author found that the coefficient of variation of the heart ratios of 120 cats was about 15, and Sato (1931) found the same figure in dogs. Variations of a similar or greater extent have been shown in the case of human organs. Variations in the heart ratio are obviously likely to cause variation in the M.L.D. per kilo. of the cardiac glucosides.

A considerable range of individual variation in biochemical factors has been demonstrated in several cases. For example, Clough, Allen and Root (1923) found a coefficient of variation of 16 in the blood-sugar of 100 normal rabbits. Estimations of the total reducing substance in the hearts of 72 normal frogs were made in the writer's laboratory and showed a coefficient of variation of 31. Variations such as these in the normal carbohydrate content of the blood and other organs might be expected to cause a considerable variation in the response to insulin. In the case of small organisms the number of factors that can be measured is limited, but all the evidence available indicates an extensive variation both in form and in chemical properties (cf. p. 115). The occurrence of a considerable individual variation in the response given to drugs by unicellular or multicellular organisms does not therefore call for any special explanation.

The extent of the individual variation is likely to depend on the uniformity of the population, and this has indeed been

found to be the case. Winton (1927) measured the lethal action of red squills on rats, when given by mouth, and found that female rats were twice as susceptible as males. He compared the action of the drug on ordinary stock rats and on inbred rats and found that the standard deviation in the former case was 67 p.c. of the mean and in the latter case only 21 p.c. This difference implies that ten times as many animals of a mixed stock as of rats of a genetically standardized stock would be needed to achieve the same degree of accuracy. A similar difference, which is shown in Fig. 18, was found by

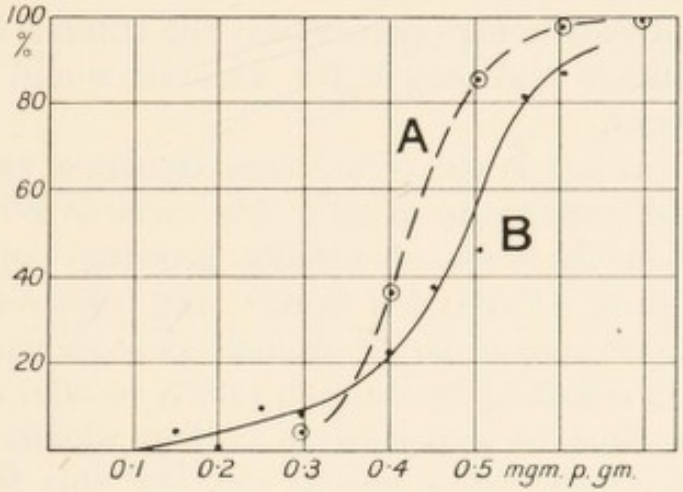

Fig. 18.—Characteristic curve of the lethal action upon mice of intravenous injections of neoarsphenamine (neosalvarsan).

Abscissa: dose in mg. per gm. Ordinate: per cent. mortality.
Curve A. Mice from inbred stock (903 used).
Curve B. Ordinary stock mice (370 used). (Durham, Gaddum and Marchal. 1929.)

Durham, Gaddum and Marchal (1929), who measured the lethal dose of neosalvarsan for mice.

Static and Dynamic Variation

Gray (1931) has pointed out the importance of distinguishing between static and dynamic variations. In the case of static variation any particular cell shows a fairly constant deviation from the mean. For example, if one cell is larger than the average and another smaller they will tend to show the same characteristics on repeated examination. On the other hand, in the case of dynamic variation a single organism will at one time rise above and at another time fall below the average. There are many cyclic functions in living cells and individuals

will show dynamic variation as regards any characteristic that depends on such a function.

An important distinction between static and dynamic variation is that in the former case there is a likelihood that the distribution of variation will approximate to the normal or bell-shaped type, whereas any form of distribution may occur in the case of dynamic variation. The œstrous changes in female rodents are a simple example of dynamic variation. In this case a count of the percentage of cornified cells present in a number of vaginal smears from a population of rats would show a U-shaped distribution of variation. The animals in œstrus would show a high percentage, the animals in diœstrous a low percentage and only a few animals would show intermediate figures.

Variation in age is the commonest dynamic variation that influences the response to drugs ; this variable is of particular importance in the case of rapidly growing populations of micro-organisms. Variations in age may however be of considerable importance in larger animals as is shown by quantitative results obtained by Janisch (1927, p. 309) on the meal moth. The time of exposure to carbon dioxide needed to kill newly hatched moths was 25 sec. and this fell to 10 sec. seven days after hatching. Similar results were obtained with carbon disulphide.

In most cases it is possible to use populations of uniform age, but in the case of bacteria the variation in the age of the population constitutes a serious complication in the interpretation of the response of such populations to drugs.

Curves relating Drug Concentration and the Incidence of Effects

The data considered hitherto have shown the individual variation in the dose of drugs required to produce a certain selected effect. The characteristic curves obtained have been in nearly all cases sigmoid curves showing a greater or less degree of asymmetry. Measurements of the relation between drug concentration and the incidence of effects in a population result in curves of a much greater variety. In some cases simple sigmoid curves are obtained, but very frequently the curves approximate more nearly to a hyperbola than to a sigmoid form.

For example, Dr. C. P. Percival has measured the minimum concentrations of certain irritant drugs that were needed to produce skin irritation in each of a number of individuals. He has kindly permitted me to quote certain of his results which are shown in Fig. 19. The curves which relate the concentrations of chrysarobin and of mercuric chloride and the percentage incidence of skin irritation cannot be described as sigmoid curves and indeed resemble hyperbolæ. Sigmoid curves are

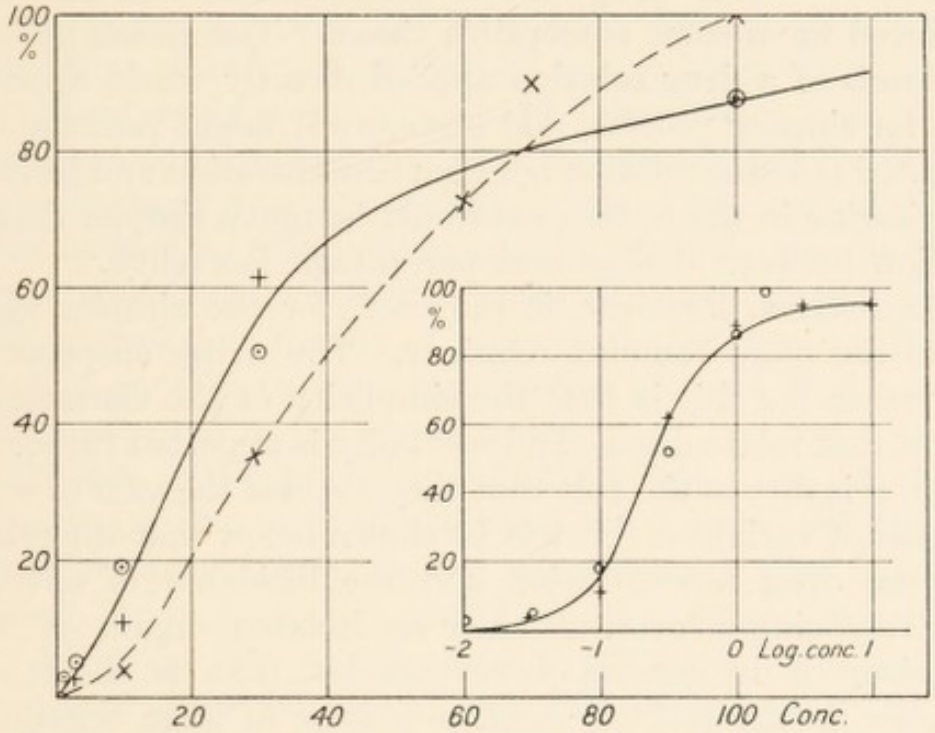

Fig. 19.—Characteristic curve of the production of response in 35 patients by skin irritants.

Abscissa : concentration. Ordinate : per cent. of population responding. Continuous line = drugs in vaseline. Conc. in parts per 10,000. + = chrysarobin ; ⊙ = mercuric chloride. Broken line and × = mercuric iodide in water. Conc. in parts per 100,000.

Inset. Abscissa : logarithm of the concentration per cent. of chrysarobin (+) and of mercuric chloride (⊙) in vaseline. Ordinate : per cent. incidence of skin response. (G. H. Percival.)

however obtained if the incidence of response be plotted against the logarithm of the concentration as is shown in the inset in Fig. 19.

In other cases the relation between drug concentration and incidence of skin irritation approximates more nearly to the usual form of characteristic curves, as for instance in the case of the effects produced by a watery solution of mercuric iodide, which also is shown in Fig. 19. The curious form of these

characteristic curves is surprising for the following reasons. The sigmoid characteristic curves relating dosage and drug action can be explained on the assumption that the population shows a normal or bell-shaped distribution of variation as regards some function that regulates the effect produced by the drug. The effect of a drug administered to an intact animal must depend upon a chain of processes, but must be regulated to a large extent by the relation between the concentration of the drug in the blood-stream and the effect produced upon some susceptible tissue. The action upon the epidermis of a drug solution applied directly would appear to be a far simpler process, and therefore it might reasonably be expected that the relation between concentration and incidence of irritation in the latter case would be much simpler than the relation between dosage and percentage mortality.

The reverse, however, is the case, for the simpler system shows the more complex relations. The writer suspects that the reason for this is that the simplicity of the characteristic curves that relate dosage and mortality is apparent rather than real and is due to the fact that they express the sum of a large number of variables. It will be shown below that the relation between drug concentration and the incidence of effects in relatively simple systems such as isolated organs or small organisms is in general more complex than is the relation between dosage and per cent. mortality of large organisms.

Hypersensitiveness to Drugs

The remarkable relations found between drug concentration and incidence of action show that care is needed in defining the terms idiosyncrasy and immunity. For example, in the case of the action of nicotine on *aphis* the following figures were obtained by Tattersfield and Gimingham (v, 1927):

Mols per 100 litres.	0·01	0·25	1·2
Per cent. population moribund or dead . .	2	48	98

Two per cent. of the population are therefore so susceptible that they are killed by 1/25 of the median lethal concentration, whilst 2 p.c. are so resistant that they are not destroyed by five times the median lethal concentration, and the ratio between the concentrations needed to kill the 2 p.c. at each extreme is no less than 120/1.

Dr. G. H. Percival's figures for skin irritation show a similar scatter. In the case of chrysarobin the following figures were obtained:

Parts per 10,000	4	23	300
Per cent. of population showing skin irritation .	5	50	95

The figures obtained with *aphis* and with the human skin show a similar scatter, and in both cases continuous curves were obtained expressing the variation in individual susceptibility.

It seems unreasonable to draw lines through such curves and say that all individuals outside certain limits show hypersensitiveness or immunity. On the other hand, any person who made a few observations on a population and found that some individuals were affected by 1/100 of the concentration of drug needed to affect others would probably ascribe these effects to some form of specific immunity or hypersensitiveness.

This remarkable spread of individual variation is also of interest in another respect, namely, the problem of the factor of safety required in the use of drugs.

In the case of the action of digitalis on cats (Fig. 13) it will be seen that a dose equal to half the mean dose did not produce death in a single animal in 573 experiments. The standard deviation (σ) in this case was 12·15 p.c. of the mean and Table XI shows that a dose equal to $100 - 4\sigma = 100 - 48{\cdot}6 = 51$ p.c. of the mean would only kill one animal in 10,000.

In the case of dysentery toxin, on the other hand, Fig. 17 indicates that a dose equal to 10 p.c. of the median lethal dose would kill at least 5 p.c. of a population of mice, whilst in the case of contact insecticides a concentration of nicotine which was only 4 p.c. of the median lethal dose killed 2 p.c. of *aphis*.

These results suggest that the factor of safety probably varies enormously in the case of different drugs, and that the term hypersensitiveness in many cases only means that there is a wide scatter as regards the individual variation in response to the drug employed.

Characteristic Curves obtained with Isolated Organs

Curves of this type can be obtained by determining the concentration of drug needed to produce a certain selected effect in each of a sufficient number of experiments. The

method is extremely laborious because about 100 experiments are needed to establish the shape of a curve. The amount of evidence available is very limited, but is sufficient to show that the shape of the characteristic curves varies very widely.

In certain cases simple sigmoid curves are obtained. For example, Takahashi (1925) measured the response of isolated sartorii of frogs to varying concentrations of caffeine and obtained the following figures :

Concentration of Caffeine in parts per 10,000 .	0·25	0·286	0·33	0·4	3·3	4	5	6·7
Percentage of muscles showing—								
(*a*) Histological change	0	14	78	100	—	—	—	—
(*b*) Destruction of cells	—	—	—	—	0	47	80	100

In the case of both the changes measured the concentration producing a 100 p.c. effect is less than twice the lowest concentration that produces any effect, and therefore the range of individual variation is unusually small.

In certain other cases the relation between the concentration of the drug and the percentage of isolated organs showing a selected response is of an exponential rather than a sigmoid character. Curves of this type, which suggest a widely scattered skew variation, have been obtained with acetyl choline acting on the frog's isolated heart (Fig. 34), and adrenaline acting on the isolated uterus of the rabbit (Fig. 37).

Characteristic Curves obtained with Small Organisms and Cells

Curves of this type can easily be obtained by measuring the percentage mortality produced by a series of concentrations. The amount of drug taken up by each organism is unknown and hence there are two variables, namely the individual variation in the uptake of the drug and the individual variation in the amount of drug needed to produce the effect measured.

The curves relating concentration and percentage effect vary from the steep and symmetrical sigmoid form to the exponential form. Fig. 29 provides an example of a symmetrical sigmoid curve. This expresses the relation between the concentration of ethyl urethane and the percentage of sea-urchin eggs paralysed. An almost identical curve was

obtained by Shackell (1925) for the paralysis of *Limnoria* by ethyl alcohol. In this case the probable variation was 9 p.c. of the median. White (1929) obtained a similar curve for the destruction of this organism by changes in salinity. In this case the probable variation was 18 p.c. of the median. The hæmolysis of red blood-corpuscles by various simple agents shows a similar sigmoid relation between concentration and

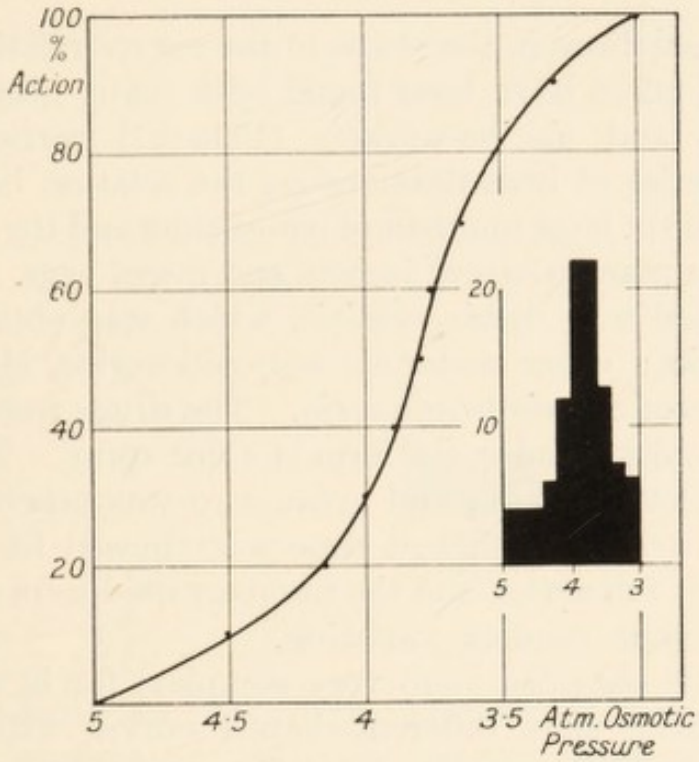

Fig. 20.—Characteristic curve of the hæmolysis produced by hypotonic saline.

Abscissa : saline concentration expressed as atmospheres of osmotic pressure. Ordinate : per cent. hæmolysis.

Inset. The distribution of variation in the resistance of the red blood-corpuscles. (Mond. 1927.)

percentage of hæmolysis. This relation was found by Handowsky (1912) for hæmolysis by alkalies, and by Snapper (1912) and by Mond (1927) for hæmolysis by hypotonic solutions (cf. Fig. 20).

Other hæmolytic agents such as saponins show much more complex relations between concentration and effect. Ponder (1930) measured the percentage hæmolysis produced by saponins when sufficient time was permitted for equilibrium to be established (time = 7 hours). He found that there was an extreme variability in the frequency distribution of the

resistance of the cells. The frequency polygon varied between two extremes, namely :

(1) a very skew hump with a long tail to the right, and

(2) a very flat curve with less skewness but a considerable tail.

The first of these distributions if integrated would show a hyperbola as the relation between dose of saponin and amount of action.

Similar variations in the shape of the curves relating concentration and effect have been found with many other systems. Tattersfield and his co-workers (1925–27) carried out an extensive series of investigations on the relation between the concentration of large numbers of insecticides and the percentage mortality in populations of insects and insect eggs. The chief material used was *Aphis rumicis*, which was obtained from inbred stocks ; other materials were silkworms, cheese mites and the eggs of *Selenia tetralunaria*. The drugs were dissolved in benzole and applied in the form of a fine spray. The solvent exerted no lethal action and great care was taken to ensure uniform spraying. Sufficient time was allowed for the drugs to exert their full action and the numbers used were sufficiently large to exclude random variation.

The results obtained were very complex, for in some cases the same drug gave different-shaped curves with different populations, whilst a single population gave curves of a wide variety of shapes with different drugs. For example, the characteristic curve of the action of pyridine vapour on cheese mites (Jewson and Tattersfield, 1922) was the steep sigmoid curve shown in Fig. 21, which has a probable variation 22 p.c. of the median, and a similar curve was obtained with *Aspergillus niger* immersed in pyridine solution (Jewson and Tattersfield, 1922). On the other hand, the characteristic curve of pyridine applied in a benzene spray to *aphis* (cf. Fig. 21) was nearly linear, a shape which suggests a very widely scattered variation. (Tattersfield et al, v, 1927.)

A few representative curves are shown in Fig. 22. It will be seen that the characteristic curve of di-nitro-o-cresol is sigmoid and symmetrical in the case of *aphis* (Tattersfield et al, III, 1925), but markedly skew in the case of insect eggs (Tattersfield et al, III, 1925, and Gimingham, Massee and

Tattersfield, 1926). Variations of this kind make it difficult to generalize, but the results taken as a whole indicate that insecticides of feeble intensity such as phenol show fairly steep sigmoid characteristic curves, and the same is true of the fatty acids and their soaps, of which a long series ranging from acetic to oleic acid were tested. (Tattersfield et al, VI, 1927.)

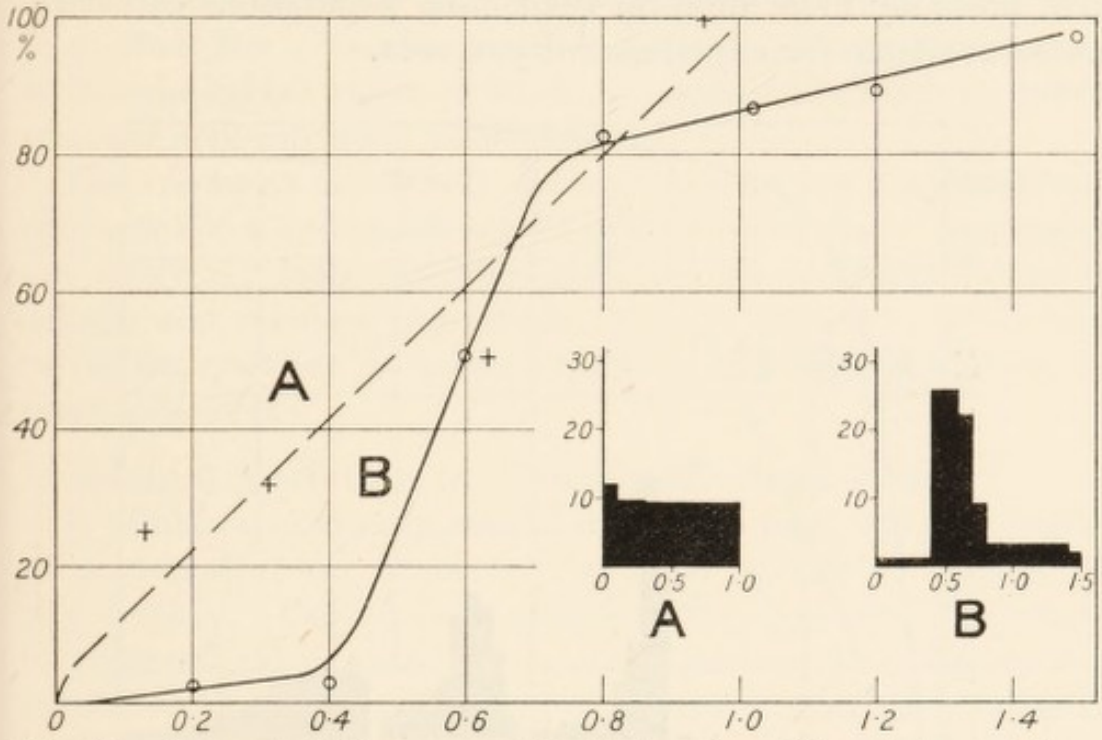

Fig. 21.—Characteristic curves of the destruction of insects by pyridine.

ABSCISSA : concentration. ORDINATE : per cent. mortality.

CURVE A. Pyridine dissolved in benzene (conc. in gm. mols per litre) and sprayed upon *Aphis rumicis*. *Inset A*. Distribution of variation. (Tattersfield, Gimingham et al. V, 1927.)

CURVE B. Pyridine vapour (conc. in gm. mols per 10^4 litres of air) acting on cheese mites. *Inset B*. Distribution of variation. (Jewson and Tattersfield. 1922.)

Powerful insecticides show exponential characteristic curves more frequently than sigmoid. The characteristic curve of nicotine that is shown in Fig. 22 is of particular interest because the figures are the average of a large number of experiments. It will be seen that its shape shows more resemblance to an exponential curve or to a hyperbola than it does to a sigmoid curve. A similar curve was obtained for the poisoning of silkworms by the toxic resin of *Derris elliptica* (Tattersfield et al, V, 1927), and for the poisoning of *aphis* by tephrosin (Tattersfield et al, II, 1925) and by pyrethrum (Fryer, Tattersfield and Gimingham, 1928).

This extensive series of experiments proves conclusively that the curves relating the concentrations of various drugs and the percentage of deaths in a population of a single type may vary in shape from a steep sigmoid curve to a form that approximates to a hyperbola. The populations of *aphis* were of uniform age and came from an inbred stock and therefore were probably more uniform than most populations of vertebrates available for experimental purposes.

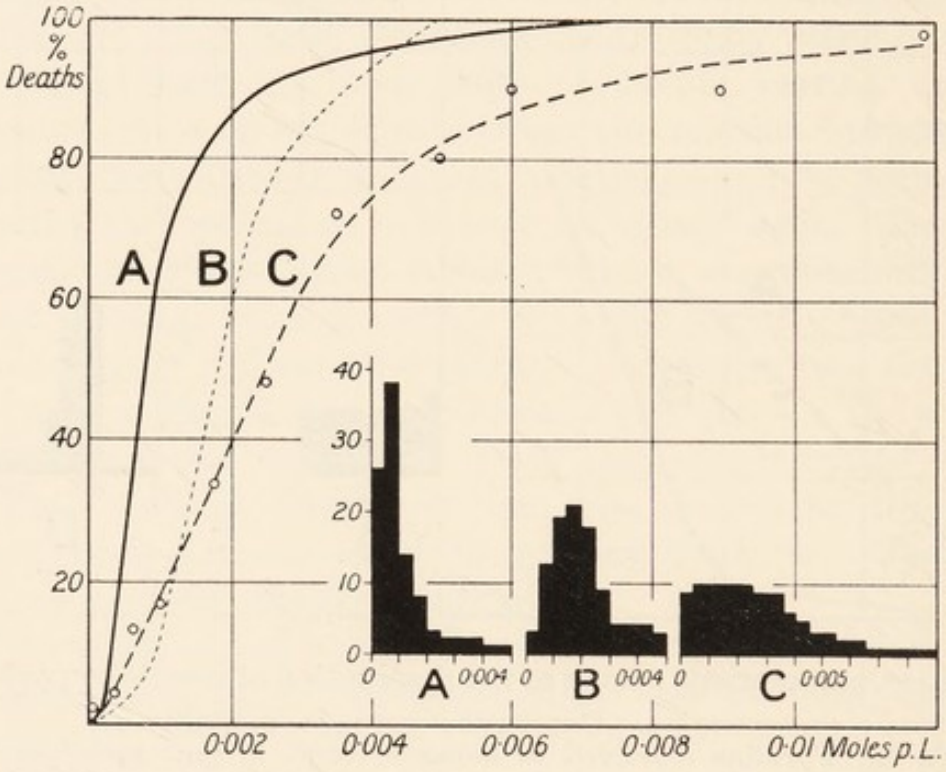

Fig. 22.—Characteristic curves of the destruction of insects and their eggs by insecticides.

Abscissa : concentration in gm. mols per litre benzene (solutions applied as a spray). Ordinate : per cent. mortality.

Curve A. Di-nitro-o-cresol acting on the eggs of *Tetralunaria*. (Tattersfield, Gimingham et al. III, 1925.)

Curve B. Di-nitro-o-cresol acting on *Aphis rumicis*. (Tattersfield, Gimingham et al. III, 1925.)

Curve C. Nicotine acting on *Aphis rumicis*. (Tattersfield, Gimingham et al. V, 1927.)

The insets show the distribution of variation in the corresponding curves.

The experiments on insects described above are exceptionally complete and extensive, but results that are similar although not so complete have been obtained with other systems. Baker (1932) studied the lethal action of a large number of drugs on spermatozoa, and found that the range of variation of the resistance of the population differed greatly in the case

of different drugs. This range of variation can be expressed by the ratio $\frac{C_2}{C_1}$, where C_1 is the highest concentration that produces no effect and C_2 is the concentration that just produces a 100 p.c. mortality. In the case of hydroquinone this ratio was as low as 2, in a large number of cases the ratio was 4 or 8, whilst in the case of zinc sulphocarbolate the ratio was more than 256. The low ratios indicate a steep sigmoid curve, whilst the higher ratios indicate an exponential form of curve relating concentration and action.

The examples quoted show that the relations between drug concentration and incidence of effects upon small organisms are distinctly more complex than are the relations between dosage and incidence of effects upon vertebrates. This fact raises the question as to the extent of individual variation in small organisms.

Individual Variation in Unicellular Organisms

It would be obviously absurd to suggest that any population of vertebrates was absolutely uniform in size or indeed in any quality. Unicellular organisms are, however, too small for their individual variation to be easily susceptible of measurement. One popular theory of drug action which is discussed in Chapter VIII assumes that the reaction between drugs and cells follows the course of a monomolecular reaction, and that there is no extensive static variation in the susceptibility of cells to drugs. It therefore is necessary to consider the evidence regarding the extent of the individual variation known to occur in cells.

In actual practice the obtaining of a sufficiently uniform population is recognized as one of the chief experimental difficulties in protozoology and bacteriology, for in order to obtain uniform results it is necessary to work with inbred populations that have been reared under carefully controlled conditions.

Adolph (1931, Table 5) has collected figures for the coefficient of the variation of certain characters in a number of micro-organisms. The most uniform population measured was a clone of *Trypanosoma lewisi* obtained at the same time from one host ; in this case the coefficient of variation of the length was only 3, but the same figure for the breadth was 15. Other populations of protozoa (one clone and one culture) showed

coefficients of variation of length of about 15. In the case of certain bacteria (*B. coli* and *B. megatherium*) this figure was as high as 25. These figures were all taken from populations in which special care had been taken to ensure uniformity, and under ordinary conditions the variations in form are much greater than those mentioned. Age is the most important variable in unicellular populations. In the case of populations that do not multiply in numbers (e.g. red blood-corpuscles, bacterial spores or eggs) the age distribution is likely to show symmetrical variation. In the case of a population of bacteria which divides by fission the age distribution must be highly skewed.

Variability of Age in Bacterial Populations

The rate of growth of *B. enteritides Gaertner* was studied by Lane Clayton (1909), who found that after an initial lag the rate of multiplication remained constant for about 10 hours, after which it decreased rapidly. Reichenbach (1911) made similar measurements for *B. paratyphosus* at 37° C. He found that from $2\frac{1}{2}$ to $6\frac{1}{2}$ hours the population increased about sixfold per hour, after which the rate declined rapidly, and the population became stationary at 12 hours and subsequently declined. Hence in a young culture growing at its maximum rate the age distribution must be of the extreme skew type. The proportion more than an hour old must be much less than 1/6, and more than half must be less than 20 min. old.

In the case of the older populations in which the population is either stationary or declining, the proportion of young cells cannot be so great, but in any population that reproduces by asexual division the age distribution is extremely unlikely to show symmetrical variation, and as long as active division is occurring there will be a preponderance of young forms.

Variations in Bacteria correlated with Age

A considerable number of properties of bacteria are known to vary with age. Adolf (1931) quotes measurements made by Dawson (1919) which show that the water content of *B. coli* falls steadily with the age of culture. After implantation for 24 hours the bacilli contain 97 p.c. of water, and this falls steadily until the figure of 74 p.c. has been reached on the 10th day. Other observations quoted by Adolf show that

the size of bacteria in a culture changes rapidly as the age of the culture increases. Clark and Ruehl (1919) found that when *B. subtilis* was implanted into new broth the average body length increased from 2 micra to 4 micra in 2 hours, and decreased to 1·2 micra after 12 hours. Henrici (1923) obtained similar variations in lengths of *B. coli* when implanted in agar. Henrici showed that the size was largest when the rate of multiplication was most rapid. These observations show that a bacterial population is likely to show wide variations of a skew type.

The Influence of Age on the Resistance to Drugs

Reichenbach (1911) studied the relation between the age of cultures of *B. paratyphosus* and the rate at which this organism was killed by heating to 49° C. His figures show that 5 minutes' exposure to this heat kill 85 p.c. of a 5½ hours culture, 57 p.c. of a 13 hours culture and 5 p.c. of a 28 hours culture. The fact that cultures of different ages show great variations in their resistance to drugs is well known. For example, Chick (1908) found in the case of 0·6 p.c. phenol acting on *B. paratyphosus* that the time needed for 90 p.c. destruction was about 2 min. with a 24 hours culture and about 15 min. with a 3 hours culture.

Similar variations in resistance have been found with stationary populations of varying ages; for instance, Henderson Smith (1921) found that 6-day-old botrytis spores were killed about twice as rapidly as were 9-day-old spores. Handowsky (1912) concluded that young erythrocytes were more resistant than old to saponin hæmolysis.

It is evident that a skew distribution of variation of response to drugs is very likely to occur in the case of freely growing micro-organisms. Such populations will contain an excess of young forms, and since these are more susceptible to drugs than are older forms, there is bound to be a skew distribution of variation in the response to drugs.

Unfortunately it is almost impossible to obtain curves relating drug concentration and percentage of destruction of bacteria by disinfectants because the lowest effective concentration of a disinfectant will, if allowed sufficient time, ultimately produce complete destruction. The skew distribution in the age and in the resistance of bacteria is however of great importance

in the interpretation of time-concentration curves, a problem that is discussed in Chapter VIII.

There is, however, a tendency to assume that the proper distribution of variation in a population is of the " normal " bell-shaped type, and it is of interest to note that one of the commonest variables in populations and one which influences profoundly their response to drugs is likely to be frequently, if not usually, distributed in a markedly skew fashion.

Skew distributions of variations in age will not, however, provide a general explanation for skew distribution of variations in response to drugs because in many cases these are obtained with populations of uniform age (e.g. Figs. 21 and 22).

Variations in the Resistance of Bacteria to Heat

Williams (1929) observed that the heat resistance of spores of *B. subtilis* varied extensively. Desiccated spores were more resistant than young moist spores. The resistance of spore populations could be increased by selective breeding from resistant cells. Variation in the nutritive substratum also altered the resistance profoundly.

The capacity for variation in bacteria is well illustrated by the results that have been obtained by the selection of heat resistant forms through several generations. Magoon (1926A) found that the resistance of spores of *B. mucoides* to heat was not a fixed property but increased with age ; for example, spores 1 day old were killed three times as quickly as spores 30 days old. He also found (1926B) that by selecting the resistant survivors from a series of experiments he could obtain a strain whose spores showed a resistance to heat at least twenty-five times that of the original spores.

The Significance of Sigmoid Characteristic Curves

Characteristic curves such as those shown in Figs. 14 and 15 are remarkable for their steepness and symmetry. There is, moreover, a general agreement that these characters are most likely to be obtained when care is taken to use as uniform a population as possible (cf. Fig. 18).

On the other hand, it is interesting to note that a reduction in the number of variables does not necessarily alter the shape of a characteristic curve. For example, the characteristic curve relating insulin dosage and incidence of convulsions in

mice (Trevan and Boock, 1926) resembles the curve relating insulin dosage and the extent of the fall in blood-sugar in rabbits shown in Fig. 49 (Marks, 1926). There is, however, an extensive individual variation as regards the amount of fall in blood-sugar necessary to produce convulsions, and therefore the second method which eliminates this variable ought to show a steeper characteristic curve than does the former method. The steepness and symmetry of the characteristic curves obtained with some drugs is indeed very different to explain, because the measurement of most morphological or functional variables shows a wider and less symmetrical variation.

The normal "probability" curve or Gaussian curve of error is obtained when each measurement is affected by a considerable number of independent factors. The curve then expresses the chances of the occurrence of the various possible combinations of these factors. The curve does not show that all the factors have a symmetrical action (i.e. are capable of influencing the measurement equally in a positive or negative manner), but a symmetrical curve is unlikely to be obtained if a few of the factors are of greater weight than the remainder and have an asymmetrical action. The shape of curve B in Fig. 18 would appear to be due to the introduction of some factors that vary extensively in an asymmetrical manner. The shape of the curves in Fig. 19 suggests that the whole of the factors influencing the measurements have their variation distributed in a geometrical manner, and the possible reasons for this are discussed below.

According to this hypothesis the symmetrical characteristic curves occurring with mammals or other large organisms should approximate to the "normal" probability curve. This can be tested by plotting results on probability paper. This paper, which was described by Whipple (1916), has the ordinates so adjusted that the characteristic sigmoid curve obtained by integration of a normal bell-shaped frequency distribution is converted into a straight line. Fig. 23 shows some results obtained with this method. The characteristic curve of frogs poisoned with ouabain is exactly linear, whilst that of cats poisoned with digitalis shows a symmetrical deviation. On the other hand, the characteristic curves of

cocks poisoned with ergot and *aphis* poisoned with nicotine deviate markedly from the linear.

The Interpretation of Skewed Characteristic Curves

The evidence already presented in this chapter shows that the curves relating concentration of drugs and the incidence of response in a population are frequently of an exponential rather than of a sigmoid shape. Such a curve suggests a skew

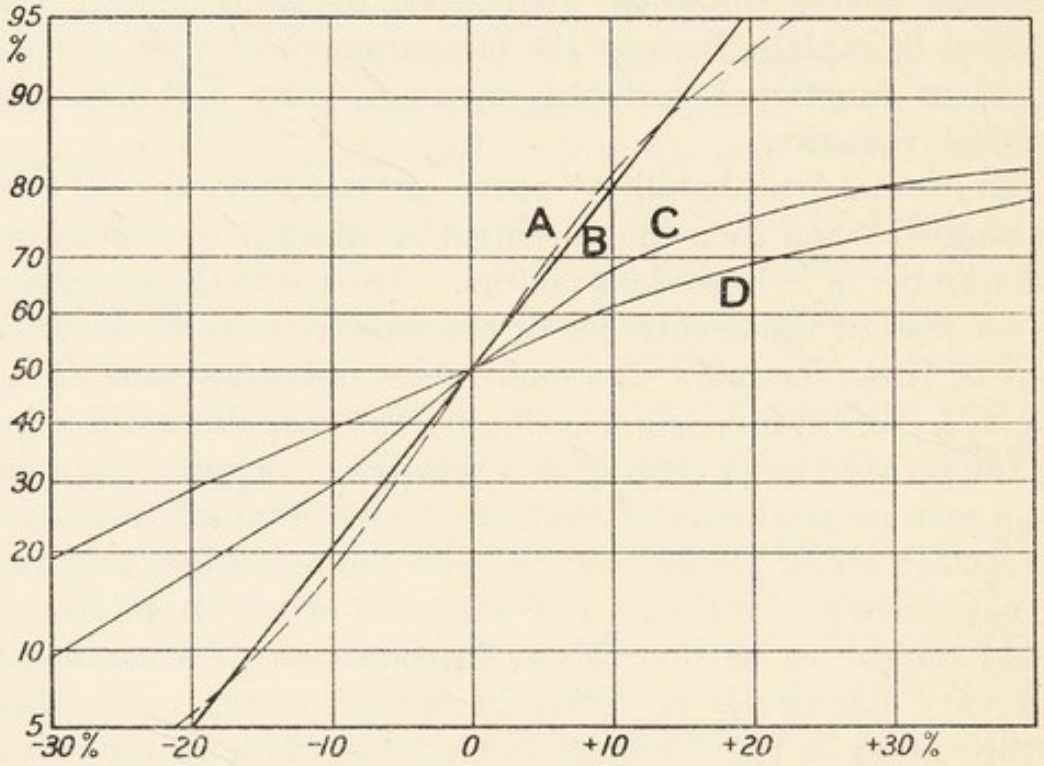

Fig. 23.—Characteristic curves plotted on "probability" paper.

Abscissa: amount of variation in effective dose expressed as per cent. of the median. Ordinate: per cent. responses.

Curve A. Digitalis on cats (Fig. 14).
Curve B. Ouabain on frogs (Fig. 15).
Curve C. Ergot on cocks (Fig. 16).
Curve D. Nicotine on *aphis* (Fig. 21).
In curve D the deviation observed has been divided by 2.

type of variation with a high proportion of susceptible individuals and a tail of resistant individuals spreading out over a wide range of variation.

The occurrence of skew variations appears probable in the case of micro-organisms dividing by fission, but it appears improbable in the case of insects of uniform age or in the case of isolated organs. Exponential characteristic curves have, however, been found in both of the last-mentioned cases. It is

therefore necessary to find other reasons than variation in age for these exponential characteristic curves.

The interpretation of exponential characteristic curves must depend upon probability rather than upon direct proof, and therefore it is of interest to consider the conditions that result in skew variations in non-living systems.

Extreme skew or J-shaped distributions of variations have been shown to occur frequently in emulsions. Sibree (1930)

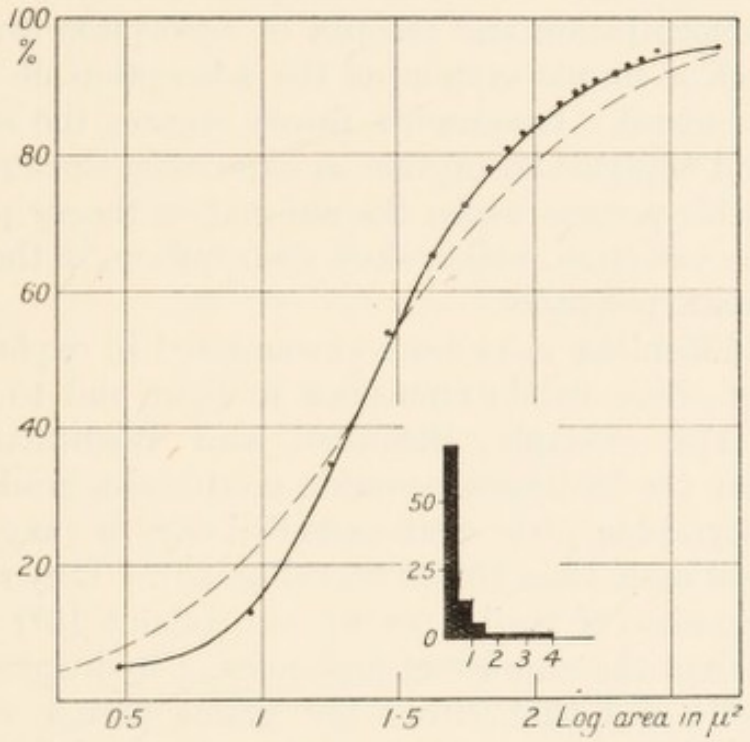

Fig. 24.—Characteristic curve of the area of particles in a silver halide emulsion.

ABSCISSA : logarithm of the area of particles (in sq. μ).

ORDINATE : per cent. of particles with an area equal to or less than any particular value.

The inset shows the distribution of particles as regards their area in sq. μ.

The broken line is drawn to the formula $kx = \frac{y}{100 - y}$.

(Wightman and Sheppard. 1921.)

found this type of distribution of particle size in an emulsion of paraffin in a solution of soap, a similar distribution was found by Smith and Grinling (1930) in an old emulsion of cod-liver oil, and it also has been found in silver halide emulsions (cf. Fig. 24). (Wightman et al, 1921, 1923 and 1924.)

A skew distribution of catalytic activity also has been demonstrated in the molecules on the surface of adsorbing agents.

Various authors have studied the adsorption of oxygen by charcoal (Blench and Garner, 1924 ; Garner and McKie, 1927 ; McKie, 1928 ; Keyes and Marshall, 1921 ; Cameron, 1930). The results suggest that the distribution of activity amongst the molecules on a charcoal surface is somewhat as follows. About 1 per cent. are highly active, another 20 p.c. show a rapidly decreasing activity and the remaining 80 p.c. show a very slowly decreasing activity.

There are therefore two lines of explanation for the relation between concentration and amount of adsorption even in the case of such a simple system as the adsorption of a gas by charcoal or metal. Langmuir's theory regards the surface as uniform and explains adsorption as expressing the equilibrium of a reversible process whilst the alternative theory postulates a very wide variation, with a skew distribution, in the activity of the surface receptors.

Similar difficulties have been encountered in explaining the response of silver halide emulsions to light and to chemical agencies. For example, Sheppard and Wightman (1923) showed that the hydrogen peroxide produced a graded action on a photographic plate that extended over a range of concentration of more than 10,000 fold (Fig. 25). They calculated that the number of grains per sq. cm. (about 10^9) was very much less than the number of molecules of hydrogen peroxide ($10^{16} - 10^{21}$) and that either the grains had a very wide range of individual variation or else that only very small proportions of the molecules of peroxide were capable of producing a reaction.

There is also an unsettled controversy as to how the individual variation of emulsion grains affects the response to light of halide emulsions. (Sheppard, Trivelli and Loveland, 1925.)

Since physical chemists are not yet in agreement regarding the phenomena observed in the simple systems with which they work, it is unnecessary to apologize for an even greater uncertainty in the case of drugs acting on living cells. It is possible to explain any curve by individual variation, and the very facility of this form of explanation makes it unsatisfactory. The following argument appears important to the writer. If concentration-action curves are an expression of individual variation in a system, then the tendency should be for any

particular cell system to show similar concentration-action curves with a variety of drugs. If, however, concentration-action curves owe their form to some relation between drug concentration and fixation, then the same drug tested over a series of preparations should show similar characteristic curves. The evidence appears to be definitely in favour of the latter hypothesis.

The examples of hæmolysis, injury to sea-urchin eggs and

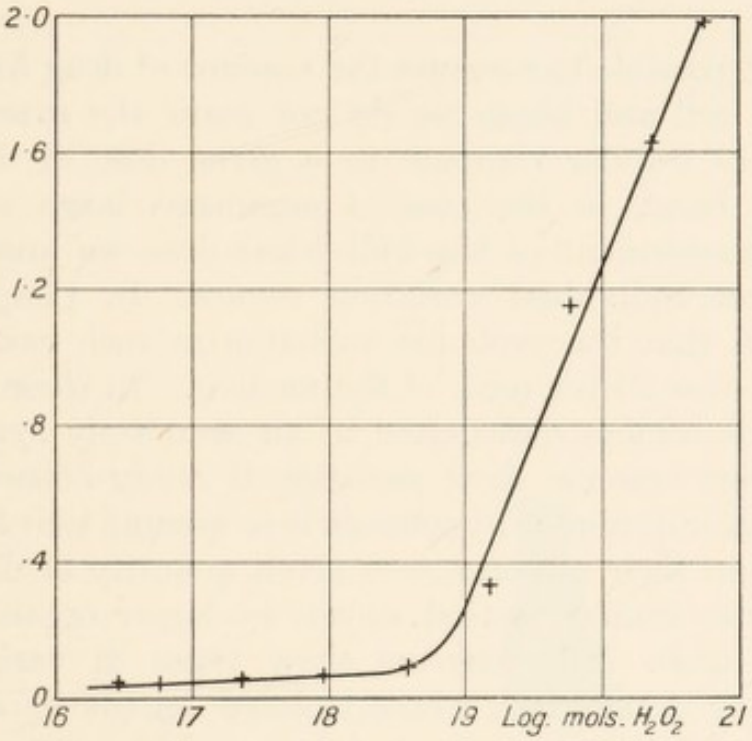

Fig. 25.—The relation between the concentration of hydrogen peroxide and the effect produced on a silver halide emulsion.

Abscissa : logarithm of the number of molecules of H_2O_2 present per cm^2. Ordinate : amount of chemical change in emulsion. (Sheppard and Wightman. 1923.)

destruction of *aphis* all show that both sigmoid and exponential characteristic curves can be obtained with different drugs from a single population. On the other hand, drugs that show exponential curves relating concentration and percentage incidence of response in a population often provide curves of similar shape when concentration is plotted against graded action and even when the progress of an action is measured (time-action curves). The writer believes that the exponential type of characteristic curve does not really indicate an extreme

skew distribution of variation but is due to the distortion of a more or less symmetrical variation by the conditions of the experiment. This may occur in the following manner.

All populations of living organisms vary, and hence we may assume that all relations between dosage and effect are distorted by this variation. Individuals exposed to a drug solution may, moreover, vary in two respects : (1) as regards the amount of drug they fix, and (2) as regards the amount of action produced by a given quantity of drug after it has been fixed.

It is not possible to measure the amount of drug fixed by an individual cell and hence we do not know the extent of the variation of cellular response to a given dose of drug. On the other hand, in the case of organisms large enough to permit measurement of the individual dose we know that a considerable individual variation occurs. In Chapter V it was shown that the probable variation in such cases ranged from 8 to over 20 per cent. of the median. In these cases the variation is seldom distributed in an accurately symmetrical manner, but extreme skew variation is rarely observed.

The least improbable hypothesis is to assume that individual cells vary in their response to a given quantity of drug fixed, in a manner similar to that shown by larger organisms to a measured dose. The extreme skew types of variation are observed in those cases where isolated organs or organisms are immersed in solutions of drugs, and the concentration-action curves indicate that the relation between drug concentration and drug fixation follows a hyperbola. In the case of acetyl choline, for example, it will be shown in Chapter VII that concentration (x) and the amount of graded action (y) are related by the formula $\mathrm{K}x = \frac{y}{100 - y}$ (cf. p. 146). If we assume that the action is a measure of drug fixation, then the relation between concentration (x) and drug fixed (y) will follow the same formula.

Dr. A. C. White has kindly communicated to the author unpublished measurements of the individual variation of mice to acetyl choline. These results (cf. Fig. 26) show a probable variation of 21, which corresponds to a standard deviation of 31.

For the purposes of the present argument it will be assumed

that this indicates the range of individual variation to acetyl choline.

The problem therefore is as follows. If the relation between concentration (x) and uptake of acetyl choline (y) by the frog's

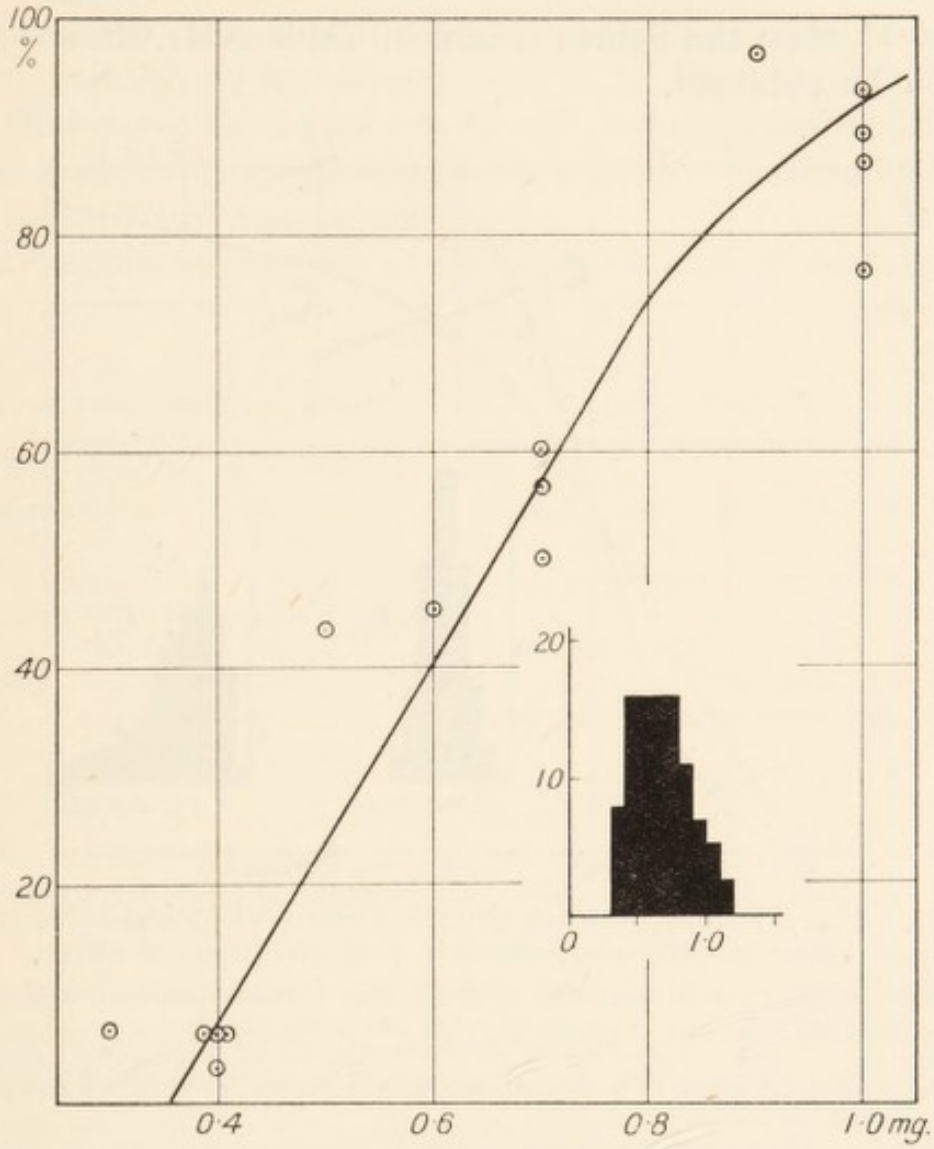

Fig. 26.—Characteristic curve of the lethal action of acetyl choline when injected intravenously into mice.

Abscissa: dose in mg. per 20 gm. mouse. Ordinate: per cent. mortality. About thirty mice were used to determine each point, and the total number used was 450. (A. C. White. Unpubl. results.)

heart follows the formula $Kx = \frac{y}{100 - y}$, and if the hearts show an individual variation in respect to y which is distributed with a standard deviation of 30, then what will be the individual variation of the hearts in respect of the concentration of acetyl

choline (x) needed to produce a given effect, e.g. 50 p.c. inhibition ?

If the relation between the concentration of a drug (x) and the amount fixed by cells (y) follows the formula $kx = \frac{y}{100 - y}$ ($k = 0{\cdot}1$), then the figures shown in Table XIII, lines A and B, will be obtained.

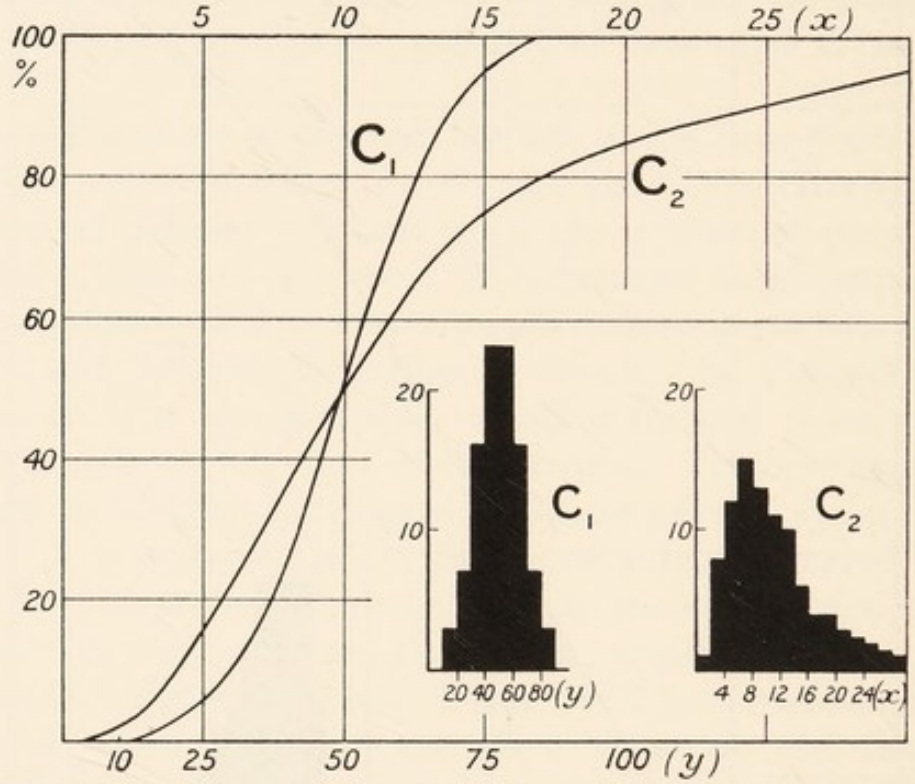

Fig. 27.—The relations between drug fixation and incidence of effect and between drug concentration and incidence of effect.

Upper abscissa : concentration of drug (x). Lower abscissa : amount of drug fixed by cells (y). Ordinate : per cent. response.

Curve C_1. Characteristic curve of the incidence of response to varying quantities of drug fixed in a population which shows a standard deviation of 30 per cent. of the mean.

Curve C_2. Characteristic curve of the incidence of response to varying concentrations of a drug when the relation between drug concentration (x) and drug fixation (y) follows the formula $kx = \frac{y}{100 - y}$.

Insets : the apparent distribution of variation in the two cases.

If now it be assumed that the population varies in respect to the amount of drug that must be fixed before a certain selected effect is produced, and that the distribution of variation is symmetrical and has a standard deviation equal to 30 p.c. of the mean, then the relation between the amount of drug fixed and the incidence of the occurrence of the selected

response will be as shown in curve C_1, Fig. 27, and in Table XIII, line C. The relation between drug concentration and drug fixation is, however, a hyperbola, and hence the relation between drug concentration and incidence of response differs greatly from the relation between the amount of drug fixed and the incidence of response. These two relations are shown respectively in curves C_2 and C_1 in Fig. 27. It will be seen that curve C_2, which relates concentration and incidence of effect, shows more resemblance to an exponential curve than to a symmetrical sigmoid curve.

The argument applied in the case of acetyl choline will

TABLE XIII

A. Drug concentration (x) .	1	3	5	7·5	10	15	20
B. Drug fixed (y) (per cent. of maximum)	9	23	34	43	50	60	66
C. Per cent. of population showing some selected response	—	0	4	14	32	50	75
A. Drug concentration (x) .	30	40	50	75	100	200	300
B. Drug fixed (y) (per cent. of maximum)	74	79	83	88	92	95	97
C. Per cent. of population showing some selected response	85	94	97	98	100	—	—

also apply to any case where the relation between drug concentration and drug fixation is not linear, for in all such cases the relation between concentration and incidence of a response is likely to follow an exponential curve.

For example, in the case of hæmolysis by ammonia, Stadler and Kleeman (1911) found that the adsorption of ammonia by red blood-corpuscles followed the formula $k.c_1^{0.4} = c_2$, where c_1 = concentration in solution and c_2 = concentration inside the corpuscles. This formula gives a nearly linear relation between the amount of drug fixed and log. concentration over most of the experimental range of concentration. If we assume that the cells show a symmetrical variation with a standard deviation of 20 p.c. of the mean as regards the amount of drug fixed (y) that is required to produce

hæmolysis, and the value of y_2 needed to produce 50 p.c. hæmolysis be taken as 2, then the following figures are obtained :

Concentration of drug in solution (x) .	1	1·8	2·7	3·9	5·5	7	9	12	16
Amount of drug fixed (y_2) . . .	1	1·25	1·5	1·75	2	2·25	2·5	2·75	3
Per cent. of cells hæmolysed . .	1	4	11	28	50	73	88	97	99

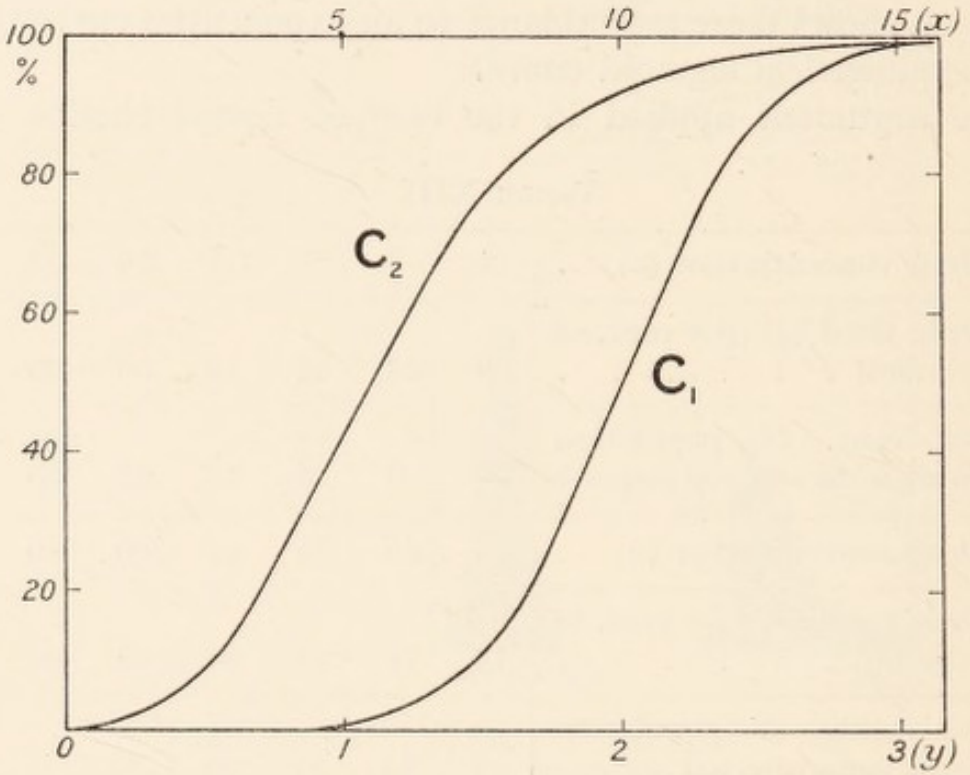

Fig. 28.—The relations between drug fixation and the incidence of hæmolysis, and between drug concentration and the incidence of hæmolysis.

Upper abscissa : concentration of hæmolytic (x). Lower abscissa : amount of hæmolytic fixed by cells (y). Ordinate : per cent. hæmolysis.

Curve C_1. Characteristic curve of hæmolysis with varying quantities of drug fixed when the standard deviation is 30 per cent. of the mean.

Curve C_2. Characteristic curve of incidence of hæmolysis and drug concentration when the relation between concentration and fixation of drug follows an adsorption formula.

The curves in Fig. 28 show the way in which a symmetrical distribution of variation as regards the dose of drug needed to produce hæmolysis would be distorted into a skew relation between concentration and percentage hæmolysis.

The chief objection to the explanation outlined above is the following. If the characteristic curve of a drug expresses chiefly some reaction between the drug and cells, then the same

curve should be obtained with any one drug, whatever the nature of the population used. A comparison of the characteristic curves obtained with acetyl choline acting on mice (Fig. 26) and on frogs' hearts (Fig. 33) shows that this assumption is untrue. The fact that the characteristic curve of the more complex system (the mice) approximates to the simple curve of error, suggests that in this case there are so many independent variables that the influence of the fundamental chemical reaction is obscured.

Conclusion

In the case of organisms sufficiently large to permit the measurement of individual dosage, the characteristic curves relating dosage and incidence of effect are usually sigmoid, and can be assumed to express the individual variation of the population. In many cases the uniformity of the population as regards its response to drugs is very remarkable. Indeed, the variation in this respect is less than the variation found for many morphological and biochemical factors.

The curves relating drug concentration and incidence of response, on the other hand, show a remarkable variation in shapes. In many cases exponential curves are obtained which at first sight suggest that there is an extreme skew distribution in the susceptibility of the population to drugs. The alternative explanation suggested is that the relation between concentration and action expresses two relations: firstly, the relation between concentration and drug fixation, and secondly, the variability of the organisms as regards the amount of drug that must be fixed in order to produce a given effect. This explanation permits the interpretation of the exponential curves relating concentration and incidence of action without the assumption of any extreme skew distribution of variation.

According to this theory the relation between concentration and incidence of any selected effect in a population will differ in the case of different drugs. In those cases where there is a linear relation between drug concentration and amount of drug fixed by the cells, the characteristic curves will approximate to the symmetrical sigmoid type. Such curves are obtained for hæmolysis produced by hypotonic saline (Fig. 20), for ethyl urethane acting on sea-urchin eggs (Fig. 29), and pyridine acting on cheese mites (Fig. 21).

In the first case there is probably a linear relation between the reduction of the osmotic pressure and the amount of water entering the corpuscles, and in the second case the relation between concentration of narcotics and the amount entering cells is known to approximate to the linear. On the other hand, extreme skew characteristic curves have been obtained with acetyl choline acting on frogs' isolated hearts (Fig. 33), with adrenaline acting on rabbits' uteri (Fig. 36), and with nicotine and other powerful insecticides acting on *aphis*. In the first two cases there is reason to believe that the drugs are fixed by adsorption, and the relations between concentration and graded action would be almost impossible to explain if there were a simple linear relation between drug concentration and drug fixation.

The theory put forward therefore agrees fairly well with the facts observed, and explains many of them without involving any improbable assumptions.

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CHAPTER VII

EQUILIBRIA BETWEEN DRUGS AND CELLS

In a large proportion of cases it is possible to determine the action produced by a drug on cells after sufficient time has been allowed for equilibrium to be established. The measurement of such effects produced by a series of concentrations permits the construction of a concentration-action curve. This method eliminates many of the uncertainties and errors associated with measurements of rate of action, but a number of possible sources of error remain which require careful consideration.

1. It is easy to measure the action produced by a drug but it is much more difficult to measure the quantity of drug fixed by the cells. Unfortunately, even if this latter measurement is obtained, its significance is doubtful, because, as was explained in Chapter II, the cell is a complex structure and probably the action produced by the drug is due to only a portion of the total quantity that is fixed. In many cases the shapes of the curves obtained can be interpreted rationally on the assumption that the drug reacts with receptors, and that the action produced is directly proportional to the number of receptors occupied. Direct proof cannot however be obtained for such a hypothesis. Hence we must be content with balancing probabilities and cannot hope for formal proof.

2. The chief difficulty in interpreting concentration-action curves is to decide whether the curve obtained expresses some form of chemical equilibrium or some form of individual variation. This difficulty varies in different systems. In some cases it is possible to say at once that the results produced must depend wholly or in part on individual variation. This is true when the effect measured is of an "all or none" character as regards the individual cell or organism. Examples of such effects are production of death, inhibition of fertiliza-

tion, paralysis of nerve-fibres. In other cases the effect produced may be measured either as an all-or-none or as a graded effect. The experiments of Herzog and Bertzel, shown in Fig. 2, provide an extreme example of the difference in the relations between concentration and action that can be obtained in a single system by different methods of measurement. These authors measured first the uptake of phenol by the yeast-cells and obtained a relation that was approximately

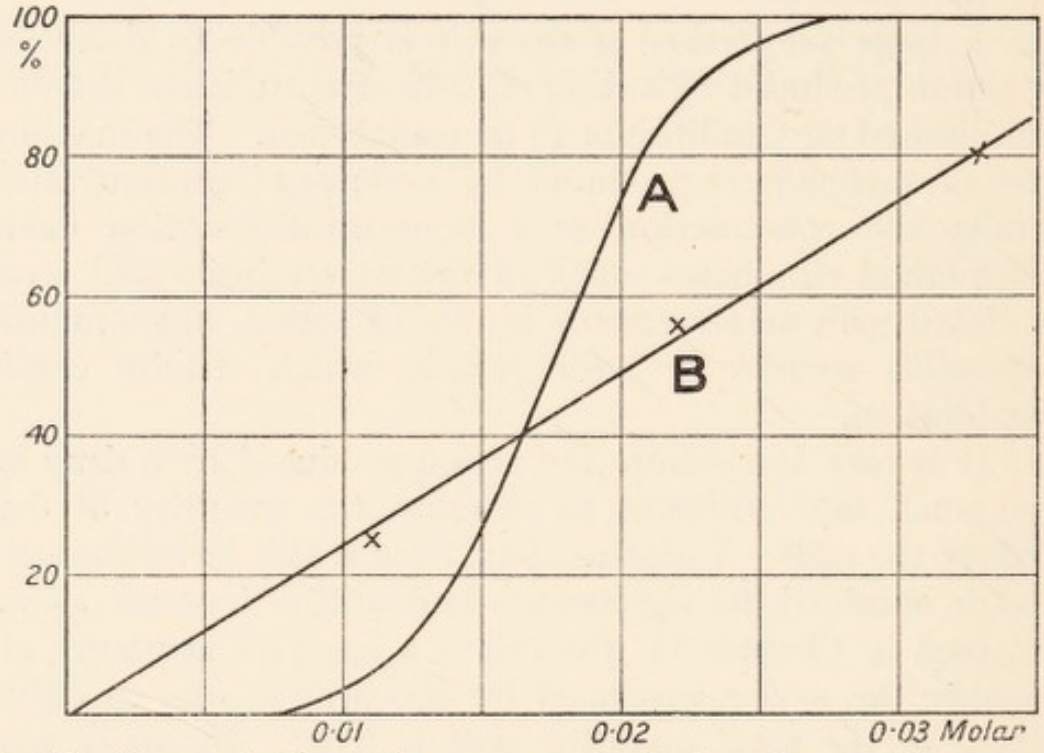

Fig. 29.—Concentration action curves of ethyl urethane acting on sea-urchin eggs.

Abscissa : molar concentration. Ordinate : per cent. mortality.
Curve A. The inhibition of division. (All-or-none effect.) (Scott. Unpubl. results.)
Curve B. The inhibition of respiration. (Graded action.) (Meyerhof. 1914a.)

linear over a considerable range of concentration. On the other hand, when the action produced by phenol on the cells was estimated by measuring the percentage of deaths in the population, a steep sigmoid curve was obtained. A concentration of 0·5 p.c. killed 5 p.c., whilst 86 p.c. of the cells were killed by 0·6 p.c. phenol. Fig. 29 shows another example of the differences in concentration-action curves that may be produced by measuring two different activities of the cell. The curves show the action of ethyl urethane on sea-urchin

eggs. In one case (A) the effect was measured by measuring the percentage of the population in which fertilization was inhibited. This is a typical all-or-none effect and a sigmoid curve is obtained. In the other case (B) the effect of the drug in reducing oxygen consumption was measured and a linear relation was obtained between concentration and action. This linear relation is the typical concentration-action curve obtained whenever the action of narcotics in producing a graded effect is measured (e.g. response of muscles, etc.).

Any graded action may be used to measure individual variation by measuring for each individual the concentration needed to produce some particular action, e.g. 50 p.c. inhibition of some function. On the other hand, the problem arises whether all graded actions do not express some form of individual variation. This is a difficult problem to settle because any curve can be explained as an expression of individual variation provided we are allowed to assume any distribution of variation that is convenient. A partial answer to this question is given in Fig. 29, for it is not reasonable to assume more than one form of distribution of variation in a single drug-cell system. In this case curve A undoubtedly expresses individual variation, and hence curve B, which shows a completely different relation between concentration and action cannot be an expression of individual variation. A complete proof of this would be afforded by the measurement of the effect of urethane on the oxygen consumption of a single cell. This measurement is not available, although it is probable that it would show a graded action similar to that shown in curve B.

Systems suitable for the Measurement of Graded Actions

Measurements of graded actions can be made much more accurately upon some systems than upon others. The effect of a drug upon the oxygen consumption of cells or upon the mechanical response of a muscle are examples of favourable systems. On the other hand, the action of drugs upon the conduction in nerves or indeed upon conduction in general is an example of an unfavourable system, because the effect measured is of an all-or-none character, namely whether or not the drug has destroyed the power of the tissue to conduct

impulses, and there is very little gradation between full conduction and complete obstruction. The systems of choice for this type of measurement are those in which the widest possible range of graded effects can be most easily and accurately measured.

These properties are in marked contrast to those required when it is desired to measure the incidence of a certain response in a population, for in this case it is desirable to select some sharp all-or-none effect so that all responses can be easily classified as either positive or negative.

In the second place graded responses can be measured much more easily when they are reversible than when they are irreversible. In the former case a series of experiments can be made upon a single preparation, and errors due to individual variation of preparations can thus be excluded. In the case of irreversible actions each point in a curve must be determined by performing a sufficient number of experiments to get averages, the standard deviation of which is not too high.

Measurement of Irreversible Actions

The difficulties attending the study of irreversible actions upon isolated tissues are illustrated by the following experiments which were performed by the writer in order to determine the relation between the concentration of caffeine and

TABLE XIV

The relation between concentration of caffeine and the contraction produced in isolated frogs' muscles. In each experiment the isometric tensions produced by a pair of muscles was measured, and the sub-maximum response was expressed as the percentage of the maximum response. (Clark. Unpublished results.)

Concentration of caffeine.	Sartorius.		Rectus abdominis.	
Per cent.	Number of experiments.	Response.	Number of experiments.	Response.
0·1	10	100	2	100
0·08	22	89	6	95
0·06	8	52	13	72
0·05	8	18	4	14

the amount of contraction produced in the isolated muscles of the frog. Pairs of muscles were taken and one member was immersed in a solution of caffeine sufficiently strong to produce a maximum contraction, whilst the other member was immersed in some weaker solution. The contraction

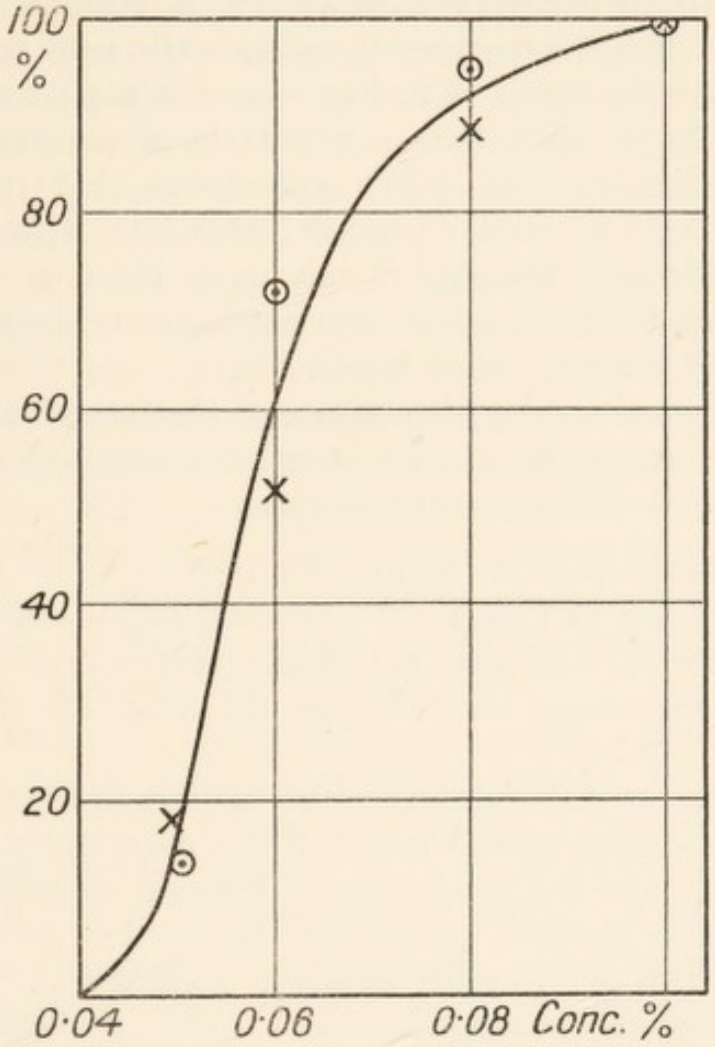

Fig. 30.—The concentration-action curve of the isometric response to caffeine shown by isolated frogs' muscles. (× = Sartorius; o = Rectus abdominis.)

Abscissa: caffeine concentration per cent. Ordinate: isometric response expressed as the percentage of the maximum response. (Clark. Unpubl. results.)

produced by the sub-maximum concentration was expressed as the percentage of the maximum contraction.

This method of control partly eliminated errors due to individual variation, and the results which are shown in Table XIV and Fig. 30 appear to give a fairly smooth curve. The number of experiments made was 73, but statistical

analysis showed that this number was quite insufficient to provide reliable results. For example, 22 experiments were made to determine the effect of 0·08 p.c. caffeine on the sartorius. The average response was 89 p.c. of the maximum, and the standard deviation of this mean was $\pm$ 6. This figure, although not very satisfactory, is permissible, but in the case of the 8 experiments made with 0·06 p.c. caffeine the standard deviation of the average (52 p.c.) was as much as $\pm$ 11. It is clear that so uncertain an average has but little significance. The writer calculated that in order to obtain the eight averages shown in Table XIV with a standard deviation for each average of not more than $\pm$ 5, it would be necessary to make about 200 experiments, instead of the 73 that had actually been made.

In the case of a drug such as acetyl choline, which produces a freely reversible contracture, a greater accuracy could have been obtained with 10 experiments.

Graded Responses in Single Cells

It was shown in Chapter VI that all organisms show individual variation, and that this was true not only in the case of larger organisms but also in the case of monocellular organisms.

In the case of striated muscle the individual fibre is believed to give a response that is nearly if not completely all-or-none in character, and the gradations of response to nerve stimulation shown by striated muscles are believed to be due chiefly to variation in the number of fibres contracting.

It is therefore conceivable that all graded responses given by isolated muscles to drugs are due not to variations in the extent of the response of individual cells but to variation in the number of cells responding to the effect produced by the drug. The possibility of such a hypothesis makes it important to determine whether single cells are capable of giving graded responses to drugs or to other stimuli. The accurate measurement of the response of a single cell is always a matter of difficulty and in many cases is impossible, and hence the evidence on this point is limited.

In the case of protozoa it is practically certain that these must be capable of giving graded responses to stimuli and there is also direct evidence for this conclusion.

Pantin (1924) studied the effect of varying the reaction of fluids upon the movements of individual amœbæ, and found that the speed was maximum at *p*H 8·0, that arrest was produced by *p*H 7·0, and that intermediate reactions caused a reduction of speed. Pantin (1925) found, however, that other ionic changes such as excess of magnesium or potassium, or lack of calcium, produced an all-or-none action on amœbæ, for they continued to move well until sudden arrest occurred. Chase and Glaser (1930) studied the effect of acidity on the movement of paramœcia and noted that a gradual decrease in rate of movement could occur.

In some cases it is possible to study the individual activity of the cells in isolated organs. Gray (1930) measured the movements of individual cilia of Mytilus by means of cinematography. He found that alterations of temperature caused graded changes in the activity of individual cilia.

Matthews (1931) measured the response to stimulation of single receptors in frogs' muscles, and showed that by varying the intensity of stimulus a series of graded responses could be obtained. Moreover, various ionic changes could produce graded alterations in the response.

Even in the case of striated muscle recent evidence indicates that single fibres can give graded responses. Pratt (1930), Gelfan (1930), and Brown and Sichel (1930) studied the responses of striated muscle by various micro-methods and all concluded that the responses of individual fibres showed a progressive increase with increasing strengths of stimuli.

These examples show that it is possible for single cells to give graded responses to stimuli and that drugs can produce graded effects on a single cell. This fact can only be proved in a certain number of cases. In other cases there is a strong probability that it is true, but the point cannot be proved. For example, if a drug reduces the respiration of a tissue by 50 p.c. it is almost certain that this is due to a general depression of all the cells, and not to a selective paralysis of half the population. This point could only be proved by measuring the oxygen consumption of a single cell, and this is a matter of great technical difficulty. In such cases the probabilities are so strongly in favour of the occurrence of graded effects being produced that it seems permissible to assume that they occur.

Forms of Concentration-Action Curves

Storm van Leeuwen and le Heux (1919) studied the action of a series of drugs on the reflexes of decerebrate rabbits. They found three different types of relations between drug concentration and the amount of action produced.

1. A linear relation in the case of aliphatic narcotics (i.e. action varies as concentration).

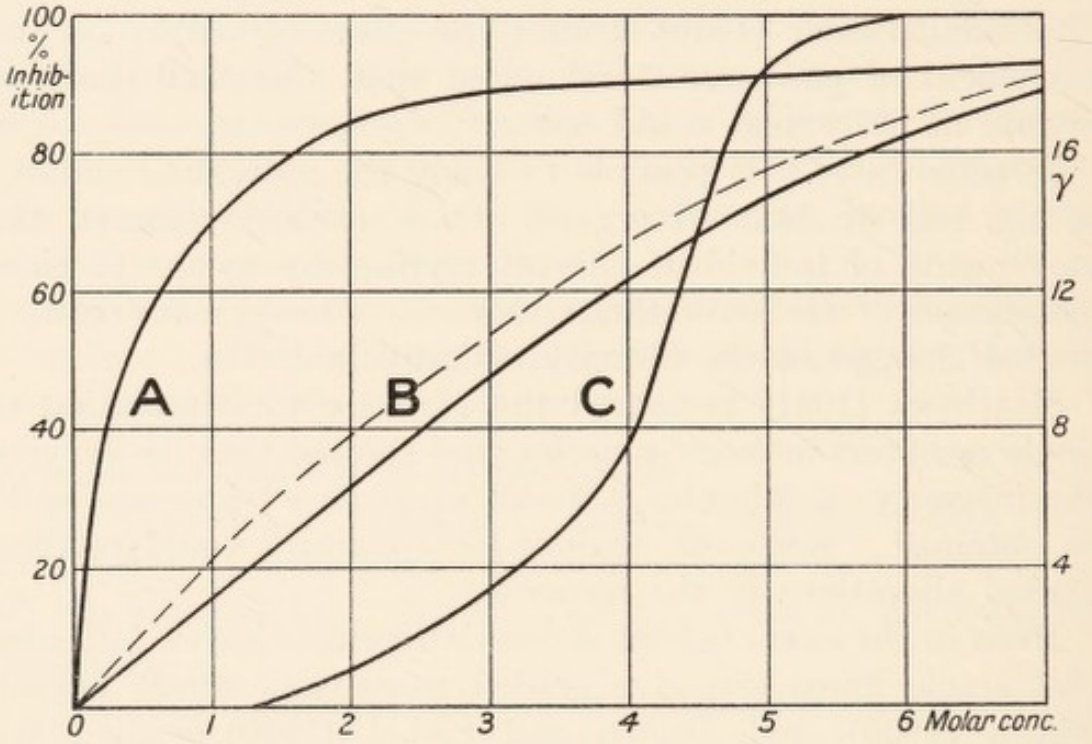

Fig. 31.—Concentration-action curves of drugs acting on frogs' isolated hearts.

Abscissa: molar concentration. Ordinate: per cent. inhibition of the isochoric response.

Curve A. Acetyl choline HCl (Conc. × 200,000).

Curve B. Ethyl alcohol (Conc. × 5).

Curve C. Potassium chloride (Conc. × 500).

Broken line: The reduction produced by ethyl alcohol (conc. × 5) in the air/water surface tension (cf. Right ordinate).

(Clark.)

2. A logarithmic relation in the case of morphine (i.e. action varies as log. concentration).

3. In the case of histamine acting on the isolated uterus they found a third type of relation, namely a sigmoid curve.

The author has studied the action of a large variety of drugs on the isolated heart of the frog, and has found the same three main types of relation, which are shown in Fig. 31. The only difference between my results and those of Storm

van Leeuwen is that I believe the curve, which he regarded as logarithmic, to be really a hyperbola. The problem is to explain the reason for the occurrence of these three types of curves.

Concentration-Action Curves obtained with Non-living Systems. The differences in shapes of curves relating drug concentration and amount of effect produced on a living organism can be paralleled to a certain extent by results obtained with non-living systems.

In Chapter II it was shown that the relation between concentration and uptake of drug might follow at least three different curves, namely linear, sigmoid and exponential. The action of drugs upon catalysts shows a similar variety of relations. Rideal and Wright (1926) measured the effect of various drugs in inhibiting the oxidation by charcoal of organic acids. Potassium thiocyanate gave a sigmoid concentration-action curve, whilst amyl alcohol showed a linear relation between concentration and action, which became discontinuous at 80 p.c. inhibition.

Rona studied the action of several poisons on various ferments and found three different types of curve relating concentration and the extent of the inhibition of the ferment activity. The inhibition of invertase by quinine (Rona and Bach, 1920) followed the usual adsorption formula, for there was a linear relation between the logarithms of the concentration and of the action. In the case of the inhibition of serum lipase by atoxyl (Rona and Bach, 1920), and by quinine (Rona and Reinecke, 1921), there was a linear relation between the action and the logarithm of the concentration. Finally, in the case of the inhibition of invertase by *m*- and *p*-nitrophenol (Rona and Bach, 1921) there was a sigmoid relation between concentration and action. Complete inhibition was produced in this case by a concentration only four times the minimum concentration that produced a demonstrable action.

These examples show that the relation between the concentration of drugs and their action on ferment activity may vary when the same drug acts on different ferments or when the same ferment is acted upon by different drugs. The case of a drug acting on a ferment is far simpler than the case of a drug acting on a living cell, and therefore it is unlikely that

the relation between drug concentration and the effect produced on cells will be absolutely constant for any one drug or for any particular cells. As a general rule however a drug gives similar concentration-action curves even when it acts on a wide variety of tissues. It is therefore of interest to consider the probable physico-chemical reasons for the occurrence of the chief types of concentration-action curves that have been described.

Sigmoid Curves. The examples given in Chapter VI show that sigmoid curves are very frequently obtained when the relation measured is that between the concentration of a drug and the incidence of some particular response in a population of cells. The sigmoid form of concentration-action curve may be expected when the action measured is of an all-or-none type as regards the response of individual cells. All-or-none effects are particularly likely to be produced when a drug interferes with the conduction through an excitable tissue, and it is reasonable to explain the action of potassium chloride on the frog's heart as an effect of this nature. According to this hypothesis, curve C in Fig. 31 expresses the individual variation in the cells of the conducting tissues in the frog's ventricle as regards the concentration of potassium chloride needed to paralyse their power to conduct impulses.

Certain tissues are particularly likely to give all-or-none responses to most drugs. For example, the action of drugs in paralysing a nerve-trunk always approximates to the all-or-none type of effect. The isolated uterus of the guinea-pig also is an organ that appears to have an explosive type of response. Up to a certain concentration drugs produce no action and a slightly increased concentration produces maximum contraction. Sigmoid concentration-action curves have been obtained by most authors with this organ, although Cameron and Mackersie (1926) describe an exponential relation between pituitary dosage and uterine response.

A sigmoid relation between concentration and response is obtained under the following conditions :

1. It is the commonest type of curve obtained when the method of measurement simply measures the presence or absence of a given effect in the individual cell, e.g. death of cells, failure to divide, failure to conduct impulses (nerves).

2. When the mechanical conditions of the experiments tend to express the cell response as an all-or-none effect; e.g. in the case of the isolated frog's heart a delicate isometric lever will measure gradations of action accurately, but a heavy isotonic lever will tend to convert a graded effect into one that is apparently all-or-none.

3. Certain tissues have a tendency to give an all-or-none effect; any stimulus sufficient to excite them tends to produce a nearly maximum effect, e.g. the guinea-pig's uterus.

Linear Relation. This is the simplest relation observed between concentration and action and is fairly constantly given by aliphatic narcotics. The most probable reason for this relation in this case is that narcotics are adsorbed on cell surfaces and the action they produce is directly proportional to the amount of their adsorption.

The effect of narcotics upon surface tension is a simple measure of their adsorption at an air/water surface. The relation between concentration and fall of surface tension is a hyperbola as is shown in curve B, Fig. 32. In the case of actions that are less than 20 p.c. of the maximum the relation between concentration and surface action is nearly linear as is shown in Fig. 31 (broken line, curve B).

In the great majority of cases studied the relation between concentration of aliphatic narcotic and the amount of action produced on living cells is nearly linear. This relation is shown in curve B, Fig. 31, which expresses the inhibition of the mechanical response of the frog's heart by ethyl alcohol, and the same relation was found between concentration and inhibition of the oxygen consumption of the heart. (Clark and White, 1928.) Other examples of this same relation are the inhibition of the respiration of *Nitrosomas* (Meyerhof, 1917), and of sea-urchin eggs (Fig. 29, curve B). Meyerhof (1916A) found that in the case of the inhibition of the respiration of *Nitrobacter* by narcotics the concentration-action curve was of a sigmoid shape.

The distinction between the linear and the sigmoid shape in these cases depends chiefly, however, on the accurate determination of the feeblest actions produced by the drug. These values are particularly difficult to determine accurately

and therefore these sigmoid relations may be considered somewhat doubtful.

In a few cases the concentration-action curve is exponential (Fig. 32), and the same type of relation has been found for the action of narcotics on contact catalysts (Meyerhof, 1914B). In regard to this point it must, however, be remembered that the relation between concentration of narcotic and its uptake

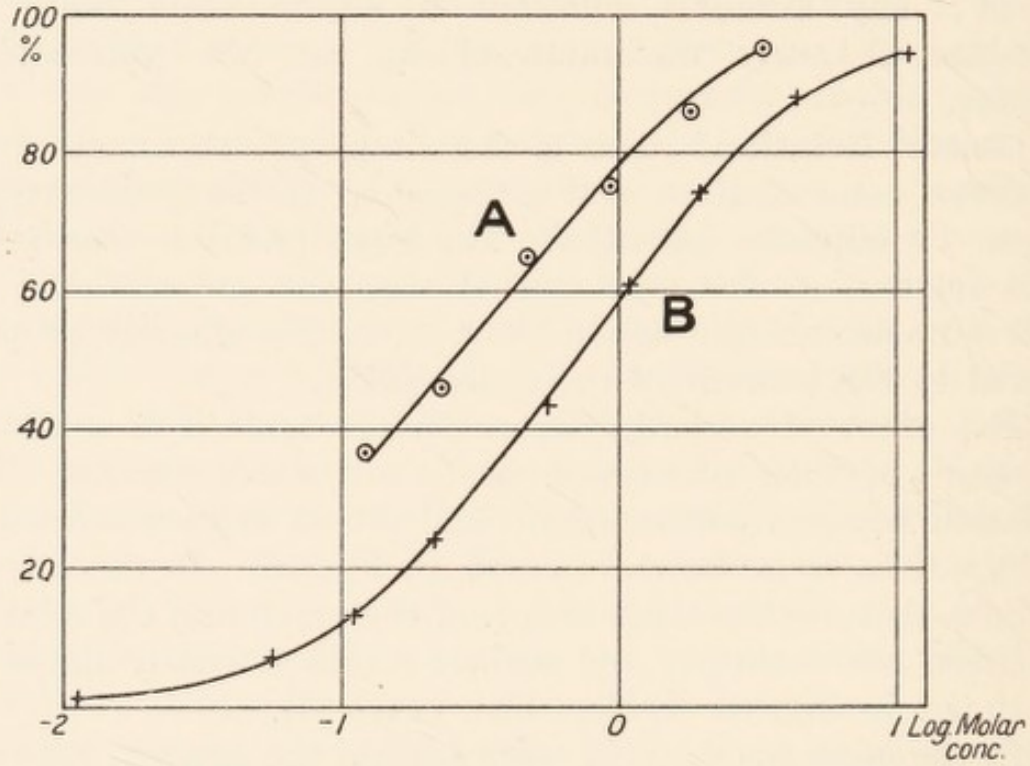

Fig. 32.—Concentration-action curves of urethanes.

ABSCISSA: logarithm of molar concentration. ORDINATE: action produced expressed as per cent. of maximum possible.

CURVE A. The inhibition of the production of CO_2 by *Chlorella* by phenyl urethane (Conc. × 1000). (Warburg. 1920.)

CURVE B. The reduction of the air/water surface tension by ethyl urethane (Surface tension of saturated solution = 36 γ). (Landolt-Börnstein. 1931.)

by charcoal is a matter of dispute, for some workers have found an exponential relation whilst others describe a discontinuous linear relation (cf. p. 53).

Since the mode of action of these drugs on a simple catalyst such as charcoal is not completely understood, it is unlikely that conclusive evidence will be obtained in the case of living cells.

Isonarcotic and Isocapillary Actions

Traube pointed out the remarkable parallel between the action of narcotics on cells and their power of reducing surface

tension. This parallel has been shown to hold with surprising accuracy for a wide variety of living tissues, providing that the comparison is made between members of a single class of narcotic. This subject has been discussed fully by Winterstein (1926, p. 327).

Table XV shows certain results obtained by the author

TABLE XV

Isonarcotic and isocapillary concentrations of normal alcohols and of various other narcotics. (Clark, 1930.)

Drug.	Molar concentrations which reduce response of the frog's ventricle to half normal.	Molar concentrations which reduce the surface tension of water to 63 Dyn./cm.
Normal alcohols :		
Methyl	1·6	1·5
Ethyl	0·65	0·5
Propyl	0·17	0·14
Butyl	0·05	0·043
Amyl	0·02	0·018
Heptyl	0·000,7	0·002,4
Octyl	0·000,2	0·000,6
Decyl	0·000,03	0·000,094
Dodecyl	0·000,006	—
Tetradecyl	0·000,1	—
Urethanes :		
Methyl	0·17	0·76
Ethyl	0·13	0·26
i-Amyl	0·001,4	—
Ethyl ether	0·081	0·066
Chloral hydrate	0·006	0·4
Butyl chloral hydrate	0·001,5	0·04
Chloroform	0·002	—

on the isolated frog's heart, and these illustrate this rule and its exceptions. The rule holds with very fair accuracy for all the normal alcohols ranging from methyl to decyl, although the equi-active concentrations vary 300,000 fold. On the other hand, chloral hydrate has three times as strong a biological action as has amyl alcohol, but as regards action on surface tension amyl alcohol is twenty times as strong as

chloral hydrate. The work of Rideal and Wright (1925) provides a possible explanation for this difficulty. They measured the action of amyl alcohol in inhibiting the auto-oxidation of charcoal and found that complete inhibition was produced when the quantity of amyl alcohol adsorbed was sufficient to cover only 0·38 p.c. of the surface. This proves that the alcohol can be adsorbed selectively by a particular set of receptors without covering the surface as a whole. In the case of living cells there is evidence that the activities of the cell depend upon certain active patches on the surface. It seems possible that those narcotics whose biological activity is greater than would be expected from their general action on surface tension may be selectively adsorbed by the active patches on the cell surface. The complexity of the results obtained in the case of narcotics acting on charcoal suggests a large number of possible reasons why there should not be an accurate parallel between the intensity of action of these drugs on an air/water surface and their action on a system as complex as a living cell. It is indeed both surprising and remarkable that the parallel between the isocapillary and isonarcotic concentrations should be as complete as it has been found to be.

The Concentration-Action Curve of Acetyl Choline

The curve relating concentration and amount of graded action is a hyperbola in the case of many drugs of great pharmacological interest, e.g. acetyl choline, adrenaline, nicotine, etc. The interpretation of this relation is a matter of considerable theoretical interest and therefore the evidence regarding the mode of action of acetyl choline will be considered in some detail because such evidence is more complete in the case of this drug than in the case of the other drugs mentioned.

The writer found that the relation between concentration and action of acetyl choline on both the frog's heart and *rectus abdominis*, followed a hyperbola (Fig. 31, curve A, and Fig. 33), and the relation could be expressed by the simple formula $Kx = \frac{y}{A - y}$ where x = concentration ; A = maximum action and y = action observed. This is the formula

that expresses the relation between concentration and chemical change when two substances combine to form a freely reversible combination and when one substance is in such a great excess that its concentration is not altered significantly even when the other substance has been exhausted. For example, this formula expresses the relation between oxygen pressure and the formation of oxyhæmoglobin. It also is the type of relation particularly likely to obtain when any substance present in excess is adsorbed by a limited surface. Langmuir showed that this equation expressed the simplest form of adsorption of a gas by a metal surface ; namely, the case in which one molecule of gas united with one receptor on the surface. It also expresses the adsorption of a surface active substance on an air/water surface as is shown in Fig. 32, curve B.

A fair amount of information has been accumulated regarding the action of acetyl choline on the isolated heart and muscles of cold-blooded animals. It is therefore worth while considering in detail whether the evidence existing conforms to the hypothesis that the action of the drug depends upon it forming a freely reversible combination with a limited number of receptors in the tissue and that the extent of its action is proportional to the proportion of receptors occupied. This hypothesis postulates the relation $Kx = \frac{y}{100 - y}$ between concentration (x) and p.c. of possible action (y).

Fig. 33 shows two typical concentration-action curves of acetyl choline. With regard to this figure it may be remarked that acetyl choline inhibits the isolated frog's heart, and whilst it is not possible to measure accurately a very small degree of inhibition, yet it is possible to distinguish very small residual movements and hence differences between 90 and 100 per cent. inhibition can be measured accurately. The upper half of curve A is therefore more accurate than the lower portion. In the case of the *rectus abdominis* the drug produces contracture and the maximum amount of contracture that can be produced cannot be measured very accurately. On the other hand, a delicate lever will record accurately the slightest degree of contracture. Hence the bottom half of curve B is more accurate than the upper portion. The two curves A

and B in Fig. 33 together record fairly accurately the general shape of the concentration-action curve of acetyl choline.

In general the figures lie along hyperbola and can be fitted by the formula $Kx = \frac{y}{100 - y}$ and are difficult to fit with any other formula. For example, the extreme values very definitely diverge from the relation action varies as log. concentration, although this fits the central part of the curves.

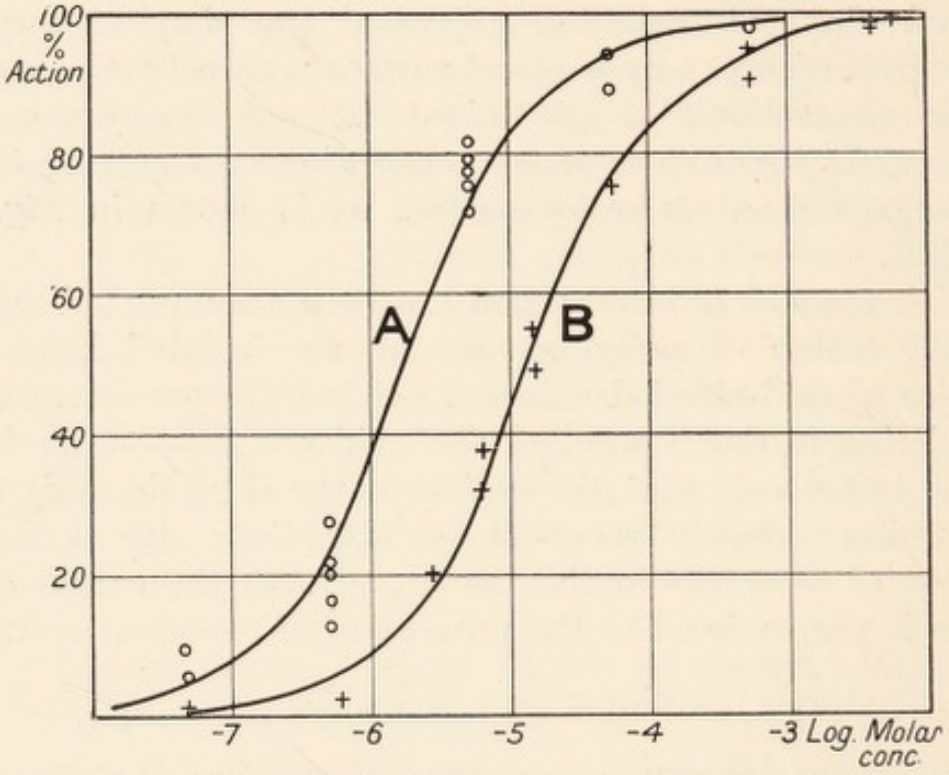

Fig. 33.—Concentration-action curves of acetyl choline.

Abscissa: logarithm of molar concentration. Ordinate: action produced expressed as per cent. of maximum.

Curve A. Inhibition produced on frogs' isolated hearts.

Curve B. Contraction produced in frogs' isolated *Recti abdominis*. (Clark. 1926.)

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Evidence regarding the Nature of the Reaction. (i) *Amount of drug fixed by tissue.* The author (Clark, 1927) has calculated this approximately both on the frog's heart and the *rectus abdominis*. The results show that a 50 p.c. action is produced by the fixation of 2×10^{-8} mgm. drug per mgm. tissue which corresponds to about 200,000 mols per heart-cell. This quantity is of course extremely small in comparison with the quantity present in any solution bathing the tissue.

The fixation of drug by the tissues can only be demonstrated if special precautions are taken. Any given concentration of acetyl choline will produce practically identical effects on a heart whether 1 c.c. or 10 c.c. of fluid are circulated, and in order to demonstrate depletion the volume of perfusion fluid must be reduced to a fraction of a cubic centimetre.

The amount of drug fixed by the tissues does not therefore affect the concentration of drug in the surrounding fluid unless special precautions are taken and the fluid is reduced to a very small quantity, hence it is permissible to regard the concentration of drug in the solution as remaining constant during the course of an experiment. The amount of drug fixed is too small to cover more than about 1/10,000 of the cell surface, and this in itself is strong evidence that the drug acts on specific receptors.

(ii) *Rate of action of acetyl choline.* The author (Clark, 1927) has shown that acetyl choline produces half its maximum action in a period of not more than 1 or 2 seconds and that the rate of washout is of similar duration. This is in agreement with the conception that the action of the drug is proportional to the number of receptors that are maintained in occupation by the drug, for such a theory postulates a rapid action which is freely reversible.

(iii) *Destruction of acetyl choline.* Loewi and Navratil (1926) showed that the tissues contained a ferment that rapidly destroyed acetyl choline. The writer (Clark, 1927) found that nine-tenths of a moderate dose of acetyl choline could be destroyed in ten minutes. These figures agree with results obtained by Engelhart (1930) in showing that 0·1 gm. of frog's heart can destroy about 2·5γ acetyl choline from a 10^{-5} molar solution. The amount of drug that produces the action at this concentration is probably about 2×10^{-7} mgm. per mgm. or 2×10^{-5} mgm. per 0·1 gm. heart, and this quantity must be destroyed every 5 seconds.

It therefore is possible that acetyl choline does not form a reversible compound with the receptors on the heart surface but that the drug is broken down almost as rapidly as it is fixed, and that this is the reason for the rapid recovery on washout. The physico-chemical explanation advanced for the shape of the concentration-action curve was based on the

assumption that the drug formed a freely reversible compound with receptors on the heart surface, but it would apply equally well if the drug was broken down nearly as rapidly as it was fixed.

(iv) *The antagonism of acetyl choline by atropine.* The general problem of drug antagonism is discussed in Chapter XI, but the following points may be mentioned as throwing light on the mode of action of acetyl choline. The form of the antagonism between acetyl choline and atropine suggests at first sight that the two drugs compete for the occupation of common receptors. The curves relating the concentrations of acetyl choline and atropine and the effect produced by the former drug show a close resemblance to the curves relating the concentrations of oxygen and carbon monoxide and the amount of oxygen fixed by hæmoglobin. This theory is, however, untenable because acetyl choline in excess does not displace atropine from the heart. The most probable assumption is that the two drugs act at different points in a chain reaction. Examples of such effects can be found in the case of drugs acting on the respiration of cells.

(v) *Relations found between dosage and action in intact animals.* Acetyl choline produces graded effects over a very wide range of doses in intact animals. Clark and White (1927) found that in the intact cat intravenous injections of acetyl choline produced a graded fall in blood-pressure when the dosage ranged from $4{\cdot}25 \times 10^{-6}$ to 0·425 mg. per kg., and produced a graded effect on the pulse rate over a range of dosage from $1{\cdot}4 \times 10^{-3}$ to 0·425 mg. per kg. In both cases the figures showed an approximately linear relation between the logarithm of the dosage and the action produced. The minimum effective dose in this case was rather large, for Hunt (1918) found that a dose of $2{\cdot}4 \times 10^{-9}$ mg. of acetyl choline produced a demonstrable effect on the cat's blood-pressure. The full range of effective dosage may therefore be greater than that found by Clark and White.

White (Fig. 26) found, however, that the relation between dosage of acetyl choline and the percentage mortality of mice followed an ordinary slightly skewed frequency curve, which showed a probable variation of 21 per cent. of the median.

This result shows that it is possible to obtain a fairly sharp

all-or-none effect with a fairly narrow range of individual variation, even in the case of a drug that can produce graded effects over a very wide range of dosage.

Possible Explanations of Acetyl Choline Concentration-Action Curve

There are three possible interpretations of the concentration-action curves observed in the case of acetyl choline.

1. That the action follows the Weber-Fechner law.

2. That the concentration-action curves are the expression of some form of individual variation distributed in an extreme skew fashion.

3. That the curves express the adsorption of the drug.

(i) *The Weber-Fechner law.* There has been a general tendency to explain concentration-action curves as logarithmic relations, and to adduce them as examples of Weber's law. It will be shown in Chapter IX that the evidence for the truth of the Weber-Fechner law is very doubtful, and that it certainly is not a correct statement of the relation between stimulus and effect in the case of several of the sense organs. The writer has found that in practice the relations between concentration and action in the case of most potent drugs that produce a reversible action follow a hyperbola more nearly than they do the relation action varies as log. dosage. Furthermore, the hyperbola can be interpreted as the expression of a simple chemical reaction, whereas there is no simple explanation of the logarithmic relationship. On the other hand, it must be admitted that the hyperbola could be interpreted as a logarithmic relationship distorted by individual variation in the cells.

(ii) *Individual variation.* Individual variation may be regarded as a fundamental law characteristic of living matter and moderately skew distributions of variation are not uncommon. The explanation of relations by assuming individual variation is, however, unsatisfactory in that there is no type of curve that cannot be explained in this manner provided that any form of distribution of variation that is desired is assumed.

The hearts of different frogs show an extremely wide variation as regards their sensitivity to acetyl choline, and the distribution of this variation is of an extreme skew form as is shown in Fig. 34. Therefore it is tempting to try to explain the

concentration-action curve of acetyl choline as an expression of skew variation. This hypothesis cannot be disproved, and the only thing to consider is whether it involves improbable assumptions.

The concentration-action curve of acetyl choline has a similar shape in the case of the frog's ventricle, auricle and *rectus abdominis*. If these curves are to be interpreted as expressions of individual variation it is therefore necessary to assume that in all three cases some factor varies over a similar range, and that the same extreme skew distribution of variation

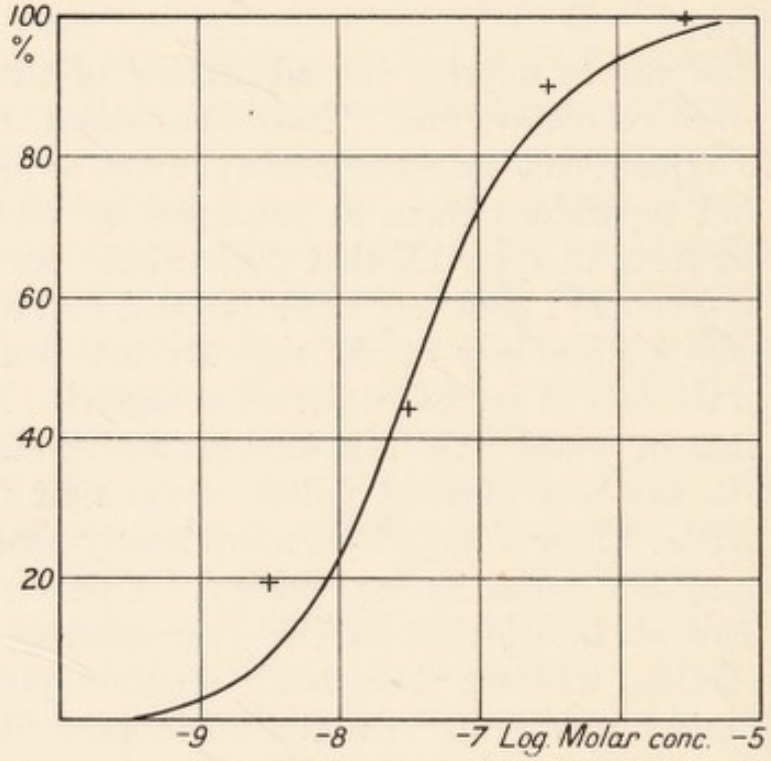

Fig. 34.—Characteristic curve of frogs' isolated hearts responding to acetyl choline.

ABSCISSA : logarithm of molar concentration. ORDINATE : per cent. of hearts showing 50 per cent. inhibition. (Clark. 1927.)

occurs in each case. If the heart alone were concerned it would be possible to assume variation between one heart-cell and another, but it is very improbable that the fibres of a striated muscle such as the *rectus abdominis* would vary in exactly the same manner as the cells of the heart. It seems necessary therefore to assume that the individual cell receptors vary. The assumption of a skew distribution of sensitivity amongst the cell receptors would explain the concentration-action curves observed and also would explain very simply the phenomena

of drug antagonism observed with atropine and acetyl choline.

A variation of this type has actually been postulated for the receptors on the surface of charcoal. Cameron (1930) measured the gramme-molecules of oxygen adsorbed by charcoal and the calories produced per mol. oxygen adsorbed. His results show that a small fraction (about 1 p.c.) of the total charcoal surface has a high activity and the remainder is much less active. The figures show an approximately linear relation between the quantity of heat produced and the logarithm of the amount of oxygen fixed. The assumption that there is a very great individual variation in the activity of cell receptors can therefore be supported by analogous cases in simpler systems.

Such an assumption would not however help to explain the individual variation found between one heart and another. The number of cell receptors in a heart must be more rather than less than 10^{12}, and the fact that the individual members of such a population vary widely, will not account for wide variations in the average sensitivity of a series of populations of this size.

The concentration-action curves can therefore be explained as expressions of individual variation in the activity of cell receptors, but this explanation will not account for the characteristic curve relating concentration and incidence of effect in a population of hearts. On the other hand if we assume that the concentration-action curve expresses some fundamental chemical reaction, then, as was shown in Chapter VI, the characteristic curve can be explained as an example of a moderate variation in sensitivity distorted by the relation between drug concentration and drug fixation.

(iii) *Adsorption hypothesis.* The hypothesis that the concentration-action curve of acetyl choline expresses an adsorption process of the type described by Langmuir appears to the writer to involve fewer improbable assumptions than any alternative hypothesis. It is, of course, unproven and rests on the assumption that the action produced varies directly as the amount of drug that is fixed by the cell receptors. The complexity of the living cell makes it impossible to prove or disprove this assumption. Even if we could measure

accurately the amount of drug fixed we still would not know how much was being fixed by the receptors and how much was being neutralized by inert material. The writer therefore accepts the hypothesis that the concentration-action curve of acetyl choline expresses the course of a reversible chemical reaction or adsorption. The justification for this assumption is that it is not inherently improbable and that the alternative hypotheses involve improbable assumptions.

Concentration-Action Curves of Various Potent Drugs

The evidence regarding acetyl choline has been considered in some detail and the following facts have been shown :

1. The amount of drug producing the action is very small.
2. The action is very rapidly produced and is equally rapidly reversed on washing out.
3. The drug produces a graded action over a range of concentration more than 1000 fold and approaching 10,000 fold.
4. The concentration-action curve follows a hyperbola.
5. The characteristic curve expressing the distribution of individual variation also follows a hyperbola.

These facts can also be demonstrated in the case of various other drugs that produce a specific action. The evidence is most complete in the case of drugs that produce a rapidly reversible and easily measured mechanical response.

Adrenaline

Wilkie (1928) found that the relation between the concentration of adrenaline and the amount of contraction produced in a strip of sheep's carotid artery followed the formula $kx = \frac{y}{100 - y}$, as is shown in Fig. 35. Hunt (1901) and Molinelli (1926) both measured the increase of blood-pressure produced in the dog by adrenaline given over a wide range of dosage. Their results, which are shown in Fig. 36, can be fitted by the formula $kx^2 = \frac{y}{a - y}$, where a is the rise of blood-pressure produced by maximum doses. The calculated and observed lines are only in rough agreement, but inspection shows that the most generally favoured alternative relation action varies as the logarithm of the concentration would not even approximate to the observed figures.

Langecker (1926) measured the concentration of adrenaline needed to cause a certain contraction in the isolated uteri of 172 rabbits. His figures are as follows :

Molar conc. of adrenaline $\times 10^{-6}$. .	0·05	0·15	0·3	0·6	1·2	2	3	6
Per cent. of uteri in which action was produced . .	5	9	27	59	85	94	97	100

The curve relating these results, which is shown in Fig. 37, follows the formula $Kx^2 = \frac{y}{100 - y}$. The only objection to

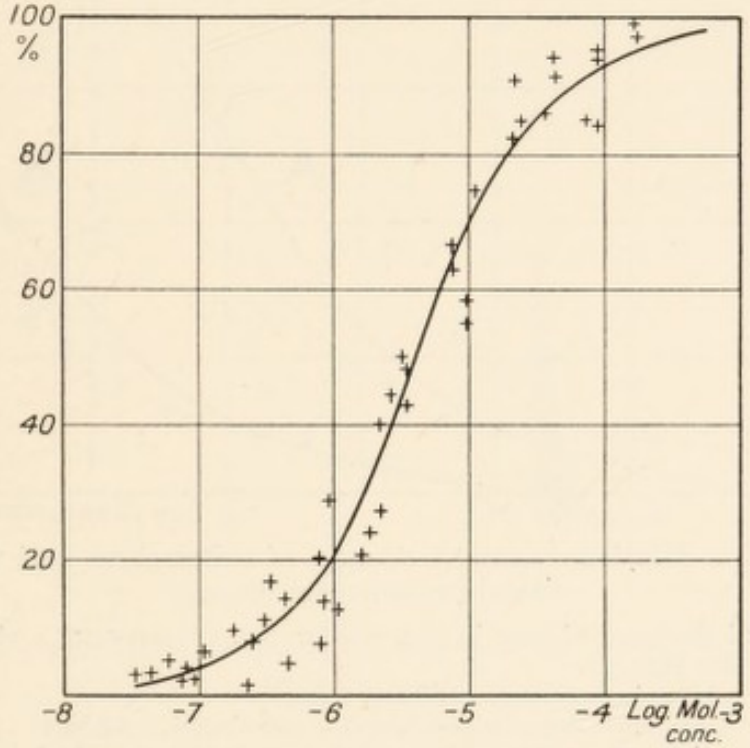

Fig. 35.—Concentration-action curve of adrenaline acting on strips of sheep's carotid arteries.

Abscissa : logarithm of the molar concentration (x). Ordinate : action expressed as per cent. of maximum possible (y).

The curve is drawn to the formula $kx = \frac{y}{100 - y}$. (Wilkie. 1928.)

this example is that the rabbit's uterus is an organ that is extremely variable both morphologically and functionally. Wilkie did not study a sufficient number of carotid strips to estimate their individual variation, but he did not note any wide individual variation in the quantity of adrenaline needed to produce a given response.

Schultz (1919) determined the toxicity of adrenaline when given subcutaneously to mice. The figures obtained with a total of 122 mice were as follows :

Dose adrenaline mgm. p. k. .	1	3	6	8	10	16	25
Percentage of deaths . .	0	21	12	57	57	74	100

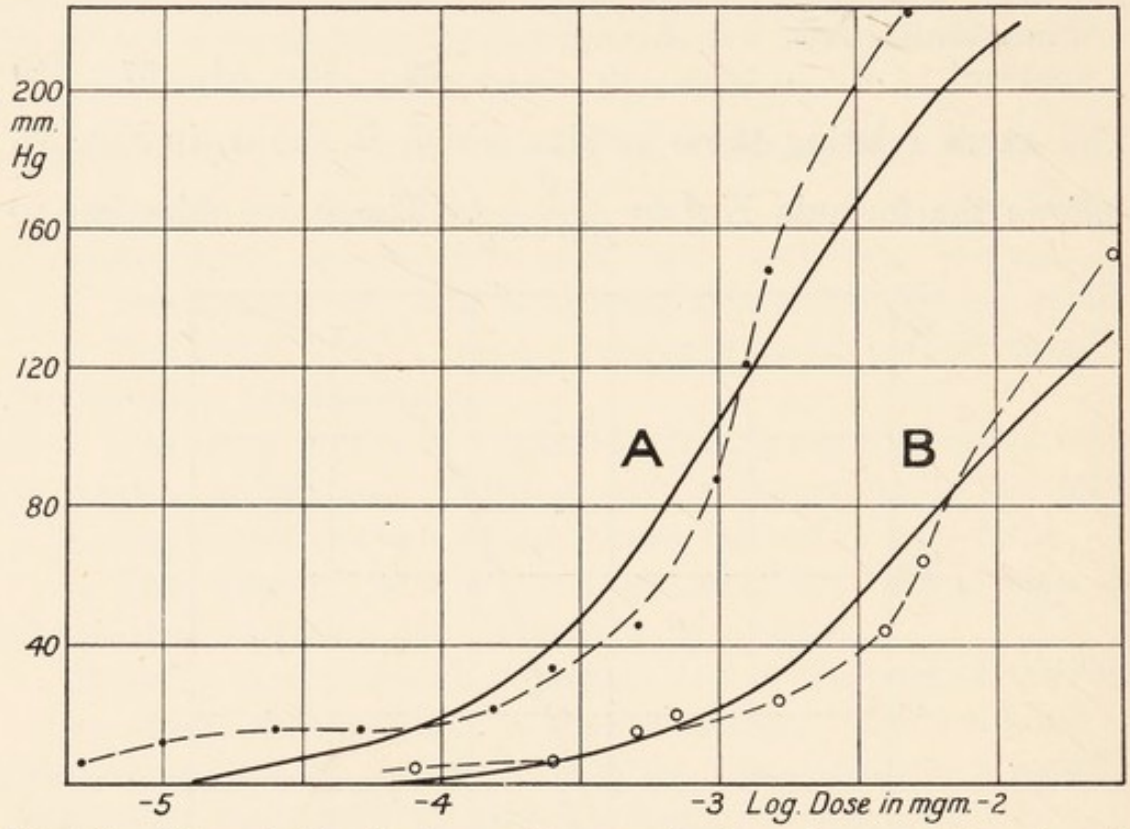

Fig. 36.—The relation between dosage of adrenaline and the rise of blood-pressure in dogs.

Abscissa : logarithm of dose in mg. per kg. (x). Ordinate : rise of blood-pressure in mm. Hg. (y).

Curve A. Atropinized dog. (Hunt. 1901.)

Curve B. Dog with denervated heart. (Molinelli. 1926.)

The broken lines show the observed figures. The continuous lines have been drawn to the formula $kx^2 = \frac{y}{A - y}$. (Curve A. $k = 6{\cdot}3 \times 10^5$, $A = 240$. Curve B. $k = 2{\cdot}5 \times 10^4$, $A = 160$.)

The figures are extremely irregular but they indicate an approximately symmetrical variation with the values for the dosage of about 5 and 14 for the lower and upper quantiles respectively. The relation between dosage or concentration of acetyl choline and adrenaline and the incidence of all-or-none effects therefore follows a sigmoid characteristic curve in some cases, whilst in other cases the characteristic curves thus obtained resemble hyperbolæ.

Nicotine

Similar curves also are obtained with nicotine. In this case the relation between concentration and action in producing contraction of the frog's *rectus abdominis* follows approximately the formula $Kx = \frac{y}{100 - y}$. The mortality produced by nicotine sprays on *aphis rumicis* also follows a hyperbola, but in this case the relation is $Kx^2 = \frac{y}{100 - y}$.

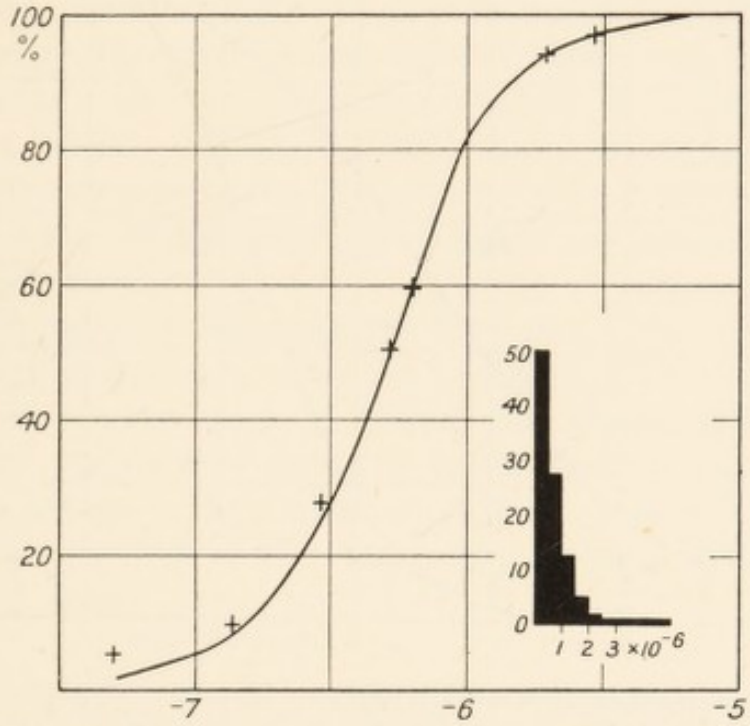

Fig. 37.—Characteristic curve of the response of rabbits' isolated uteri to adrenaline.

Abscissa: logarithm of molar concentration (x). Ordinate: per cent. of uteri showing contraction (y).

Inset. Distribution of individual variation.

Curve drawn to formula $kx^2 = \frac{y}{100 - y}$. (Langecker. 1926.)

These relations are shown in Fig. 38. Nicotine therefore provides another example of a drug the concentration-action curves of which follow the course of a hyperbola not only when the drug produces a graded effect but also when it produces an all-or-none effect.

Cyanide

Cyanide inhibits the oxygen uptake of many systems both living and non-living, and acts at very low dilutions. It

appears therefore to be a very favourable drug with which to study the relations between concentration and action. The evidence on this point is, however, very conflicting.

Warburg (1921) found that the adsorption of cyanide by charcoal followed Freundlich's formula and that there was a linear relation between the logarithms of the concentration and of the amount adsorbed. On the other hand, he found a

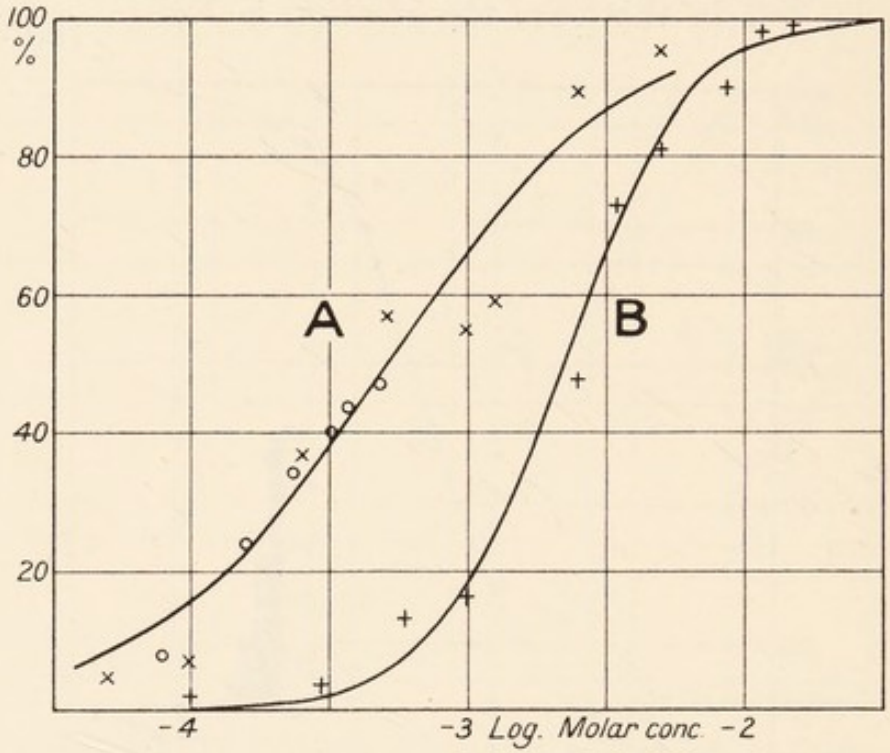

Fig. 38.—A concentration-action curve (A) and a characteristic mortality curve (B) given by nicotine.

Abscissa: logarithm of molar concentration. Ordinate: action expressed as per cent. of maximum.

Curve A. Concentration-action curve of nicotine (conc. × 1000) causing isotonic contraction of frogs' *Recti abdominis*. (o = Hill. 1909. × = Clark. Unpubl. results.) The curve is drawn to the formula $kx = \frac{y}{100 - y}$.

Curve B. Per cent. mortality produced in *Aphis rumicis* by nicotine dissolved in benzene and applied as a spray. (Tattersfield, Gimingham et al. v, 1927.) The curve is drawn to the formula $kx^2 = \frac{y}{100 - y}$.

linear relation between the concentration of cyanide and the inhibition of cystin oxidation by charcoal. In this simple system there was therefore no simple relation between the quantity of cyanide adsorbed and the amount of inhibition of oxidation. Warburg had previously shown (1910) that there was a linear relation between the concentration of cyanide

and the inhibition of the oxygen uptake by birds' red blood-corpuscles. Meyerhof (1916B) also found a linear relation between cyanide concentration and the inhibition of the oxygen uptake of *nitrobacter*. Warburg (1925) found on the other hand an exponential relation between cyanide concentration and the inhibition of the assimilation of CO_2 by *Chlorella*.

Lipschitz and Gottschalk (1920A) measured the power of minced tissues to reduce *m*-di-nitro phenol. This action was inhibited by cyanide and in the case of frogs' muscles there was a linear relation between concentration and effect. In the case of earthworm tissues (1920B) there was however a linear reaction between the logarithms of the concentration and of the effect. Pickford (1927) found an approximately linear relation between the logarithm of the concentration of cyanide and the inhibition produced in the mechanical response of the frog's heart.

These divergent results suggest that the action of cyanide in inhibiting respiration must be complex. This conclusion is supported by the work of Dixon and Elliott (1929), who found that cyanide inhibited the respiration in some tissues and not in others. They also found that cyanide could only inhibit about one-half of the respiratory activity of the tissues on which it acted.

Summary

The actions of only a few drugs on a few systems have been discussed in this chapter. One reason for this is that accurate measurements relating concentration and action can only be obtained in a relatively few cases. The example of cyanide shows how variable this relation may be in the case of a drug which produces an action that can be measured accurately and which appears at first sight to be of a simple nature. The favourable examples selected suffice, however, to show that the relation between concentration and effect produced differs very widely indeed in the case of different drugs, and that at least three distinctive types of relations can be recognized.

The simplest explanation that I have been able to devise for these various concentration-action curves is that they express a chemical reaction distorted to a greater or less extent by individual variation. I do not advance any theory as to

whether the receptors in the cell with which drugs unite are uniform or variable as regards their affinity for drugs. It is highly probable that they vary. The number of receptors per cell is, however, assumed to be large, i.e. 10,000 or more; hence it is assumed that the average sensitivity of receptors in any cell of a population of similar cells is approximately uniform, and this also applies to multicellular organisms. The response of cells or organisms to a given stimulus (i.e. combination with a definite amount of drug) is assumed to vary.

When the method of measurement records accurately all gradations in response, then the concentration-action curve may be assumed to measure the fundamental chemical reaction, although even in this case the individual variation distorts the curves at two ends, because the action recorded does not commence or end with the commencement or ending of the action on the average individual, but falls off to cover the most sensitive and least sensitive cases.

Most of the drugs in which concentration-graded action curves have been worked out accurately are those which produce a reversible action. It is, of course, far easier to get adequate controls in such cases because many experiments can be repeated on a single individual. The commonest relation the writer has found for potent drugs producing a reversible reaction has been a hyperbola. The narcotics are exceptional, for their concentration-action curves are approximately linear. There is, however, a striking resemblance between the concentration curves of narcotics on tissues, and the curves obtained by measuring the reduction produced in the air/water surface tension by narcotics at concentrations similar to those which produce a biological action.

In the case of organisms large enough to permit the measurement of the individual doses sigmoid characteristic curves are obtained relating dosage and incidence of effect. When organs or organisms are immersed in solutions and concentration instead of dosage is measured the curves relating concentration and incidence of effect are sometimes sigmoid but frequently have an exponential form.

Sigmoid curves are obtained when there is a linear relation between drug concentration and drug fixation, but when there is an exponential relation between drug concentration and

drug fixation, then the same relation is usually found between drug concentration and incidence of effect. Such curves probably are produced by the distortion of a variation distribution curve.

The writer's explanation of these curves is that there is a variation with an ordinary type of distribution as regards the amount of drug that must be fixed in order to produce the effect and that this relation is distorted by the exponential relation between drug concentration and drug fixation.

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CHAPTER VIII

THE SIGNIFICANCE OF TIME-ACTION CURVES

Historical Review of Problem

The most obvious method of measuring the mode of action of some lethal influence is to measure the rate at which it kills a fairly uniform population. This type of measurement has naturally been chosen very frequently for a wide variety of agents and there is a particularly abundant data available regarding the rate of action of disinfectants, of hæmolytics and of radiations on unicellular populations. There is a very general agreement regarding the shape of the time-action curves obtained, but unfortunately there is no sort of agreement regarding their significance, and there have been vigorous controversies on this subject in all of the three cases mentioned above. Sweeping conclusions have been drawn from the time-action curves obtained with a single class of agent, with little regard to the evidence provided by other time-action curves and with no regard to the evidence provided by the rest of pharmacology.

The trouble started when Henri (1905) noted that there was a linear relation between the logarithm of the survivors and the time of action, in the case of fowls' red blood-corpuscles hæmolysed by dogs' serum. He concluded that this proved that the hæmolytic process was a monomolecular reaction and followed the formula $Kt = \log \frac{a}{a - x}$ (t = time, a = initial population of corpuscles and x = number hæmolysed).

Geppert in 1889 had attributed the shape of time-action curves obtained in hæmolysis to what appears to be the most obvious cause, namely the individual variation in the populations studied, and Mioni (1905) pointed out that Henri had neglected the fact that there was an extensive individual variation in the resistance of the corpuscles, and that therefore

his conclusions were invalid. Dreyer and Hanssen (1907) and Salomonsen and Dreyer (1907) showed that there was an initial period of lag, during which no hæmolysis occurred, and that after this the rate of hæmolysis followed the monomolecular law. Madsen and Nyman (1907) showed that the rate of disinfection followed the formula expressing the monomolecular reaction, and this conclusion was confirmed by Chick (1908 and 1910) who also showed that figures obtained previously by Paul and Krönig (1891) and by Krönig and Paul (1897) were fitted by the same formula.

A number of observers at once pointed out that it was practically certain that organisms varied extensively as regards the time needed for their destruction and that this variation, if it had a skew distribution, might account for the approximately linear relation found between the logarithm of the survivors and the time. Reichenbach (1911) showed that the destruction of bacteria by heat furnished time-action curves similar to those obtained with drugs, and that cultures of different ages gave time-action curves of different shapes. He put forward the following objection to the theory that the time-action curves expressed the progress of a molecular reaction.

"The molecules are so small compared to the bacteria and their number is so much greater, that doubtless even in very dilute solutions every bacteria is surrounded by a similar number of molecules and therefore is exposed equally to the action of the disinfectant. And still less can one say that the bacteria arrive one after another to the condition necessary for destruction by temperature. Therefore if the bacteria are of equal resistance one cannot understand why one should die quicker than another."

The frequency with which an initial lag period was observed at first troubled the upholders of the monomolecular theory, but this difficulty was met by Yule (1910) who pointed out that the assumption that death was caused not by one event, but by a few events, would explain those curves which showed first a lag period and then a linear relation between log. survivors and time. These calculations have been repeated independently by a number of authors. Rahn (1930) attempted to calculate the number of molecules that produce

death in any individual from the shape of the time-action curves, and in recent years a number of observers have made similar calculations for the number of quanta needed to kill cells exposed to radiations.

The monomolecular theory of disinfection received great encouragement from the support given to it by Arrhenius (1915), but he differed from most writers who have supported this theory, in that he appreciated the difficulties there were in assuming that the death of an organism was caused by a single event, namely the combination with a single molecule of drug. He advanced the following argument (*loc. cit.*, p. 79). "The proteids contained in the living protoplasm are amphoteric electrolytes. Only a thousandth part (we suppose this figure for simplicity) of the proteid molecules has split off its H ions, and perhaps only a millionth part its OH ions. Then probably one part in a thousand millions has split off both its H ions and its OH ions. Perhaps it is only living protoplasm containing one or two such ions which is able to react with the poison. At every moment only this small fraction is open to attack, and at this moment a molecule of poison must be present for the cell proteid to be destroyed. Probably the cell only dies after a certain number of its proteid molecules have entered in reaction with the poison."

Arrhenius's defence of the monomolecular theory is quite coherent. There is of course no need to assume that the cell is killed by a single event, and the assumption that a cell is killed by a few events, permitted the interpretation of most of the time-action curves that are encountered. It is not possible in physical chemistry to find an example of a single molecule producing such extensive effects, but an analogy can be found in the impregnation of an ovum, which may be surrounded by millions of sperm of which only one produces fertilization. Chick (1910) showed that monomolecular or logarithmic curves were obtained equally readily when bacteria were destroyed by disinfectants, heat or drying, and put forward (1930) an alternative theory, namely that the organisms undergo rhythmical changes and are only vulnerable during a certain phase. It must also be assumed that the effect is of an all-or-none nature and either kills the organism or else leaves it unaffected. Curiously enough, this theory of

cyclic variation also finds an analogy in mammalian reproduction, although it is difficult to find one in physical chemistry.

The controversy started in 1905 is still proceeding and in recent years has acquired increased importance because many authors have attempted to estimate the number of quanta of radiations needed to kill a cell from a study of the shape of time-action curves. The literature is very extensive, but fortunately it has been summarized recently by Chick (1930) and by Rahn (1931). Hence the writer will only refer to the work that appears to him to be of particular importance.

Forms of Time-Action Curves

There is a very general agreement concerning the facts of the dispute outlined above, for all authors agree that some time-action curves are sigmoid and that others are logarithmic, and that there are two obvious lines of explanation which are as follows :

1. All the curves can be explained as expressing individual variation, provided that any form of individual variation desired is assumed.

2. All the curves can be interpreted as expressions of the chance of occurrence of a single event or a few events.

These facts are however of little significance because there are few curves expressing the action of drugs that could not be explained by either of these hypotheses.

Rahn (1931) has applied the statistical method to the evidence available. Three types of relations are found when the logarithm of the number of survivors is plotted against time. These were described by Eijkman (1908) and are as follows :

(*a*) A linear relation, which indicates a constant rate of destruction (curve A, Fig. 39).

(*b*) A convex curve, which indicates an increasing rate of destruction (curve C, Fig. 39). In many cases it is possible to interpret this as an initial lag followed by a constant rate of destruction.

(*c*) A concave curve, which indicates a decreasing rate of destruction (curve B, Fig. 39).

Rahn found the following distribution in the literature.

In 32 cases a constant rate of destruction, in 83 cases an increasing rate and in 39 cases a decreasing rate. As regards the mode of explanation adopted by the various writers, this appears to depend chiefly on environment and training. Most physicists and chemists have adopted the monomolecular or quantum theory of action of drugs and radiations. Most biologists have explained their results as expressions of individual variation. Rahn admitted that the "concave" type

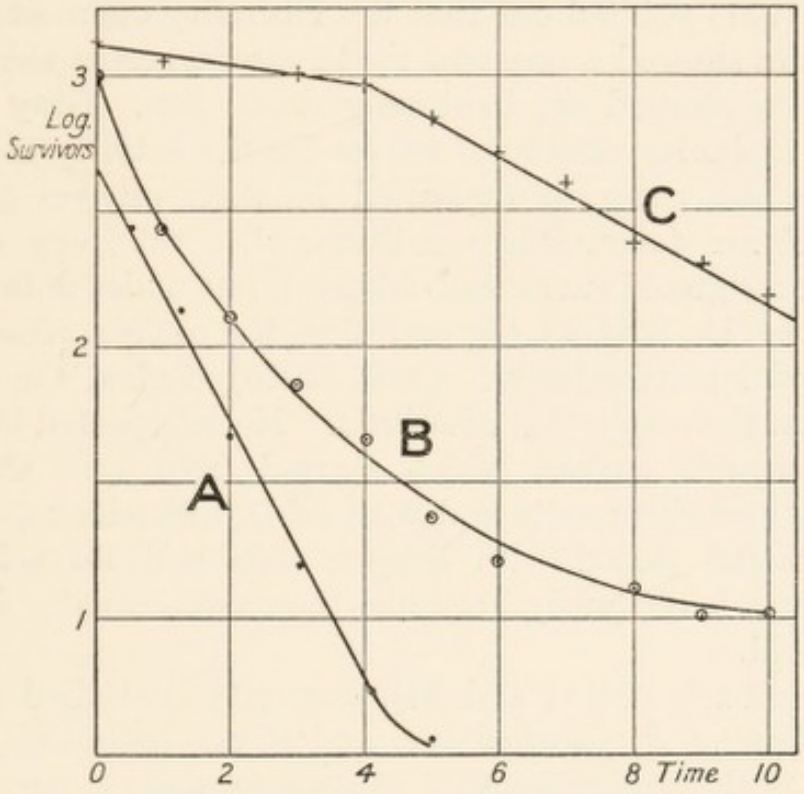

Fig. 39.—Time-action disinfection curves obtained with phenol.

Abscissa: time. Ordinate: logarithm of number of surviving bacteria.
Curve A. Destruction of anthrax spores by 5 per cent. phenol at 33·3° C. Time in hours. (Chick. 1908. Table II.)
Curve B. Disinfection of a three hours culture of *B. paratyphosus* by 0·6 per cent. phenol at 20° C. Time in minutes. (Chick. 1908. Table XI.)
Curve C. Disinfection of *Staph. pyogenes aureus* by 0·6 per cent. phenol at 20° C. Time in minutes. (Chick. 1910. Table VI.)

of curve could only be explained on the assumption that the population varied and that it contained a large proportion of susceptible individuals. He believed, however, that the linear relation indicated a monomolecular reaction, and that the convex curves indicated that death was caused by a few events.

Variations in Time-Action Curves obtained with a Single System

Rahn (1930) has shown that the most varied forms of time-action curves have been obtained by different workers. In a number of cases, however, a single worker using a single system has by varying the conditions obtained a range of curves ranging from concave to convex.

This difference of the general shape of curves has been observed even in death-rates due to no specific toxic agent. Szabo (1931) pointed out that the mortality curve of vestigial drosophila showed a constant death-rate, whereas that of wild drosophila showed an increasing death-rate. Gray (1931A) showed a similar variety of curves for the activity of spermatozoa in sea water, as measured by their oxygen consumption. Under favourable conditions, i.e. with egg secretion present, a sigmoid curve was obtained, but under unfavourable conditions, i.e. without egg secretion, the oxygen consumption decreased logarithmically. Cook (1925) studied the effect of metals on the respiration of *Nitella*. He interpreted his results as logarithmic curves, which started from zero time with mercury and silver salts and with acids, but which showed an initial latent period with copper, iron and tin salts. The latter form is obviously very like the curves usually described as sigmoid.

Reichenbach (1911) and Eijkman (1908) studied the heat destruction of *B. paratyphosus* and *B. coli communis* respectively. Both authors found that a whole range of curves could be obtained varying from concave through linear to convex when the logarithm of the survivors was plotted against the time. As a general rule the more rapid the action the less the sigmoid character of the curve appeared. Reichenbach showed that rapidly growing cultures which contained a preponderance of young forms were most likely to give a linear relation (curves A and B, Fig. 40). Eijkman found that the shape of the time-action curves could be altered merely by varying the density of the suspension of bacteria (curves C and D, Fig. 40).

Henderson Smith (1921) studied the action of phenol on botrytis spores. He showed that under most conditions sigmoid curves were obtained but that a strong concentration of phenol acting on young (6 days old) and sensitive botrytis

spores produced a true logarithmic time-action curve. He showed that the essential fact was that when the rate of death was increased the death-rate of the more sensitive portion of the population increased more rapidly than did that of the less sensitive organisms. For example, when the concentration

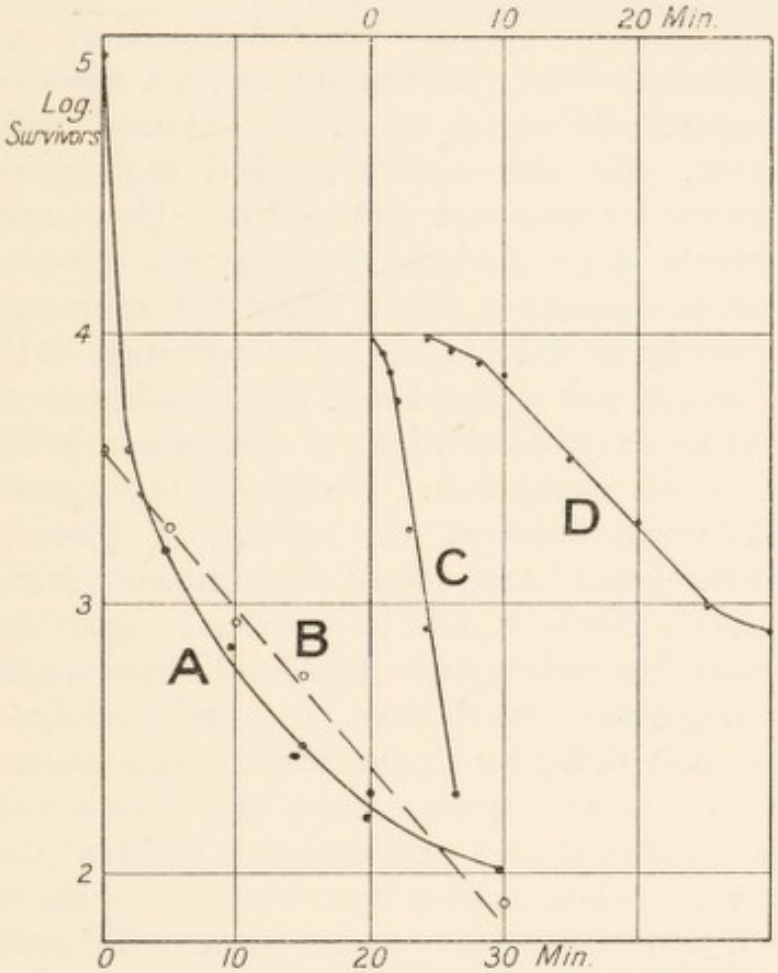

Fig. 40.—Time-action curves of disinfection.

Abscissa: time in minutes. Ordinate: logarithm of number of survivors.

Curves A and B. Destruction of *B. paratyphosus* by hot water. (Reichenbach. 1911.) A. Young culture (5½ hours) at 49° C. B. Old culture (13 hours) at 48° C.

Curves C and D. Destruction of *B. coli communis* by boiling water. (Eijkman. 1908.) C. 7 organisms per c.mm. D. 370 organisms per c.mm.

of phenol was increased from 0·4 to 0·7 per cent. the times taken to kill 25 p.c. were in the ratio of 100 to 28, but the times taken to kill 75 p.c. were in the ratio of 100 to 53. Henderson Smith showed that whenever the rate of action of phenol on the spores was increased, the increase was associated with a differential change in the rate of action of the drug which

resulted in a consecutive transition of the time-action curve from a sigmoid to a logarithmic form. He showed this to be true when the following factors were varied: concentration of phenol, temperature, age of spores, and amount of spores introduced. He pointed out that if some change in the resistance (R) occurred so that $\frac{R}{\text{log. time}} = \text{constant}$, then if a sigmoid curve were obtained with a weak concentration, a logarithmic curve would be obtained with a high concentration. He also (1923) studied the destruction of botrytis spores by heat and obtained a series of symmetrical sigmoid curves when various temperatures were employed. The highest temperature (50·3) killed the spores about 260 times as rapidly as did the lowest temperature (31° C.), but the rapid action was sigmoid and not logarithmic and hence the same ratio was obtained for the destruction of 25 p.c. and of 75 p.c. of the population. These results suggest a fundamental difference between the actions of phenol and of heat. On the other hand, many other authors (e.g. Eijkman (1908) and Reichenbach (1911)) obtained logarithmic curves with the heat destruction of bacteria and hence the distinction found by Henderson Smith does not always apply.

The essential difficulty about the interpretation of time-action curves is that a single system may give a wide variety of curves. It is irrational to postulate a different distribution of variation of a population for every variation of the conditions. Similarly it is irrational to suggest that a different number of drug molecules or of quanta are needed to kill cells whenever the conditions are varied.

There has been so much controversy about the meaning of these curves that the simple facts have been rather obscured. It is not sufficient to find an explanation for the occurrence of logarithmic or of sigmoid curves; what is needed is an explanation for the occurrence of both types of curves in a single system.

Time-Action Curves of Hæmolysis

There is an extensive literature concerning time-action curves of hæmolysis, but these curves present certain special difficulties. These can best be appreciated by a consideration of curve A in Fig. 41, which shows the times of occurrence of

hæmolysis after U–V irradiation. This curve shows a striking resemblance to the true time-action curves, shown in curve B, Fig. 41, but the two curves are totally different in nature. The time-action curve measures the injury produced in a

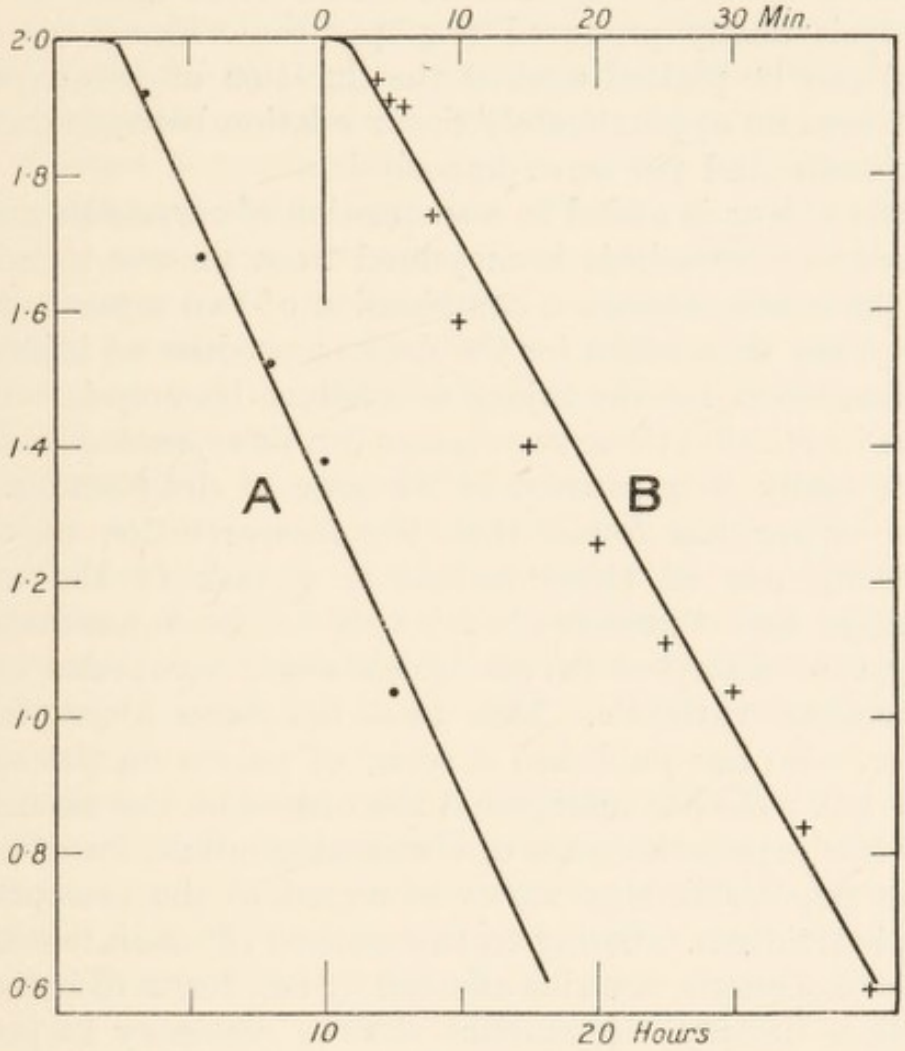

Fig. 41.—Similarity of time-action curves measuring (A) lag in biological response and (B) rate of production of injury.

Abscissa : time. Ordinate : logarithm of number of survivors.

Curve A. Time in hours at which hæmolysis appears in fowl's red blood-corpuscles after exposure for a few minutes to U-V light. (Woerz, quoted from Rahn. 1931.)

Curve B. Duration in minutes of exposure of *Drosophila* eggs to X-rays and resultant death-rate. (Packard. 1931.)

certain time ; the injury is estimated by determining the proportion of the population that is viable after various periods of exposure, and no account is taken of the time at which the organism happens to die. The hæmolysis curve (curve A, Fig. 41), on the other hand, shows the time at which death,

or rather hæmolysis, occurs subsequent to some injury. A time-action curve for the hæmolysis produced by ultra-violet light can be constructed from the figures given by Woertz (Rahn, 1931), for he measured the hæmolysis resulting from various lengths of exposure to ultra-violet light, and the hæmolysis finally produced (e.g. per cent. hæmolysis at 22 hours) can be plotted against the duration of the exposure. This shows an approximately linear relation between duration of exposure and per cent. hæmolysis.

When a drug is added to a suspension of corpuscles and the incidence of hæmolysis is measured from minute to minute, then the results express a combination of two separate times, namely, the time taken for the drug to produce an injury and the time taken for the injury to produce hæmolysis. In the case of irradiation these two factors can be separated, but they cannot easily be separated in the case of drug action.

The writer has found that the interpretation of curves expressing one of these factors is a task of the utmost difficulty, and therefore believes it to be unprofitable to attempt to interpret curves which are a composite of two independent variables. This task has been attempted by Ponder, who has published a series of papers on the subject (1923–32). He has interpreted the curves on the assumption that they express the rate of chemical combination of a drug with a population that varies in regard to the susceptibility of its individuals. Owing to the method of measurement the form of the curves must be affected by two forms of individual variation, namely, the amount of drug necessary to produce hæmolysis in a cell, and the rate at which hæmolysis is produced in a cell that has received a dose of drug sufficient to cause hæmolysis.

Time-Action Curves of Physical Agents

The destruction of bacteria and other cells by physical agents has been studied extensively. The chief physical agents used have been drying, heat, ultra-violet light, X-rays and beta and gamma rays. The outstanding facts are that similar curves relating duration of exposure and percentage of survivors are obtained whatever be the nature of the physical lethal agent, and that the curves obtained with physical agents show all the essential characteristics of the curves obtained with

drugs. It is therefore no matter for surprise that the same controversy that has arisen regarding chemical time-action curves has also arisen regarding physical time-action curves. Chick (1910) pointed out that the destruction of bacteria by hot water, sunlight and drying followed the course of a monomolecular reaction. Measurements of the process of destruction of organisms by X-rays gave sigmoid curves which could be interpreted as commencing with a period of lag and thereafter showing a constant death-rate. Blau and Altenburger (1922) advanced the theory that cells or organisms did not vary as regards sensitivity to X-rays, that each died when it had absorbed a certain number of quanta, and that consequently the shape of the curves relating the amounts of exposure and action indicated the number of quanta needed to kill the organism.

This suggestion has resulted in a controversy that has proceeded on almost exactly the same lines as the controversy regarding the meaning of the time-action curves in disinfection. The quantum theory of the action of radiations was apparently put forward in ignorance of the large mass of evidence regarding the course of action of drugs, heat and ultra-violet light on organisms.

The action of heat deserves special attention, for this cannot be explained on the quantum theory, and on the other hand it differs from drug action in the following respect. Drugs such as disinfectants enter cells relatively slowly and probably the quantity of drug fixed by the cell increases steadily during the period of exposure. When cells are exposed to physical agencies such as heat or radiations, their physical condition is changed abruptly but remains unaltered throughout the period of exposure. For instance, when bacteria are dropped into hot water, their temperature rises almost instantaneously and thereafter remains constant.

The remarkable fact is that the time-action curves obtained with chemical and physical agencies are so very similar. This is strong evidence in support of the view that the physical agents set up slow secondary chemical changes and the time-action curves indicate the rate at which these secondary changes occur. This fact is almost self-evident in the case of destruction of bacteria by drying ; for in this case death must be due to

loss of water. The destruction of bacteria by heat also shows a striking similarity to the heat coagulation of proteins. Chick and Martin (1910–12) studied the heat coagulation of proteins and concluded that the action proceeded in two stages, namely a chemical process of denaturation which was followed by a physical process of agglutination, and they found that the latter process was much more rapid than the former. Chick and Martin (1910–12) and Hartridge (1911) showed that the process of denaturation of proteins followed accurately the course of a monomolecular reaction.

The destruction of protoplasm by heat therefore resembles a chemical reaction rather than a physical process. Rahn (1931) has analysed the results of Reichenbach (1911), Eijkman (1912) and of Sattler (1928), who all studied the destruction of bacteria by heat and found that out of 94 curves 14 showed a constant death-rate (logarithmic curve), 68 showed a decreasing death-rate (sigmoid curve) and 12 showed an increasing death-rate.

Henderson Smith (1923) found a difference in the time-action curves of botrytis spores killed by phenol and by heat. In the first case he found a progression from sigmoid to logarithmic curves, and in the second case he found that all the curves were sigmoid. This distinction has not been found by other workers, for logarithmic death-rates have been found as frequently with heat as with drugs. Moreover, slow destruction with heat gives sigmoid curves and rapid destruction with heat usually gives logarithmic curves.

The first question is whether the fact that the heat coagulation of proteins happens to follow the monomolecular formula is proof that a monomolecular reaction occurs. The literature of colloidal chemistry shows that die-away curves occur very frequently in such systems. For example, Gann (1916) found that the precipitation of aluminium hydrate sol by potassium chloride gave curves of which two are shown in Fig. 42. Weak concentrations of KCl gave sigmoid time-action curves and stronger concentrations gave curves which show a fairly accurate linear relation when log. survivor is plotted against time (Fig. 42, inset). Logarithmic relations are particularly likely to occur in such a process as precipitation of proteins because the rate is probably regulated by the diffusion of water

from one phase to another, and the kinetics of a diffusion process resemble those of a monomolecular reaction. The fact that heat coagulation of proteins follows a monomolecular curve does not therefore prove that it is a monomolecular reaction and still less does it prove that the heat death of bacteria is due to such an effect.

The destruction of cells by radiations is a more difficult

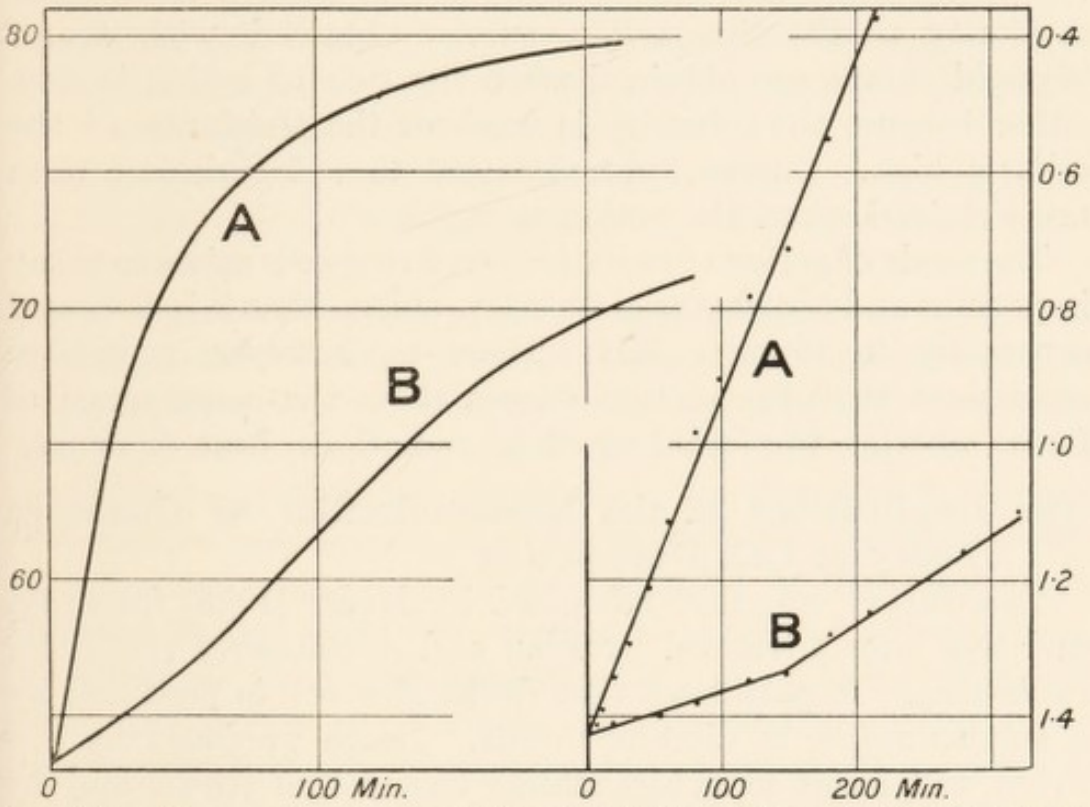

Fig. 42.—Time-action curve of the coagulation of a sol of $Al(OH)_3$ by KCl.

Abscissa : time in minutes. Ordinate : increase in viscosity.
Inset shows logarithm (maximum viscosity—viscosity observed) plotted against time.
Curve A. 80 m.molar KCl.
Curve B. 60 m.molar KCl. (Gann. 1916.)

subject than destruction by heat because the action of radiations is steadily cumulative and hence equilibrium studies cannot be obtained. The only curves available are those relating amount of exposure (intensity $\times$ duration) with effect. The Bunsen-Roscoe law states that the same photo-chemical effect will be produced however the intensity and duration are varied as long as the product $I.t$ remains constant. This law is approximately true over a considerable range in the case

of biological effects. Consequently the curves relating exposure and effect can be regarded as either concentration-action or as time-action curves. If the duration of exposure is short in comparison to the time that elapses before the biological effect appears then the curves obtained resemble concentration-action curves, but if the duration of exposure lasts until the action appears then the curves resemble time-action curves. The interesting point is that these curves show a remarkable similarity to the time-action curves obtained with drugs. Sigmoid curves are obtained when the rate of action is slow either because the intensity is weak or the resistance of the cells is high. Curves approximating to a logarithmic form are obtained when the action is rapid.

The mode of action of radiations on living cells raises so many important and controversial special problems that it is discussed separately in Chapter XII. There is, however, a general agreement that time-action curves show the same range of forms whether the lethal agent be radiations, heat or drugs.

The Implications of the Monomolecular or Quantum Theory of Cell Destruction

This theory was first advanced by Henri (1905) for drugs and was later advanced by Blau and Altenburger (1922) for radiations. It postulates that death of a cell is produced by a small number of discrete events. Death produced by one event will result in a logarithmic time-action curve, and by a few events in sigmoid curves.

Curie (1929) and Rahn (1930) have calculated the curves that express the deaths in a population when death is due to a few discrete events. When the logarithms of the survivors are plotted against time the following curves are obtained. When death is due to one event : a linear relation starting from zero time, e.g. Fig. 40, curve B. When death is due to two or three events : an initial period of lag followed by a linear relation, e.g. Fig. 41, curve A. When death is due to several events (10 or more) : a long period of lag followed by a steep fall. When the number of the survivors is plotted against time these curves show every gradation from the logarithmic to the steep sigmoid form.

These formulæ provide curves of such a wide variety of shape that almost any time-action curve can be fitted by them. They

also provide excellent fits for many cases in which the formula can have no meaning. For example they fit curve A in Fig. 41 which measures the rate at which hæmolysis occurs after the radiation has ceased and hence cannot possibly measure the rate of absorption of quanta. This is an example of the fact that quantitative biological data often are fitted approximately by formulæ that have no theoretical significance.

The chief postulates of Henri's theory are as follows :

1. That cell populations may be regarded as uniform.
2. That death is produced by a few molecules of drug or a few quanta of radiant energy.
3. That the action of drugs or of radiations is non-cumulative and that a cell is unaffected until the lethal event occurs.

These consequences of the theory were pointed out by Brooks (1919) in the following words : "The acceptance of such an explanation makes it necessary to assume that loss of viability like the breaking up of a single molecule of saccharose during inversion takes place in a single step ; in other words, that the disinfectant cannot have any cumulative effect on the viability of individual cells." The mere statement of these postulates appears to the writer to constitute a *reducio ad absurdum.* Variation is one of the fundamental laws of living material ; every population of organisms shows extensive variation and the variation of unicellular organisms is certainly as great as that of larger organisms. The conception of death being due to a few molecules of drug is opposed to the quantitative evidence available, for this proves that in most cases a single cell fixes millions of molecules of the drug.

It is equally absurd in the case of radiations, as is shown by the work of Wyckoff. This worker studied the action of radiations on *B. coli* and obtained logarithmic time-action curves. He first studied cathode rays (Wyckoff and Rivers, 1930), and then X-rays (Wyckoff, 1930), and interpreted the results as proving that each bacterium was killed by a single quantum. He next studied ultra-violet light (1932) and obtained the same curves, but in this case he measured the radiant energy absorbed by the bacteria and found it to be 136,300 quanta per second. The acceptance of the quantum theory implied that only one quantum out of $4{\cdot}19 \times 10^6$ that

were absorbed produced an effect. He regarded this result as too improbable to be accepted and concluded that his experiments provided a striking example of the pitfalls that attend efforts to give detailed interpretations of biological reactions, even when quantitative data are available.

A more general objection to the quantum or monomolecular theory is that it bears no relation to the known laws of pharmacology. Most drugs when they act on cells produce a graded response, and direct evidence has been advanced (Chapter VII) that drugs can produce graded actions on single cells. In the case of a large number of drugs it is highly probable that they act on the cell surface. For example, in the case of saponin hæmolysis there is evidence that the red blood-corpuscle bursts when the cell-wall has been weakened by the adsorption and subsequent chemical action of about 100 million molecules of saponin. The monomolecular theory states that this process of adsorption has nothing to do with hæmolysis, but that this event is due to a single molecule which happens to hit the vital point of the cell and that the chances of such a collision are equal whether or no any other molecules of the drug have been fixed.

One minor difficulty is to conceive of the site of the vital spot in the cell which is destroyed by the single event. As regards the cell surface there is evidence for the existence of active patches, but these must be numbered at least in thousands per cell. As regards the interior of the cell the experiments of Chambers show that many drugs when injected inside the cell fail to produce their typical action. In the case of nucleated cells it is possible to postulate a vital centre in the nucleus, but this hypothesis involves the assumption that hæmolysis of a non-nucleated mammalian erythrocyte is a process of a different nature from the hæmolysis of a nucleated bird's erythrocyte.

The quantum theory of radiations is equally irreconcilable with data regarding graded actions. It will be shown that the most probable theory regarding the action of visible light on the retina is that it produces a primary reversible photochemical process. The relation between intensity of stimulus and response is graded with extreme accuracy over an amazing range of light intensity. This can only be interpreted on the

assumption that there is some close relation between the amount of light arrested in the retinal cells and the amount of chemical change produced. In the case of light of somewhat shorter wave length acting on bacteria, we are asked to believe that millions of quanta are absorbed by a bacteria but produce no action except in the case of one quantum and that this produces death. Henri's theory is in fact completely incompatible with the greater portion of the science of quantitative pharmacology. As soon as its implications are thought out, insuperable objections are met in every direction. On the other hand, the only reason for its adoption is the fact that it provides a fairly accurate interpretation for a number of curves.

The writer has provided numerous examples of the fact that quantitative biological data are characteristically promiscuous as regards their relations to formulæ, and that very frequently formulæ which are quite meaningless fit data in an excellent manner. The application of the quantum formula shown in Fig. 43 is a typical example of this fact.

Since the whole of the quantum argument depends on the fitting of certain curves by certain formulæ, it appears to the writer to be a valid test to see what kind of fit these formulæ give to curves in cases in which the formulæ can have no possible meaning. Rahn (1930) has calculated the curves obtained when death is caused by the occurrence of a small number of discrete events. For the case of two discrete events being required to produce an effect Rahn gives the following formula. Survivors $= aq^n + n.mq^{n-1}$. (a = initial population and m = number inactivated in time n and $q = \left(l - \frac{m}{a}\right) =$ proportion of population surviving after time n.) The form of the curves resulting from this formula when q varies from 0·1 to 0·8 suggests that they will give an approximate fit to any data depending on a slightly skew distribution of frequency. The writer tried this in the case of figures given by Greenwood (1904) for the variation of the weights of human hearts. Fig. 43 shows that the distribution of the heart weights of 1382 males can be fitted throughout its course with a maximum deviation of 5 p.c. by the formula: Hearts in excess of 6·5 oz. $= 100\,n - q(n-1)\,q^{n-1}$, where $n = \frac{\text{wt.}}{6{\cdot}5}$ and $q = 0{\cdot}2$.

The formula therefore gives a curve which approximates quite closely to the integration of a slightly asymmetrical frequency distribution. The close resemblance of such curves to logarithmic curves showing a lag was pointed out by Peters (1920), who showed that the destruction of colpidium by mercuric chloride accurately followed a logarithmic course after an initial period of lag.

The monomolecular or quantum theory of cell destruction

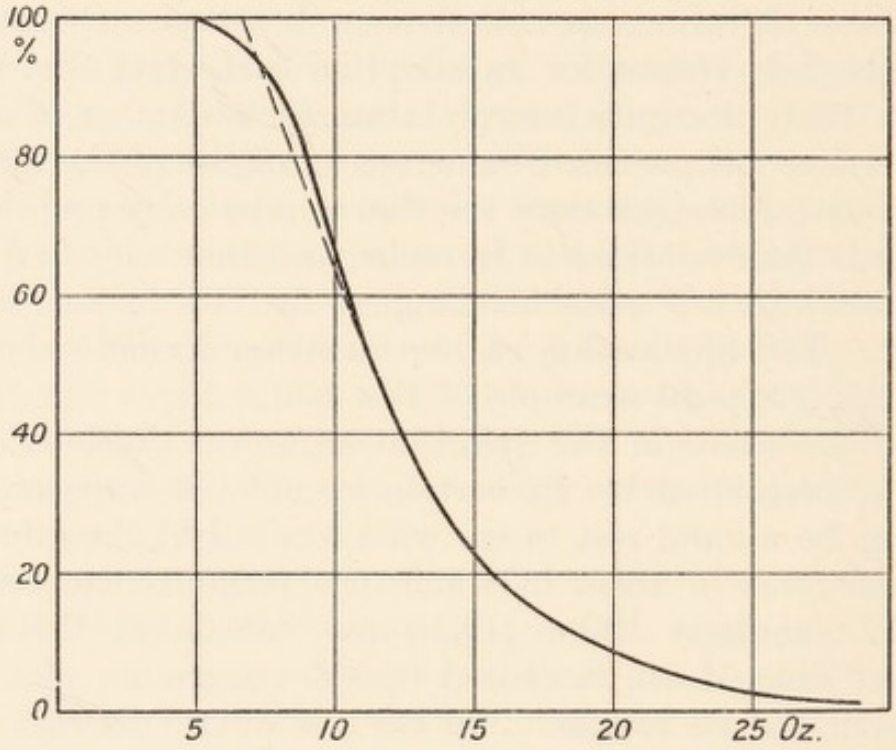

Fig. 43.—Characteristic curve of the variation of the heart weight of males found in 1382 post mortems.

ABSCISSA : heart weight in ounces. ORDINATE : per cent. of population that attains a given weight.

The continuous line shows the observed figures and the broken line follows the formula given in the text. (Greenwood. 1904.)

has therefore very little solid evidence in its support, and its application leads to conclusions so absurd that they are difficult to discuss. One amusing paradox is that the upholders of this theory regard themselves as the defenders of mechanistic doctrines against vitalist errors, and yet their implicit trust in formulæ leads them to a conclusion of an extreme vitalist type, namely, that the life of a cell is dependent on some kind of single archon amongst the millions of molecules of which the cell is composed.

Time-Action Curves as Expressions of Individual Variation

The writer finds it simplest to admit the fact that living cells in regard to their response to injurious agents vary extensively and also possess considerable powers of resistance. As regards drug action the whole of quantitative pharmacology is based on the assumption that there is some relation between the amount of drug acting on a cell and the response produced, and it is simplest to regard death as the final stage of a graded injurious process. These assumptions explain the sigmoid time-action curves obtained with drugs. It is true that many of these curves indicate asymmetrical distribution of variation, but there is no particular reason why biological variation should be distributed with exact symmetry. Francis Galton in 1879 pointed out the likelihood of skew distributions of variation occurring in biological objects and suggested that in many cases the geometrical mean and not the arithmetical mean was likely to give the most probable result.

Two points that require explanation are as follows :

1. Why do logarithmic curves ever occur ?

2. Why do some populations show sigmoid curves under some conditions and logarithmic curves under other conditions ?

The populations that have been studied can be divided into two classes, namely, freely growing bacteria and the remainder. Freely growing bacteria are unsatisfactory material for quantitative work because such a population must contain a high proportion of young forms. The age distribution cannot be uniform and may be of a highly skew character. There is direct evidence that the response of bacteria to drugs is influenced by age, and hence it is impossible to decide the extent to which the individual variation in such a population is likely to distort a time-action curve.

The study of stationary populations is therefore more profitable. Erythrocytes are unsatisfactory because the time-action curves of hæmolysis express two variables, namely, the rate of some chemical reaction and the rate of response of the cells, and hence are more difficult to interpret than such an effect as the destruction of bacterial spores, eggs, or protozoa. These latter classes are therefore the most favourable material to consider.

With regard to the occurrence of logarithmic curves in general the following considerations apply.

In Chapter VI it was pointed out that the action produced by a drug at equilibrium on unicellular organisms depended on two processes, namely, the uptake of the drug and the action of the drug after fixation. The relation measured in this case was that between drug concentration and action observed, and it was shown that unless there happened to be a linear relation between drug concentration and drug fixation the individual variation of cellular response in relation to drug concentration would be almost certainly of a skew type, and the manner in which an exponential relation could distort a normal symmetrical variation was discussed.

The simplest case of drug kinetics is when a population is exposed to a lethal influence (drugs or radiations) for measured times, and at the end of this period the power of the organisms to survive is estimated. In this case there are two processes in regard to which individual variation may occur :

(*a*) Rate of fixation of the drug by the organism ;

(*b*) The response of the organism to the dose of drug fixed.

If the rate of fixation of the drug is constant, then the dosage of drug (i.e. drug fixed) will vary directly as the time. If the cells show a normal distribution of variation, as regards the effect produced by a given dose of drug, then the cells will also show a normal distribution of variation as regards the time taken to produce the action measured.

The writer is, however, unaware of any case in which it has been proved that the rate of fixation of drugs is constant. The commonest case is that the rate of entrance of a drug into an organism is regulated by diffusion processes. If the drug is in excess and the external concentration does not alter significantly, the rate of diffusion will vary as the difference between the internal concentration when equilibrium has been obtained and the existing internal concentration, hence the curve relating time and the amount of drug that has entered will be exponential in shape. In such a case if the cells show a normal symmetrical variation as regards the amount of drug, that must be fixed before death is produced, and the amount of drug fixed varies roughly as the logarithm of the time, then the cells cannot show a symmetrical

variation as regards the time of exposure needed to produce death.

On the other hand, if we assume that the cells vary in regard to the facility with which they fix the drug, then skew variations are equally likely. In some cases the relation between concentration (c) and time (t) is simple, namely, ct = constant. In many cases the relation is much more complex, for instance in the case of phenol c^5t = constant. If the relation between the concentration and the time needed to produce an action is as complex as this, it seems very unlikely that a variation in the facility with which cells fix a drug will be represented in an undistorted manner by the time-action curve. Curves of this type cover so many complex relationships that it is somewhat surprising that symmetrical sigmoid time-action curves should ever be obtained, and the logarithmic curves are obviously capable of a considerable variety of explanations. The writer inclines to the belief that the true relation between time of exposure and amount of action is usually a skew sigmoid curve and that symmetrical sigmoid curves only result accidentally by the summation of a number of variables. The essential difficulty is to explain why one system of drugs and cells should give a range of curves varying from sigmoid to logarithmic when the experimental conditions are varied.

The conditions usually observed are that lethal agents at a feeble intensity give sigmoid time-action curves and that as the intensity of the agent is increased, the curves approximate to a logarithmic form. The most probable reason for this is variation in cellular resistance.

The Influence of Cellular Resistance

Most writers when discussing time-action curves have ignored the fact that cells are living organisms and possess considerable powers of resistance and repair of injury. Gray (1921 and 1932) studied the rate of exosmosis from single eggs of the trout, and found that feeble toxic agents gave sigmoid time-action curves, but strong toxic agents produced logarithmic curves. Gray (1931B) interpreted this effect as the result of the rate expressing two processes, namely the destruction of units on the cell surface and the resulting increased diffusion due to increased permeability. When the toxic agent was

strong all the units were destroyed rapidly and the curves expressed diffusion, which was a slower process, and in consequence were logarithmic in shape. When the toxic agent was weak the curves expressed both the variability of the protoplasm and the effect of a falling osmotic gradient between the inside of the egg and the external solution, and hence a sigmoid curve was obtained (cf. Fig. 61).

The simplest assumption that explains the facts is that the rate of entrance of a drug into the various individuals in a population of cells, or the rate of injury of cells by heat or by radiations, are all fairly uniform, but that the cells vary extensively regarding their power of repair.

This theory postulates a minimum intensity of injury at which the injury is balanced by the repair and the cell survives indefinitely although it may be unable to grow or reproduce. A threshold concentration of this type is always found in practice. Numerous examples of such thresholds for drugs were given in Chapter V. As regards heat, Henderson Smith (1923) pointed out that in the case of botrytis spores every gradation of action could be produced between the temperature of optimum growth and of instantaneous destruction, and that at some temperature about 26° C. injury and growth were approximately at a balance.

The change from sigmoid to logarithmic time-action curves when the intensity of the lethal agent is changed from moderate to strong can be explained along these lines. The cell membrane may be supposed to be capable of resisting the entrance of a drug such as phenol. As long as the resistance is maintained the drug enters slowly, but above a certain limit the membrane is destroyed and the drug enters more rapidly. With moderate concentrations of drug a sigmoid curve will be obtained that expresses the variation in the power of the cell membranes to delay the slow entrance of drugs. Above a certain concentration this resistance is swamped in the case of the less robust portion of the population and these are killed almost instantaneously, whilst the more resistant forms still follow the latter portion of the sigmoid curve obtained with moderate concentrations.

Another deviation from symmetry is to be expected when the intensity of the lethal agent approaches the non-effective

limit ; such limits exist for nearly all drugs and radiations and prove that the living cell has powers of resistance and repair. When a drug is below a certain concentration its rate of action will depend on the difference between its rate of entry and the rate of repair processes in the cells ; consequently the more resistant portion of the population will show an extreme skew distribution of their rate of death. Such phenomena have

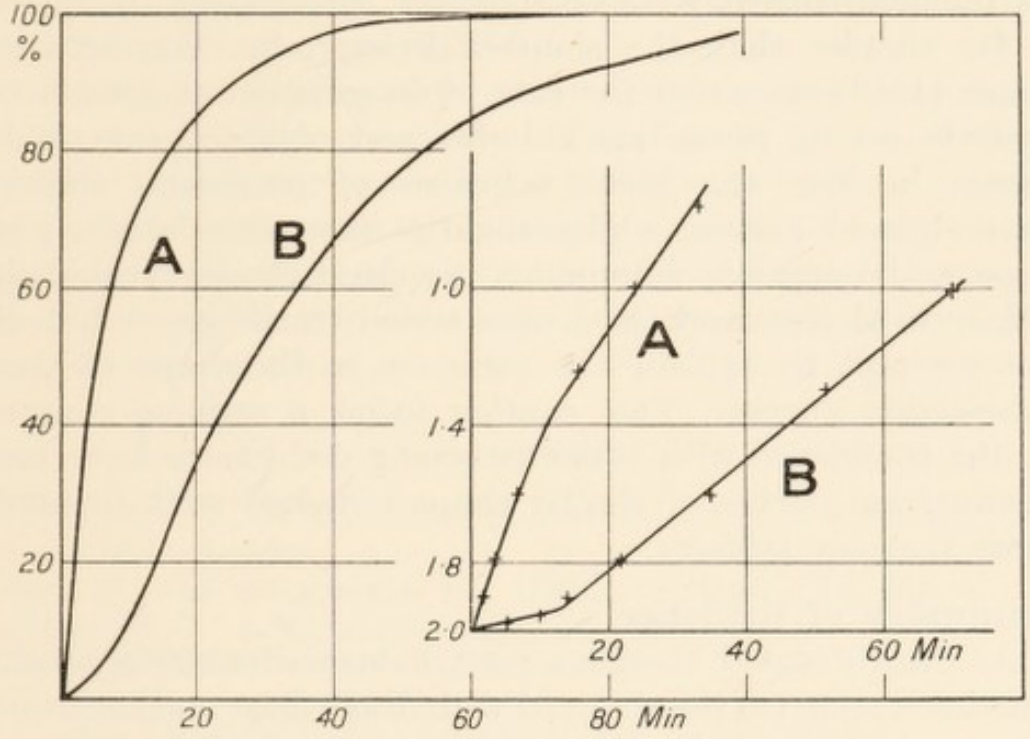

Fig. 44.—The rate of progress of a diffusion process.

Abscissa : time in minutes. Ordinate : per cent. of gas absorbed. The curves show the rate of absorption by 4 c.c. of 0·1 molar NaOH of carbon dioxide liberated in a large container. In one case (A) the container was shaken continuously and in the other case (B) occasionally.

The inset shows the logarithm of the per cent. of gas unabsorbed plotted against time. (Clark. Unpubl. results.)

been frequently reported in the case of destruction of cells by radiations of low intensity.

Finally it may be pointed out that logarithmic and sigmoid time-action curves can be obtained in very simple systems by slight variations of the conditions. The curves in Fig. 44 show the effect of lag on a diffusion process in a very simple chemical system, namely, the uptake of carbon dioxide by sodium hydrate. When diffusion was accelerated by shaking the system an exponential curve (curve A) was obtained which showed an approximation to a linear relation between time

and the logarithm of the unabsorbed gas (curve A, inset). When the system was not shaken a sigmoid curve was obtained (curve B), and this showed an initial lag followed by a linear logarithmic relation (curve B, inset).

In this case the nature of the lag that causes the sigmoid curve is obvious, but in other simple systems logarithmic and sigmoid time-action curves may both occur and the reason for the difference may not be apparent, even though the system is far simpler than the simplest living cell. For instance, Gann (1916) measured the rate of coagulation of aluminium hydrate sol by potassium chloride and obtained the results shown in Fig. 42. Weak solutions of potassium chloride gave sigmoid curves, whilst slightly stronger solutions gave exponential curves. Freundlich discussed the theoretical significance of this work in a note added to the paper but did not attempt to explain the variation in the shape of these time-action curves. This caution forms a striking contrast to the confidence with which sweeping deductions have been drawn from curves of similar shape obtained with infinitely more complex systems.

Summary of Evidence

As already stated, there is a remarkable uniformity regarding the time-action curves obtained with drugs, heat and radiations on a wide variety of cell populations.

When death proceeds slowly either because the lethal agent is weak or the cell is resistant, then obvious sigmoid curves are obtained. When death is accelerated the sigmoid curves become steeper. In a few cases symmetrical sigmoid curves have been obtained, but in the vast majority of cases the curves are asymmetrical, with a long " tail " of highly resistant individuals. In most cases the more intense the lethal agent the greater is the skewing of the curves, and in a considerable proportion of cases rapid lethal effects follow fairly exactly a logarithmic curve. The most careful workers agree that in many cases there is a definite change from sigmoid to logarithmic shape in the curves given by a single type of population when the intensity of the lethal action is increased.

Such a phenomenon cannot be explained in any simple manner by cell variation and therefore it has been necessary to consider carefully the alternative theory of monomolecular or quantum

action. It has been shown that the adoption of such a theory produces difficulties in all directions, because it is opposed to all the evidence of quantitative pharmacology. The writer has put forward alternative hypotheses that do not involve any wildly improbable assumptions. The truth is that there are so many unknown variables in a system containing living cells that it is usually possible to find several alternative explanations for any particular set of curves without any serious violation of probabilities.

The monomolecular and quantum theories of cell destruction have an attractive simplicity. This simplicity is however in itself absurd, for it involves the assumption that the response of living cells is simpler than is that of relatively simple colloidal systems. The chief objections to this theory are, however, that it is opposed to so many of the known laws of biology and of pharmacology.

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CHAPTER IX

VARIOUS THEORIES REGARDING DRUG ACTIONS

The Potential Theory of the Actions of Drugs

Quantitative pharmacology is founded on the assumptions that drugs act by entering into a chemical relation with certain receptors in the cells and that there is some simple relation between the amount of drug fixed by these receptors and the action produced. These postulates are however denied by one of the best known of the theories of the mode of action of drugs, namely the potential theory of drug action advanced by Straub. Straub's original experiments dealt with the action of drugs on the heart of aplysia. He first (1903) studied the action of veratrin and found that the action produced varied as the amount of alkaloid entering the heart. In the case of muscarine (1907) he found that the drug produced an immediate inhibition but that the heart recovered after a few hours, although it was still in contact with the drug. He showed that the drug was not destroyed and was present in both the heart-tissue and in the perfusion fluid. Moreover, the heart had acquired a tolerance that could be demonstrated by further addition of the drug. On washing out the drug, however, its action again reappeared during the process of wash-out. The heart of torpedo behaved like that of aplysia, but very little tolerance could be demonstrated in the frog's heart.

This last observation I have confirmed in the case of acetyl choline acting on the frog's heart. In this case the following variations in sensitivity are observed. At the commencement of an experiment the heart is relatively tolerant of the drug, its sensitivity increases rapidly after two or three doses have been given, and after this the sensitivity remains fairly constant. A moderate dose of the drug produces its greatest effect a minute or two after administration, and this is followed

by a slight recovery, after which the action of the drug remains almost constant for hours. The heart can, however, break down the drug and hence, unless the fluid is changed frequently, an apparent recovery will occur.

Straub explained his results on the theory that the action of drugs depended on a potential gradient between the concentrations of the drug outside and inside the cells. The aplysia heart was permeable to muscarine and when the drug attained a certain concentration inside the heart the action ceased. Washing out the drug again established a potential difference and hence the drug again produced an action. Straub found that muscarine did not penetrate the frog's heart, a conclusion I have confirmed with acetyl choline, and he attributed the continued action of the drug to the continued maintenance of a potential difference. The theory of potential action of drugs therefore postulates the following behaviour in tissues exposed to the drug. If the drug cannot enter the cell, then it will continue to produce its action as long as its concentration outside the cell is maintained. Presumably the same thing would happen if the drug were broken down as rapidly as it could enter the cells. The action of acetyl choline on the frog's heart can be taken as an example of this type of action. If, however, the drug can enter and attain a concentration within the cells, then the action it produces passes off when a certain internal concentration has been attained, and the tissue becomes tolerant to the drug. When the drug is removed from the fluid around the tissue a fresh potential difference is established and a fresh action of the drug is produced. Hence wash-out of the drug produces the same effect on the tissue as does introduction of the drug. Straub himself did not suggest that the theory of potential action was a general theory of drug action, for indeed it did not explain the results he had previously obtained with veratrin, and it is obvious that this theory cannot explain a large number of drug actions. For example narcotics can maintain their action for days and yet they freely enter the cells.

A number of writers have tried to demonstrate the acquisition of tolerance to narcotics by isolated tissues, but the bulk of the evidence is negative. Kuno (1914) reported some habituation, Ransom (1920) found no certain tolerance, Kochmann

(1921) reported some immunity in unicellular organs, Hecht (1926) stated that the isolated rabbit's gut gradually recovered in Ringer's solution in presence of 0·5 p.c. ethyl urethane; Haffner and Wind (1926) found that tadpoles showed some spontaneous recovery when immersed in most narcotics but attributed this to some non-specific biological adaptation of the organisms; Wind (1926) found no spontaneous recovery or tolerance in frogs' hearts exposed to narcotics except that there was a slight recovery with urethane. Cattell (1927) found that the isolated frog's heart became quickly habituated to weak ether solutions. Lendle (1927) exposed frogs to alcohol for prolonged periods. He found that the isolated hearts of alcoholized animals were just as sensitive to ethyl alcohol as were the hearts of normal frogs. Graham (1929) studied the action of ether, nitrous oxide and acetylene on the frog's sartorius and found a slight tolerance. This was not specific to the individual narcotic and was ascribed to physico-chemical and colloidal changes in the muscle possibly brought about by the increase of lactic acid in the muscle under the influence of narcotic.

Pantin (1929) recorded the effects of many drugs on amœbæ, which are so resistant that they can recover after being left for days in presence of drugs. Wash-out experiments indicated that the drugs entered the cells because recovery was slower after paralysis with strong than with weak concentrations in the case of such drugs as cyanides. There was, however, no evidence in the case of alcohols or cyanides of any tolerance. In the case of sodium sulphide at one concentration (10^{-4} molar) a recovery was recorded which appeared to be an example of potential action, but Pantin showed that this was due to a partial oxidation of the drug in the sea-water.

The writer has made unsuccessful attempts to demonstrate tolerance to ethyl urethane and ethyl alcohol in the isolated frog's heart. There are numerous sources of error which lead to a loss of the drug, but if these are excluded no certain tolerance can be demonstrated. The summary given above hardly indicates the nature of the evidence obtained by previous workers, for they were interested in the problem as to whether any demonstrable tolerance occurred or not and the effects they recorded as indicating tolerance were in most cases very

small. The potential theory of drug-action postulates however that marked tolerance should occur when the cell is filled with the drug and such an effect has never been obtained with narcotics.

On the other hand, the entrance of narcotics into a cell probably takes longer than the time needed for narcotics to produce their maximum action, for this may be only 5 seconds (Pickford, 1927).

Hiller (1927) showed that *Amœba dubia* when immersed in a solution of narcotic was narcotized but that narcotics injected into the amœba did not produce narcosis. The narcotic therefore appears to act on the cell surface and also to diffuse into the cell, but its narcotic action is due to its surface action and any drug that enters the cell ceases to have an action. This is probably true of many other drugs and will explain some of the facts advanced to support the theory of potential action.

Cook (1926) showed that methylene blue produced two independent actions on the frog's heart, for the dye rapidly produced a surface atropine-like effect which inhibited the action of acetyl choline and it also slowly penetrated and stained the heart. The heart when deeply stained could be rendered sensitive to acetyl choline by washing out with Ringer's fluid free from dye. A potential action could therefore be mimicked in this case by using a small amount of perfusion fluid, for under these conditions the dye would be removed from the fluid and would enter the heart, and once it had entered the heart it would cease to produce its specific atropine-like action. This experiment is inconclusive because the introduction of methylene blue in the perfusion fluid can produce its specific action on a heart deeply coloured by the dye. The example shows, however, that many of the phenomena adduced as evidence of potential action can be explained as due to two processes occurring : (1) the drug producing a specific surface action, and (2) the drug entering the cells and ceasing to produce an action after it has entered.

The theory of potential actions has found many supporters, for it explains in a simple manner the transient effects produced by many drugs. In the case of adrenaline transient actions have been observed by several authors in intact animals.

Weiss and Harris (1904), Ehrmann (1905), Meltzer and Meltzer-Auer (1903), all showed that when adrenaline was injected intravenously the rise of blood-pressure ended before the adrenaline disappeared from the blood-stream. Kretschmer (1907) showed, however, that a continued rise of blood-pressure could be produced by continuous injections of adrenaline.

Transient effects on isolated plain muscle have been observed in the case of adrenaline and many other drugs. Meyer (1908) was one of the first to note this effect in the case of adrenaline acting on arterial strips. Kuyser and Wijsenbeck (1913) found with the isolated cat's gut that exposure to a high concentration of adrenaline produced a tolerance to further doses that persisted after the original dose had been removed and the gut had apparently recovered its normal activity. A variety of authors have reported wash-out effects produced after adrenaline has been applied to isolated organs. There is indeed a whole literature describing the polyphasic effects produced by drugs such as adrenaline and pilocarpine on isolated plain muscle, particularly the rabbit's intestine. Neukirch (1912) noted that a large dose of pilocarpine produced an initial stimulation of the isolated gut, that this stimulation passed off after prolonged exposure to the drug, but that wash-out caused a second powerful stimulation. Trendelenburg (1912) noted the same effect when pilocarpine acted on isolated bronchial muscle. Similar effects have been described by Jendrassik and his co-workers for a wide variety of drugs (1924–6). Wertheimer and Paffrath (1925) and Paffrath (1931) put forward figures to prove that the pharmacological activity of choline compounds is inversely proportional to the rate at which they diffuse through living tissues.

All those who have studied isolated plain muscle are well aware of the extreme ease with which results such as those described above can be produced by experimental errors. Various authors have shown recently that many of the so-called potential actions are in reality due to experimental error. Fritz (1928) showed, for instance, that the adrenaline recovery of isolated gut was due chiefly to breakdown of the drug, and that the effects frequently recorded on wash-out were due to temperature alterations. He concluded that potential effects were chiefly a summation of experimental

errors. Rentz (1929) made an exhaustive study of the phasic actions of drugs, and particularly of the actions of local anæsthetics on frogs' blood-vessels. He concluded that plain muscles very frequently gave complex polyphasic responses to drugs and that only a few of these responses could be explained by the potential theory of drug action. Nanda (1931) showed that apparent potential actions could readily be produced by imperfect experimental technique such as heavy isotonic levers. In particular Nanda showed that in the case of acetyl choline, pilocarpine and adrenaline it was quite easy to record actions as transient when with other methods of recording the action was shown to be maintained nearly uniformly for a prolonged period. Finally, in respect of the response of plain muscle to drugs it must be remembered that Winton (1930) showed that this was a complex structure containing two mechanisms which he termed the phasic contractile and the postural contractile mechanisms. In consequence of the presence of these two mechanisms a simple mechanical or electrical stimulus can evoke a response that is apparently polyphasic.

The writer considers the following to be a fair summary of the evidence available. Straub was correct in his conclusion that in many, if not most cases, drugs outside a cell have an action quite different from the action of drugs that have entered a cell. The action of potassium ions is the most striking example of this fact, for the ratio of their concentrations inside and outside of muscle-cells is about 30 to 1. A four-fold increase or reduction of the outside concentration is however sufficient to paralyse the frog's heart. The writer interprets this as due to the fact that drugs produce their action by entering into combination with receptors on the cell surface. In certain but not all cases the entrance of a drug into a cell renders the cell tolerant to the drug. This is not true in the case of narcotics but appears to hold in the case of muscarine acting on aplysia heart. Ing and Wright (1931) have shown that this effect occurs in the case of ammonium chloride and quaternary ammonium salts acting in high dilutions. These drugs produce paralysis on frog nerve-muscle preparations, and in the case of ammonium chloride full action (60 per cent. inhibition) was produced in 10 minutes,

but even when the tissue was kept immersed in the drug solution a very considerable recovery occurred after an hour. With the quaternary ammonium salts full action occurred in an hour and recovery after four hours. In these cases errors due to drug destruction were avoided by frequent changes of the solution.

Gautrelet and Baigy (1927) noted that when an isolated piece of rabbit's gut was exposed to a weak concentration of adrenaline it recovered after a short time and was then insensitive to further doses of adrenaline until it had been thoroughly washed out with Ringer's solution. Many other writers have noted that the administration of a feeble concentration of such specific drugs as adrenaline and acetyl choline renders tissues less sensitive to the further addition of larger doses. These curious tolerance effects undoubtedly occur although only with some drugs and only over a limited range of concentrations.

The writer has no explanation to offer regarding the mechanism by which this tolerance is induced, but it appears to him to be unreasonable to base a complete theory of the mode of action of drugs on these somewhat rare and irregular effects. The potential theory of drug action moreover involves undesirable assumptions in that it postulates a mode of action unlike anything known in physical chemistry.

Arndt-Schulz Law

Martius (1923) has given a history of this law. In 1885 Rudolf Arndt put forward the suggestion that if a weak stimulus excites an organism, then any drug in sufficiently weak dose ought to do this also. This suggestion was developed by Schulz, who had leanings to homœopathy. The generalized form of the law is :

" Weak stimuli excite, medium stimuli partially inhibit and strong stimuli produce complete inhibition."

Such a law will obviously hold good in a large number of cases because any drug which acts as a stimulant in therapeutic doses will produce paralysis and death if given in sufficiently large toxic doses. Bier lent his authority to this " law," but many pharmacologists have pointed out that it expresses

no general truth. It is interesting to note that no trace of evidence in support of such a law can be found in the case of the majority of drugs. For example, acetyl choline produces a graded action that can be followed over a 10,000-fold range of concentration, in some tissues it produces an inhibitory and in others a stimulant action, but in any one case the action is of the same nature throughout the whole range of effective concentrations.

As in the case of potential actions, evidence in favour of this law can easily be obtained from experimental errors. For example, some alcohols first stimulate and then depress the isolated frog's heart, but the first effect only occurs in the case of alcohols which are applied in concentrations sufficiently high to produce a disturbance of osmotic pressure, and the apparent stimulation is probably due to this non-specific effect. Handovsky, du Bois-Reymond and von Strantz (1923) showed that histamine in low concentrations accelerated the growth of a protozoon *Balantophorus minutus* and killed it in higher concentrations. This appears to be an excellent example of the Arndt-Schulz law, but the authors showed that this action was due to the release from the dead bodies of a growth-stimulating substance, which they termed necrotine. Low concentrations killed a few cells and thereby stimulated the rest, whilst higher concentrations killed all the cells. These results were confirmed by von Strantz (1927), who found that the first action of histamine on paramœcia was to cause a partial destruction of the population, and that this was followed by an acceleration so great that the poisoned cultures outstripped the controls.

The action of poisons on yeast fermentation was adduced by Schulz (1888, 1907) as an example of the Arndt-Schulz law. Dannenberg (1930) examined the action of phenol, quinine and mercuric chloride on yeast and found that it was very easy to get any apparent stimulation with minimum concentrations, but that these effects disappeared if sufficient care were taken to exclude experimental errors.

The discussion on potential action has indicated the wide possibilities for obtaining polyphasic actions in isolated plain muscles, and by an intelligent selection from such evidence it is of course easy to produce plenty of evidence for the

Arndt-Schulz law. There is, however, no serious reason for supposing that any such general law exists. The general law that covers most of the evidence adduced in support is that complex systems usually give polyphasic responses to drugs.

The Weber-Fechner Law

Concentration-action curves in the case of many drugs, including hormones, follow approximately the relation that action varies as the logarithm of the concentration. This is often instanced as an example of the operation of a more general law that is termed the Weber-Fechner law. This law was put forward to explain the discrimination of sensory stimuli and can be stated shortly as follows.

If I and $I + \Delta I$ are the intensities of any two sensory stimuli that can just be distinguished from each other, then the relation $\Delta I/I$ remains constant over wide ranges of intensity of stimulation. Hence there is a linear relation between the amount of response of a sensory organ and the logarithm of the intensity of the stimulus. An account of the history of this law is given by Hecht (1931) from which the following details are taken. Bouguer (1760) first noted the constant relation $\Delta I/I$ in the discrimination of intensity of illumination. Weber (1835) showed the constancy of the relationship for estimation of weight and for discrimination of thickness of lines. Fechner (1858) regarded the constancy of this relation as a general law of psycho-physics, and made it the basis of speculations in psychology and philosophy. Very soon after Fechner's popularization of this general law Helmholtz (1866) showed that it was inaccurate since the relation $\Delta I/I$ was not constant in the case of vision, and at the same time Aubert (1865) published data for visual discrimination which showed the inconstancy of this relation and these results have been fully confirmed by several other workers. Fig. 45 shows the results obtained by Koenig and Brodhun (1889), and it will be seen that the relation $\Delta I/I$ is not even approximately constant, but is highest with the lowest measurable intensities, falls to a minimum as the intensity increases and finally rises slightly with high intensities. Similar results were obtained by Hecht (1931), who studied the response of Mya to light.

Houston (1932) reviewed the literature of this subject and

showed that the results of previous observers agreed in proving that the ratio $\Delta I/I$ was not constant but varied in the manner shown in Fig. 45. He also expressed these results as the reciprocal of $\Delta I/I$, namely $I/\Delta I$, and found that this value plotted against log I gave a bell-shaped curve that was fitted exactly by the Gaussian formula $y = e^{-x^2/2}$. Investigations of his own on the discrimination of the intensity of monochromatic lights gave the same results as those obtained by

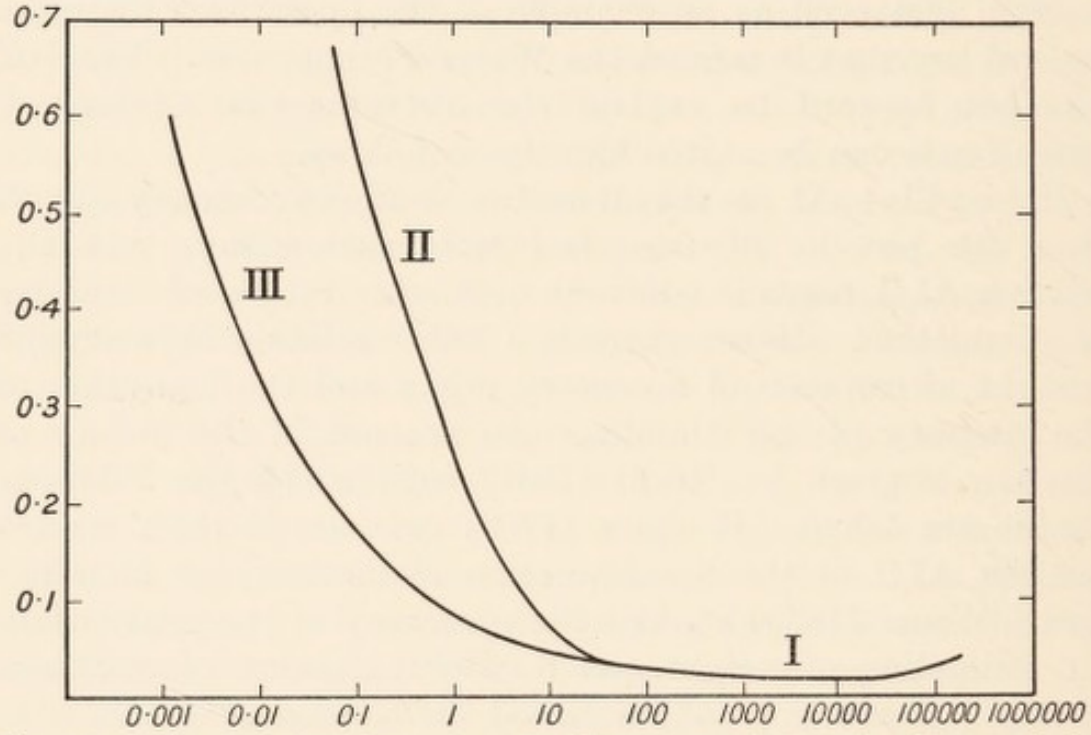

Fig. 45.—The relation between the intensity of illumination and the threshold of discrimination.

Abscissa : intensity of illumination (I) on logarithmic scale. Ordinate : $\frac{\Delta I}{I}$.

Curve II. Wave-length of light 5750 Å to 6700 Å.
Curve III. Wave-length 4300 Å to 5050 Å. (Koenig and Brodhun. 1888.)

previous workers. He calculated from this curve the relation between sensation (S) and log I, and found that it followed a sigmoid or railway curve. The writer found that an approximate fit was given to this curve by the formula $I^{0.75} = \frac{S}{1-S}$. The possible significance of this curve is discussed in Chapter XII. The point of immediate importance is that Houston's results confirm the conclusions of other authors in showing that the ratio $\Delta I/I$ is not constant but changes continuously.

A perusal of the literature shows that the Weber-Fechner law has been found to be untrue in most cases in which it has been tested carefully. In all of the investigations mentioned below the figures obtained show that when $\Delta I/I$ is plotted against log I a U-shaped curve is obtained of the general shape of that shown in Fig. 45.

This was found to hold true for the relation between drug concentration and taste sensation as measured by Keppler (1869) and confirmed by Fodor and Happisch (1922); for temperature sense measured by Pütter (1921); and for hearing as measured by Kenneth and Thouless (1930). In the case of the sensation of pressure Pütter (1918) quoted figures obtained by Stratton, and Houston (1932) quoted unpublished results obtained by Thouless which showed the same relation.

Pütter (1918) concluded that the Weber-Fechner law was completely untrue and that the true relation was that the threshold of discrimination was an exponential function of the intensity of the stimulus. The Weber-Fechner law is therefore untrue for a number of different sensations, and is merely a rough approximation that covers a fairly narrow range of intensity of stimulation.

In the majority of cases the relation found between $\Delta I/I$ and log I agrees with the hypothesis that the relation between stimulus and effect produced is a hyperbola. This is the relation to be expected if the stimulus causes some reversible chemical change in some substance, which is present in limited quantities in the sense organs. In the case of vision there is a general agreement that this expresses the primary effect produced by light on the retina. It is also probable that the sensations of taste and smell depend on a chemical change.

In other cases it is probable that the Weber-Fechner law is true. Matthews (1931) investigated the response of single receptors in frog's muscle and found an accurately linear relation between the frequency of the impulses excited and the logarithm of the load in grammes. He concluded that the sensory stimulus acted by deforming some surface membrane and thereby cause a change in the concentration of ions at the surface and hence a change in the surface polarization. The effect produced by a stimulus of such a nature would be conditioned chiefly by the diffusion away of ions from the

surface. The primary effects produced by the stimulus (weight) appears therefore to be the distortion of the muscle, and the relation between tension and length at rest in muscles often approximates to a linear relation between length and logarithm tension.

The stimulation of sensory nerve-endings can be produced therefore by at least two completely different mechanisms. In the case of vision the primary effect of the stimulus is a

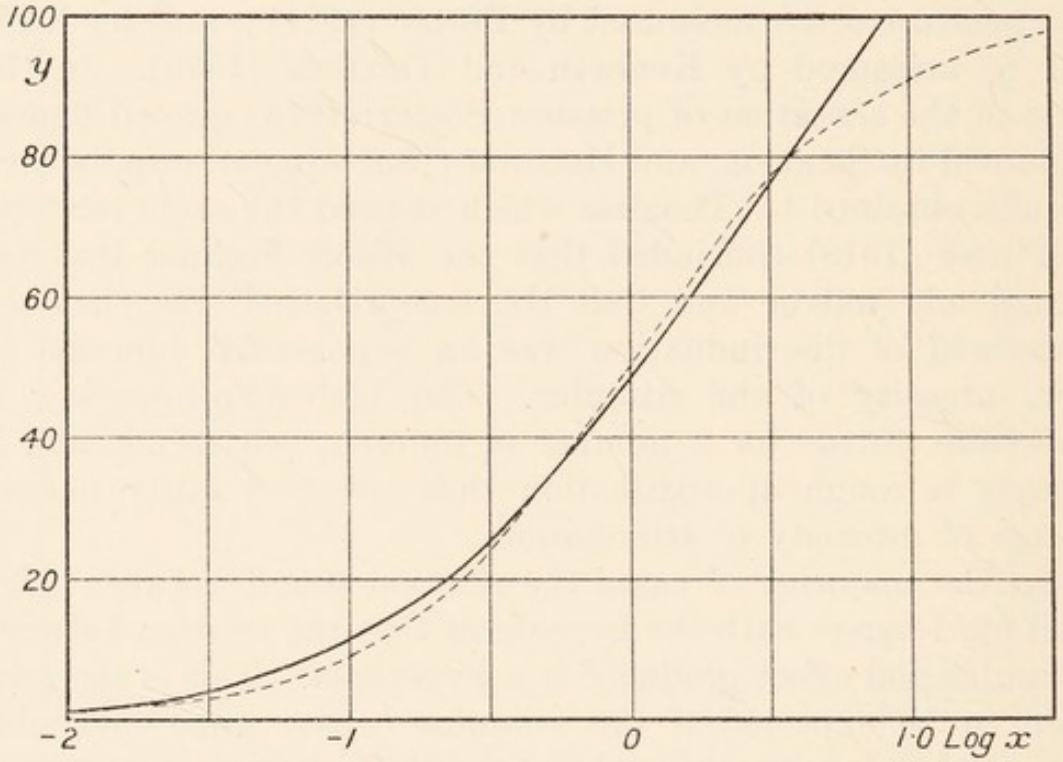

Fig. 46.—Curves following two different formulæ used to express the relation between stimulus (x) and sensory response (y).

ABSCISSA : logarithm of x. ORDINATE : y.

The continuous line follows the formula : $k_1 y = \log.(k_2 x + 1)$; $k_1 = 0{\cdot}016$, $k_2 = 5{\cdot}3$.

The broken line follows the formula $kx = \dfrac{y}{100 - y}$; $k = 1$.

photo-chemical change, whilst in the case of muscle sense the primary effect appears to be mechanical, namely a distortion of surface membranes. As far as the sensory nerve-endings are concerned it is obviously unreasonable to expect any constant relation between stimulus and effect in the different types of sensory endings which are excited by such totally different mechanisms. Fortunately it is unnecessary to discuss the psychological or philosophical hypotheses evolved by

Fechner, because in a number of cases the relation between stimulus and conscious sensation can be checked by measurements of the relation between stimulus and such objective effects as the currents set up in sensory nerves. On the whole there has been a rather surprising agreement between the results derived from subjective sensations and those derived from objective measurements of the responses of sensory nerves to stimuli.

In conclusion it must be mentioned that an approximate agreement with the experimental figures relating stimulus and effect can be obtained by a modification of the Weber-Fechner law. If the formula (A): $k_1 y = \log (k_2 x + 1)$ be substituted for the formula $ky = \log x$, then the curve shown in Fig. 46 is obtained. It will be seen that between 0 and 80 per cent. of the maximum action formula (A) gives a curve that approximates very closely to that given by the formula (B): $kx = \frac{y}{100 - y}$.

It is rarely possible to obtain experimental results ranging from 0 to 100 per cent. of possible actions and hence either of the two formulæ mentioned fit most experimental results equally well. The difference between them is that formula B expresses a probable physico-chemical process, whereas formula A has no such theoretical basis.

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CHAPTER X

MODE OF ACTION OF HORMONES AND VITAMINS

It can be argued that the study of the mode of action of hormones and of vitamins is the logical basis for the science of pharmacology, because these are drugs which the body employs to regulate its normal activities. The introduction of other drugs is an abnormal event to the organism, but in the case of hormones and vitamins we can study the response of organisms or tissues to a normal form of stimulus. Moreover by increasing the dosage it is possible to study the nature of the response produced when the stimulus is increased beyond normal limits. The hormones and vitamins show certain common characteristics in their actions. They are capable of producing an effect at extremely high dilutions. The limit of demonstrable action in several cases is of the order of less than one gramme-molecule in 10^{10} litres and the minimum doses that produce a demonstrable action are correspondingly small.

Another general characteristic of hormone action is that the effect produced is not directly proportional to the dose. If the dose of a hormone is increased tenfold the increase in the response is not nearly as great, but in many cases there is an approximately linear relation between the logarithm of the dose and the response. Most hormones produce a graded action over a wide range of dosage, and in nearly all cases the action produced is readily reversible and transient. There is nothing surprising regarding these characteristics of hormone action. It is indeed difficult to conceive how the body could regulate its functions by means of drugs with any reasonable accuracy and safety unless the drug actions obeyed these laws.

Our power to study quantitatively the actions produced by hormones and vitamins is conditioned by the effects they

produce. There are in fact very great differences in the case of the different hormones as regards the certainty with which relations between concentration and action can be established. The ideal method is to find some simple mechanical response that can be easily measured, can be rapidly produced and can be rapidly removed. In such a case we have to make one large assumption, namely, that there is some simple relation between the amount of drug fixed by the certain receptors in the cell and the amount of action that is produced. If this assumption be allowed we can, without making further assumptions, establish a considerable number of facts regarding the mode of action of such drugs as adrenaline, acetyl choline, oxytocin and vasopressin. Unfortunately only a few hormones produce responses so easy of measurement, for the majority of hormones and vitamins produce changes in metabolism or in growth, and in these cases the relation between dosage and effect is far more difficult to determine.

The easiest response to measure in such cases is the extent of the maximum change produced at any moment and such measurements may be termed measurements of intensity of action. It is, however, more logical to determine the total amount of change produced, and in this case two factors are involved, namely, intensity and duration of the action. It will be shown that there are numerous special difficulties associated with the determination of the relation between the dosage of hormone and the amount of response.

Finally, in the case of vitamins the problem is still more complex, for in most cases the effect of adding a vitamin to a vitamin deficient diet is to alter the rate of growth of cells, and hence the relation between dosage and measurable effect is extremely obscure.

Acetyl choline and adrenaline are the most favourable hormones on which to study concentration-action relations. It has been shown in Chapter VII that in both cases concentration-action curves can be obtained which approximate to a hyperbola. On the other hand, a number of authors have concluded that there is a linear relation between the logarithm of adrenaline dosage or concentration and the action produced, and this fact has been the chief evidence adduced in support of the theory that drug action follows the Weber-Fechner law.

It therefore is worth while to examine in some detail the evidence on this point.

Concentration-Action Curve of Adrenaline

The following authors have given figures which show a linear relation between the logarithms either of the concentration or of the dose and the effect produced. Van Leeuwen and le Heux (1919) and Murray Lyon (1923) who studied the action of adrenaline on the blood-pressure of the cat. Anrep and Daly (1925) who measured the acceleration of the heart in the dog's heart lung preparation. Launoy (1923) who measured the action of adrenaline on the blood-pressure of rabbits. The results obtained by Hunt (1901) and by Molinelli (1926) on the blood-pressure of the dog, which are shown in Fig. 36, prove however that although there is a linear relation between the logarithm of the dose and the effect produced over a considerable range of dosage, yet this relation does not hold outside of certain limits.

In the case of isolated organs Cameron and Mackersie (1926) concluded that there was a linear relation between the logarithm of the concentration and the contraction produced in an arterial strip, but Wilkie's results with the same system, which are shown in Fig. 35, prove that in this case also the relation over the full range of dosage is not linear.

The figures obtained by Hunt and by Molinelli show that adrenaline exerts a graded action over a range of dosage between 300 and 100 fold, and their results clearly show that the relation between the concentration of adrenaline and its action on the dog's blood-pressure follows a hyperbola. Wilkie's experiments on carotid strips show a similar range of dosage, and in this case the results follow the formula $Kx = \frac{y}{100 - y}$. The results on the dog's blood-pressure do not follow this formula but approximate more clearly to the formula $Kx^2 = \frac{y}{100 - y}$.

The relation between dosage and response cannot however be expected to be as exact in an intact animal as in an isolated organ, for the number of uncontrolled variables is much greater in the former case. Boothby and Sandiford (1924) state that there is a linear relation between the logarithm of the concen-

tration of adrenaline and its calorigenic action as measured by the increase in metabolism which it induces. This relation is, however, influenced by both the intensity of action and the duration of maintenance of action, and the significance of such results will be discussed later. The evidence shows, however, that the results of those authors who have studied the action of adrenaline on the most favourable systems and over the widest range of dosage do not support the general conclusion that drug action follows the Weber-Fechner law, a hypothesis that has been put forward by van Leeuwen (1919), by Cameron and Mackersie (1926), and by Gaddum and Hetherington (1931).

Concentration-Action Curve of Acetyl Choline

The evidence regarding the concentration-action curves of acetyl choline has already been described in Chapter VII. Experiments upon isolated organs agree well with the hypothesis that the concentration-action curve follows a hyperbola. In the case of the action of acetyl choline on the blood-pressure and pulse-rate of the intact cat the evidence is, however, inconclusive, for the figures would fit a logarithmic relation (Clark and White, 1927).

The relation between concentration and action in the case of acetyl choline and adrenaline therefore follows the relation $Kx = \frac{y}{100 - y}$ in those cases in which it is easiest to obtain exact measurements of this relation over a wide range of concentrations. The possible interpretations of this relation were discussed in Chapter VII, where it was shown that the simplest interpretation is that the curve expresses the equilibrium between a drug present in excess that reacts with a limited number of cell receptors to form an easily dissociable compound.

There are certain interesting consequences that follow from this type of relationship between concentration and action. When the action is less than 10 per cent. of the maximum the action varies as the concentration, between 20 and 80 per cent. the action varies as the logarithm of the concentration, and above 80 per cent. the action varies as about $\sqrt[3]{}$conc. Consequently very small changes in concentration can modify a slight action, but a great excess of hormone is needed to produce a toxic action. This type of relation between concentration

and action appears peculiarly suitable for drugs produced in the body for the purpose of regulating vital functions.

Concentration-Action Curves of Oxytocin and Vasopressin

Cameron and Mackersie (1926) concluded that there was a linear relation between the logarithm of the concentration of oxytocin and the extent of the response of the isolated uterus of the guinea-pig. Most other workers have found a sigmoid

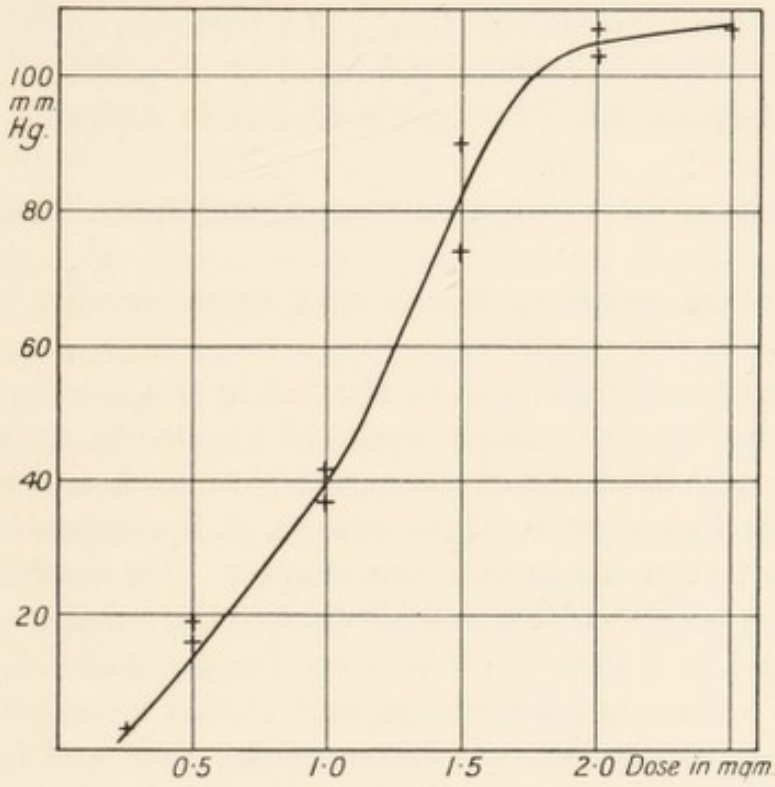

Fig. 47.—Concentration-action curve of the effect produced by posterior pituitary gland on the blood-pressure of a cat.

Abscissa : dose of dry posterior pituitary lobe in mg. per kg. Ordinate : rise of blood pressure in mm. Hg. (Hogben et al. 1924.)

relation between concentration and effect in this system. The action of vasopressin on the blood-pressure of the decapitated cat was studied by Hogben et al (1924), who found that the relation between concentration and action was a steep sigmoid curve, as is shown in Fig. 47. The results obtained with the pituitary active principles differ markedly from those obtained with adrenaline and with acetyl choline. They suggest that pituitary causes some all-or-none effect in plain muscle-cells. The special interest in these results is that they show how wide

a variety of concentration-action curves may be obtained even in the restricted field of the action of hormones upon the blood-pressure.

The evidence shows therefore that two of the active principles of the posterior lobe of the pituitary gland, namely oxytocin and pitressin, produce an all-or-none type of action on plain muscle and thereby differ from the other hormones.

The Relation between Dosage of Hormones and Amount of Action

The relation between the dosage of a hormone such as insulin and the amount of action produced is a problem of great practical importance. In such a case it is desirable to know two relations :

(*a*) Relation between dosage and maximum intensity of action.

(*b*) Relation between dosage and total amount of action produced.

For example, the amount of the fall in the blood-sugar and the incidence of convulsions produced by insulin are measures of the greatest intensity of the action produced, and are therefore similar in nature to those actions of hormones that have previously been considered in this chapter. On the other hand, the relation between dosage of insulin and the amount of glucose metabolized is a measure involving the question of duration of action. Grevenstuk and Laqueur (1925) have pointed out that these two types of measurement are quite distinct, although they have frequently been confused. It is therefore necessary to consider what types of relations may be anticipated when the amount of action is measured.

The relation between dosage of alcohol and the amount burnt in the body is a particularly simple example of measurements of this type. The figures of Mellanby (1919) show that there is a linear relation between the alcohol dosage and the concentration in the blood, and that the rate of oxidation of alcohol is constant as long as the amount in the blood exceeds a certain minimum. Finally, in the case of alcohol only a small fraction of the drug given is lost by excretion and nearly the whole of it is oxidized. Hence the amount of alcohol oxidized almost equals the amount given, and since the amount oxidized per hour remains constant, therefore the time required

to oxidize the alcohol is directly proportional to the dosage. Moreover, since there is a linear relation between the concentration of and the action produced by alcohol, therefore the total amount of any effect produced by this drug will also be directly proportional to the dosage.

The simplicity of these relations is very exceptional, and we may next consider the relations likely to occur in ordinary cases. In the first place the relation between dosage and intensity of action usually either follows a hyperbola or else is logarithmic. Secondly, it is very rare for an excess of drug to be retained in the body. In the case of all hormones except thyroxin the body either excretes or also breaks down rapidly any excess of hormone.

The Duration of Action of Drugs

The duration of the action produced by any dose of drug depends, in the first place, on the length of time for which an efficient concentration of drug is maintained in the circulation, and this depends on the rate at which the drug is eliminated from the blood. Most hormones are removed rapidly; for example, acetyl choline and adrenaline are broken down by ferments, whilst other hormones such as œstrin are excreted rapidly. The only outstanding exception to this rule is thyroxin, which is removed from the body very slowly.

As regards other drugs there are a few, such as digitalis and the heavy metals, which are removed slowly from the body and hence tend to produce cumulative poisoning, but the majority of drugs are excreted rapidly, and as a general rule the process of excretion follows a die-away curve and there is an approximately linear relation between the logarithm of the amount of drug remaining in the body and the time.

The relation between the dosage of atropine and the duration of the action produced is shown in Fig. 48, and it will be seen that there is a linear relation between the logarithm of the dose and the duration of action. This relation implies that a very large dose is required to maintain an action for a long period. Fig. 48 shows that a dose of $0{\cdot}1\gamma$ produced an action for 10 hours and that 1γ produced an action for 50 hours, and extrapolation indicates that a dose of 10γ would be needed to produce an action for 100 hours.

Burn (1931) measured the relation between the dose of

pituitrin and the delay in production of water diuresis in rats. His figures show a linear relation between the logarithm of the dose and the duration of the action. This relation suggests that the disappearance of pituitrin from the blood-stream follows a logarithmic curve.

The rate of excretion of drugs by the kidney is usually proportional to the concentration in the blood, and hence follows a die-away curve, with a linear relation between logarithm of blood concentration and time. If the breakdown of the drug happens to be a monomolecular reaction, or an enzyme process, it will follow much the same curve. In the case of most hormones, drug excess is removed fairly quickly and hence the

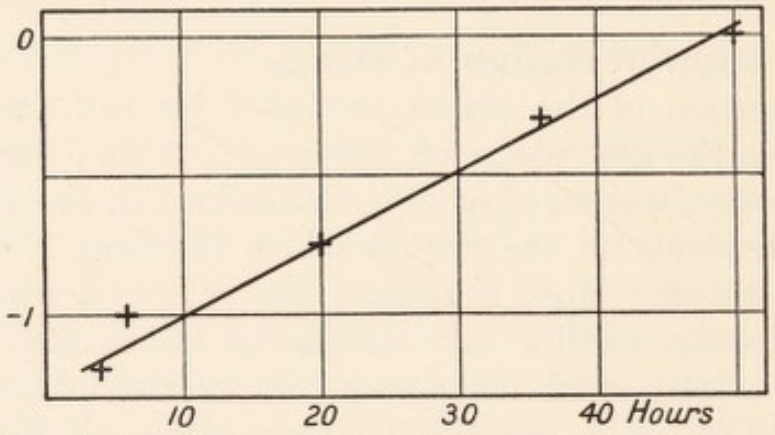

Fig. 48.—The relation between the dosage and the duration of action of atropine.

Abscissa : duration of dilatation of pupil in hours. Ordinate : logarithm of dose (in microgrammes) of atropine injected intraocularly. (Koppanyi and Lieberson. 1930.)

amount of action produced is likely to be dominated chiefly by the rate of excretion or breakdown.

The duration of action will therefore vary as the logarithm of the initial concentration. Moreover, if for the sake of simplicity we accept the approximation that the intensity of the action varies as the logarithm of the concentration then there is a simple relation between dosage, intensity of maximum action, duration and amount of action. The intensity of maximum action and the duration will vary as the logarithm of the dosage, whilst the amount of action will vary as some multiple of the logarithm of the dosage. If we assume that the threshold active concentration equals unity and that one extra unit of dosage is reduced to this threshold concentration

in 1 unit of time and produces 1 unit of action, then the following relations will obtain:

Dosage	Intensity of action	Duration of action	Amount of action
1	1	1	1
5	2·7	2·5	6·8
10	3·5	3·3	11·6
50	5·7	5·4	30·7
100	6·7	6·2	41·6

These relations show certain points of interest. In the case considered the intensity of action was assumed to vary as the logarithm of the concentration, and this relation can be taken as a first approximation in the case of several hormones, e.g. adrenaline. In such cases, although the duration of action varies as the logarithm of the dose, yet the total amount of action is almost directly proportional to the dose over a considerable range of dosage. It is quite probable that in some cases a variety of logarithmic relations might result in a fairly exact linear relation between dosage and total amount of action. On the other hand, there are certain hormones that produce an action of the all-or-none type. A certain minimum quantity is needed to produce an effect, but excess of drug above this minimum produces no further effect. In such a case the total amount of action produced by any dose will vary directly as the duration of action, and hence as the logarithm of the dose. This implies that a great increase of dosage produces only a slight increase in action. In the example worked out above a 100-fold increase in the dosage increased the duration of action less than tenfold. There is evidence that a relation of this kind between dosage and action occurs in the case of œstrin.

The Relation between Dosage and Action of Insulin

The accurate standardization of insulin is a matter of great practical importance and hence the relation between dosage and action has been studied carefully. Grevenstuk and Laqueur (1925) pointed out that the effects measured have been of two different kinds because some methods have measured the intensity of effect whilst others have measured both the intensity of effect and the duration of the action and therefore have expressed the amount of action produced.

The intensity of effect produced by insulin can be measured either by (*a*) the extent of the fall of blood-sugar or (*b*) by the incidence of convulsions. The amount of action produced by insulin can be estimated by measuring the amount of carbohydrate that a dose of insulin causes to be metabolized.

The fall of blood-sugar caused by injection of insulin is a graded action and the measurement of its extent provides a simple measure of the intensity of action. Unfortunately this method is inaccurate because some animals show a rapid fall followed by a quick recovery, whereas other animals show a slower and more sustained effect. It has been found most satisfactory to measure the average fall of blood-sugar over a period of 5 hours. The result in this case is a measure partly of intensity and partly of duration of action.

The relation between insulin dosage and the extent of the fall of blood-sugar was estimated by Marks (1926). He gave a series of doses to each of 50 rabbits and measured the mean blood-sugar over a period of 5 hours. The average results expressed as percentage of normal blood-sugar are shown in Fig. 49. The curve shows a linear relation between dosage and effect up to a dose of 3/4 of a rabbit unit per kilo., but increase of dosage above this value produces little further effect. The general shape of this curve has been confirmed by other workers. The crosses in Fig. 49 represent averages taken from tables given by the Toronto Insulin Committee (1926). Each cross represents the average of more than 20 rabbits. Macleod and Orr (1924) and Grevenstuk and Laqueur (1925) measured the maximum fall of blood-sugar produced by insulin and obtained curves of a shape very similar to that given by Marks.

Grevenstuk and Laqueur (1925) expressed the opinion that the parabola obtained in these cases had little theoretical significance because there was obviously a limit to the possible fall of blood-sugar, and an asymphotic curve was nearly certain to be obtained as this limit was approached. They also pointed out that the estimation of blood-sugar measured not only free sugar but also sugar combined with proteins, and therefore the maximum amount of sugar that could be removed from the blood was unknown. Eadie and Macleod (1924) estimated the action of insulin in inhibiting the rise of blood-

sugar produced by a fixed dose of adrenaline. They obtained a logarithmic relation between dose of insulin and inhibition of rise of blood-sugar, and their curve shows a general similarity to that obtained by Marks, and the criticism of Grevenstuk and Laqueur mentioned above could scarcely apply in this case.

There appears therefore to be no certain difference between the results which express simply intensity of effect and those which express both intensity and duration of effect. The

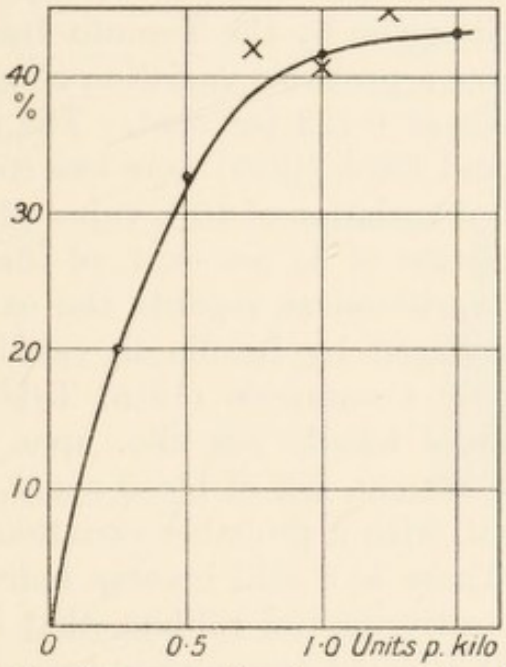

Fig. 49.—The relation between the dosage of insulin and the average fall of blood-sugar produced in 40 rabbits.

Abscissa : dose in units per kg. Ordinate : per cent. fall of blood sugar. (Marks. 1926.)

The three crosses show averages taken from figures given by the Toronto Insulin Committee (1926).

theoretical distinction between intensity and amount of action does not therefore appear to be demonstrable in practice in this case. The general shape of the curve in Fig. 49 shows that an accurate estimate of the action of insulin can only be obtained when the dose is less than 3/4 unit per kilo. because with higher doses a large difference in dosage only produces a slight difference in response.

Another method of standardizing insulin is to measure the percentage of animals which are convulsed by a given dose. In this case the incidence of an all-or-none effect is measured

and hence the populations tested must be large enough to provide statistically accurate results. This method is open to the objection that the results must be influenced by the following three independent variables : (1) The normal level of the blood-sugar. (2) The extent of the fall of blood-sugar produced by insulin. (3) The level of blood-sugar at which convulsions occur.

Individual variations in the level of the normal blood-sugar appear to be not very extensive in the case of animals kept under standard conditions. Figures for the normal blood-sugars of 70 rabbits given by the Toronto Insulin Committee (1926, Table I) show a probable variation of about 6 per cent. of the median value of 0·123 per cent. The figures obtained by Clough, Allen and Root (1923) were less constant, for they found a coefficient of variation of 16, a value which corresponds to a probable variation of 11 per cent. of the median.

The individual variation as regards the extent of the fall of blood-sugar produced by insulin in rabbits is notorious. The Toronto Insulin Committee (1916, Table I) record the effects of one unit of insulin per kilo. upon 24 rabbits, and these figures show a mean fall of blood-sugar equal to 42 per cent. of the normal, with a probable variation of 16 per cent. of the median. There is a still greater individual variation in the nervous sensitiveness of rabbits, that is to say in the level of blood-sugar at which convulsions occur. The incidence of convulsions in relation to the blood-sugar level in the figures of the Toronto Insulin Committee already quoted is as follows :

Blood-sugar level	Incidence of convulsions	Per cent. incidence
0·059 or less	5 out of 9	55
0·06–0·069	5 out of 24	21
0·07 or more	3 out of 33	9

Clough, Allen and Root (1923) measured the incidence of convulsions and the lowest level of blood-sugar in 168 rabbits and obtained the following figures :

Blood-sugar range	No. of observations	Per cent. convulsions
Above 0·030	97	13·5
0·03–0·026	25	48
0·025–0·016	31	68
Below 0·016	15	80

These figures indicate a probable variation as regards the blood-sugar level at which convulsions occur of about 30 per cent. of the median value. Of the three variable factors which affect the incidence of convulsions nervous sensitiveness appears therefore to show the greatest individual variation.

The relation found by Marks between insulin dosage and incidence of convulsions in rabbits is shown in Fig. 50. The curve shows a wide scatter and the probable variation is about 50 per cent. of the median. This result is to be expected from a consideration of the number and extent of the variables

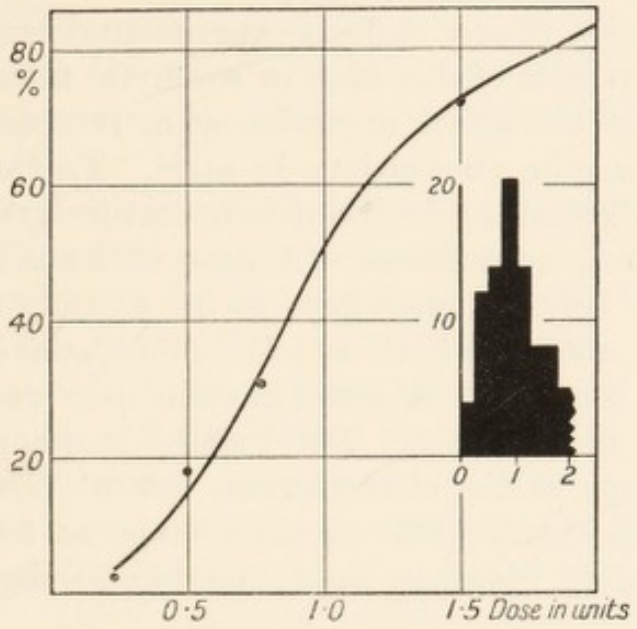

Fig. 50.—The relation between dosage of insulin and the incidence of convulsions in 50 rabbits.

Abscissa : dose in units per kg. Ordinate : per cent. of rabbits showing convulsions.
Inset. The distribution of variation. (Marks. 1926.)

influencing the response. Trevan and Boock (1926) measured the relation between insulin dosage and incidence of convulsions in mice. Their results show a sigmoid frequency curve with a probable variation of 29 per cent. of the median. This result is distinctly more favourable than that obtained by Marks.

Trevan and Boock concluded that " The lack of correlation between convulsions and hypoglycæmia would render the convulsion test much less useful than the direct measurement of blood-sugar, were it not that the number of independent

variable factors which affect the degree to which the blood-sugar falls is already so large that the effect of the addition of one other variable, namely, the variability of the convulsive response to hypoglycæmia, can be compensated by a reasonable increase in the number of animals used."

This result agrees with the general principle that if there are a considerable number of factors that vary independently, then the addition of one more may make very little difference. It has already been pointed out that the variables are just as likely to cancel each other as to summate, and the most probable final result is a bell-shaped distribution of frequency. The introduction of any definite experimental error can, however, distort results of this kind in a definite manner. This is exemplified by the effect of variation in temperature on the incidence of insulin convulsions in mice. Trevan and Boock found that a reduction in room temperature greatly reduced the incidence of convulsions. A dose of insulin which convulsed 95 per cent. of mice kept at 38° C. only convulsed 35 per cent. of mice kept at 29° C. Moreover, at the lower temperature very large doses of insulin only convulse about 60 per cent. of the mice. The change in temperature also alters the shape of the characteristic curve. This was of an approximately symmetrical sigmoid shape at 38° C., but at 29° C. the curve obtained approximated to the exponential form.

Hemmingsen and Krogh (1926), who worked with mice at 30° C., found a linear relation between the logarithm of the dose and the incidence of convulsions. The results obtained by Trevan and Boock suggest, however, that this linear relation is an accident depending on the low temperature at which the tests were conducted.

The somewhat special care that has been taken over the bio-assay of insulin has revealed a remarkably large number of possible sources of error. This is instructive from the theoretical standpoint because it shows how many variables may influence the shape of what appears on inspection to be a simple characteristic curve. The practical problem of achieving an accurate bio-assay of insulin has been solved by devising a method that eliminates as far as possible all these variables. The method adopted in the British Pharmacopœia is that

devised by Marks. The average fall in blood-sugar in rabbits over a period of five hours after insulin injection is measured. The dose given is about 1/2 unit per kilo. and the results therefore fall on the steep portion of the curve in Fig. 49. Two groups of six rabbits each are used and one group is given a standard preparation of insulin and the other group is given the preparation which is to be assayed. Three or four days later the test is repeated with the groups reversed. The average results from the two groups are compared. If the results differ by more than 10 per cent. the tests are repeated with the doses adjusted until the results differ by less than 10 per cent. This method eliminates the effects of individual variation and also the effects of periodic variation in the susceptibility of the rabbits. The method gives results accurate to ± 10 per cent.

The purpose of insulin administration is to enable the body to utilize carbohydrates and therefore the ideal method of standardization would be one based on the effect of the drug on carbohydrate metabolism. Macleod (1924) pointed out that the initial fall of blood-sugar produced by insulin was within wide limits independent of the dose administered and that the recovery process depends on a number of independent variables. The most important of these was the glycogen store in the liver, and the activity of the thyroid also had a considerable influence. The duration of action of insulin on the blood-sugar therefore is unlikely to give a direct quantitative measure of the action the drug has produced.

Allan (1924) measured the relation between the insulin dosage and the amount of glucose catabolized by a depancreatized dog. His results (Fig. 51) show a relation between dosage and effect almost identical with that obtained by Marks. Macleod (1924) quotes figures which show a very similar curve. In this case the glucose metabolized was estimated by determining the amount of sugar that a diabetic patient could take without a rise in blood-sugar being produced. Allan concluded that his figures followed the formula $g.\ (\mathrm{U})^{0{\cdot}85} = 10^{1{\cdot}86}$, where $g =$ grammes glucose metabolized per unit and $\mathrm{U} =$ no. of units. This formula implies that the total glucose metabolized varies as $(\mathrm{U})^{0{\cdot}15}$. The curve in Fig. 51 corresponds approximately to the relation amount of sugar consumed $= 10^{1{\cdot}8} \times (\mathrm{U})^{0{\cdot}2}$.

Inspection shows, however, that there is a considerable scatter of the points, and that the shape of the curve depends largely on the values obtained with the two lowest doses. The results show, however, that increasing the insulin dosage above 10 units produces a relatively slight effect on the glucose metabolism, and they suggest that up to a certain point the body has insufficient insulin and every increase of dosage produces a large effect, but that once the body has an adequate supply, then a further increase in dosage produces little effect.

The similarity in the general shape of the curves in Figs. 49 and 51 suggests that the theoretical distinction between the

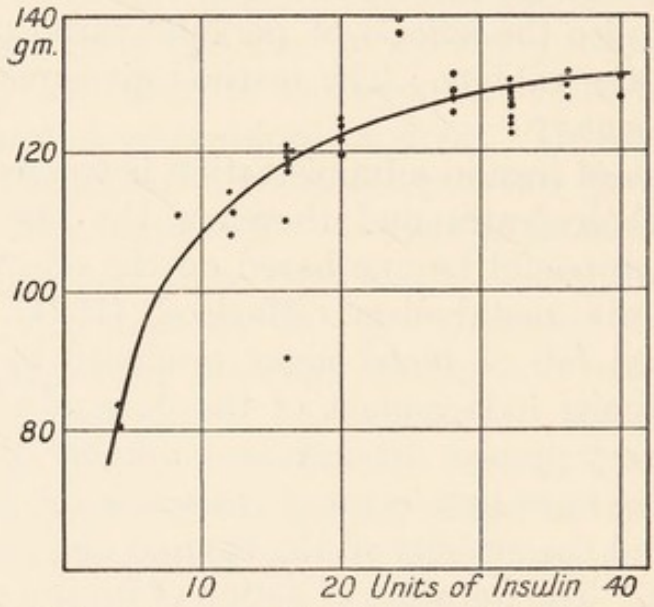

Fig. 51.—The relation between the dosage of insulin and the amount of sugar metabolized by a depancreatized dog.

ABSCISSA : daily dosage of insulin. ORDINATE : grammes of sugar metabolized daily. (Allan. 1923.)

intensity and the amount of action of insulin is not of great practical importance. It is true that the results shown in Fig. 49 measure both intensity and duration of action, but figures for the maximum fall of blood-sugar, which measure intensity of action alone, give very similar curves. The agreement between the two sets of figures is all the more remarkable because the results shown in Fig. 49 were obtained with normal rabbits in which there must have been an endogenous insulin production, whereas those in Fig. 51 were obtained on a depancreatized dog. This is another proof of the fact that the figures relating dosage and action of insulin must express

the resultant of a large number of variable factors, and cannot reasonably be regarded as a measure of any single physico-chemical process.

Dosage and Amount of Action of Thyroxin

Thyroxin is a slow-acting hormone and it is not possible to measure quantitatively any immediate effect. Boothby and his co-workers (1924, 1925) have shown that about 5 per cent. of thyroxin is destroyed or utilized daily in the body, and hence from 30 to 70 days are required for the body to lose 90 per cent. of a dose of this hormone. This rate of destruction is unlike that of any other hormone, for in all other cases hormones are rapidly eliminated. For example, 90 per cent. of a dose of adrenaline is lost in 1–4 hours. Various writers have measured the relation between daily dosage of thyroxin and the increase in metabolism produced (Mørch (1929), Gaddum and Hetherington (1931), Cameron and Carmichael (1926)). These authors all have obtained an accurate linear relation between the logarithm of the daily dose and the per cent. increase in metabolism as is shown in Fig. 52. In these cases a certain constant daily dose of dried thyroid or thyroxin was given and continued for weeks. It may be assumed that the experiments were of sufficient duration for equilibrium to be attained between daily intake and daily destruction of thyroxin. Providing that this was the case, the figures express the relation between a certain concentration of thyroid in the body and the metabolic rate. The curves therefore measure the intensity of action rather than the amount of action. The following points must, however, be taken into consideration. The metabolic rate of a thyroidless man is between 55 and 60 per cent. of the normal (Magnus-Levy, 1896). On the other hand, an increase of metabolism of 100 per cent. above normal usually is associated with toxic effects dangerous to life. The action of the thyroxin present in the normal animal is therefore equivalent to at least 25 per cent. of the maximum effect on the metabolism that can be produced by thyroxin.

The figures for the increase in metabolism caused by thyroid dosage in normal animals do not therefore represent actions ranging from zero to maximum action but really commence at about 20 per cent. of maximum action, and in the calculation of the relation between dosage and action some quantity ought

to be added to all the doses to represent the endogenous thyroxin production. Such an addition would completely spoil the exact linear relation shown between the logarithm of the dose and the effect in Fig. 52. For this reason this relation must be considered to be of very doubtful theoretical significance. The relations between thyroid dosage and the effect produced on either thyroidless animals or myxœdematous

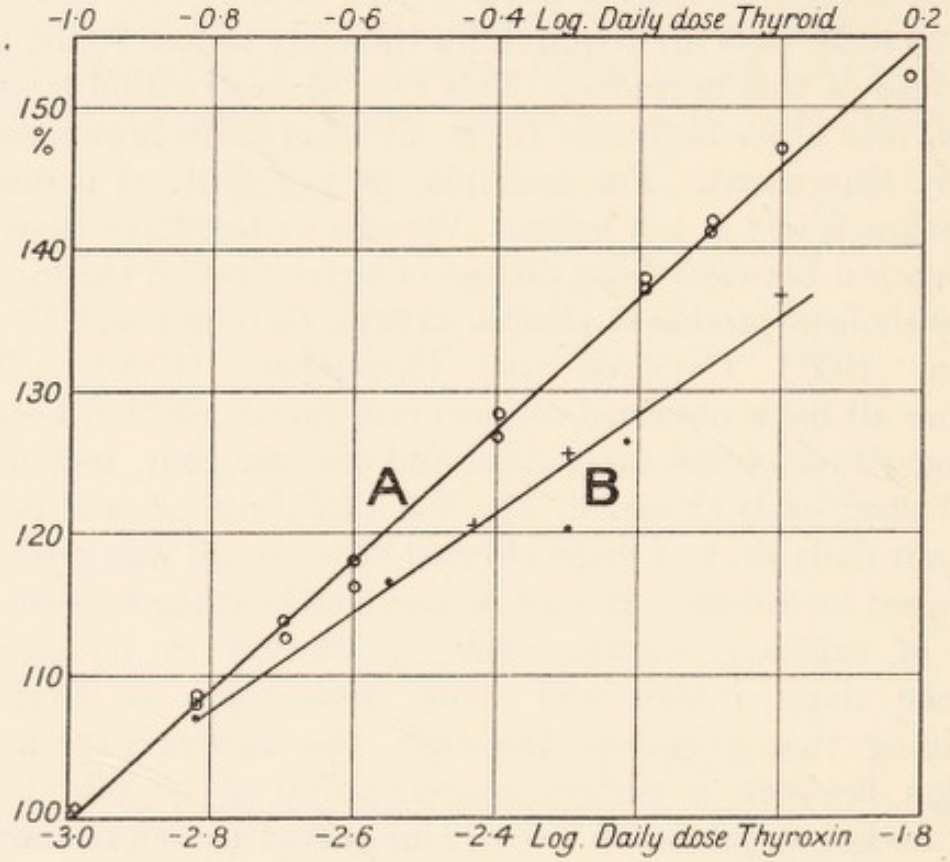

Fig. 52.—The relation between the dosage of thyroid or thyroxin and the metabolism of mice.

Upper abscissa : logarithm of daily dose of thyroid in mg. per 20 gm.
Lower abscissa : logarithm of daily dose of thyroxin in mg. per 20 gm.
Ordinate : production of CO_2 expressed as per cent. of normal.
Curve A. Effect of thyroid. (Mørch. 1929.)
Curve B. Effect of thyroxin. (Gaddum and Hetherington. 1931.)

patients are of greater theoretical significance because in this case the unknown activity of the thyroid cannot influence the result.

Boothby and Sandiford (1925) measured the effect of thyroxin on 69 cases of myxœdema. In these cases it may be assumed that very little endogenous thyroxin was present. Their results were as follows :

Daily dose of thyroxin in mgm. . . .	4·8	6·9	8·8	11·2	16	24
Per cent. increase in B.M.R. . . .	17	19	26	37	42	39
Per cent. increase in B.M.R. per mgm. . .	3·5	2·8	2·9	3·3	2·6	1·6

The average results, which are given above, show a linear relation between dosage of thyroxin and effect when the dose is less

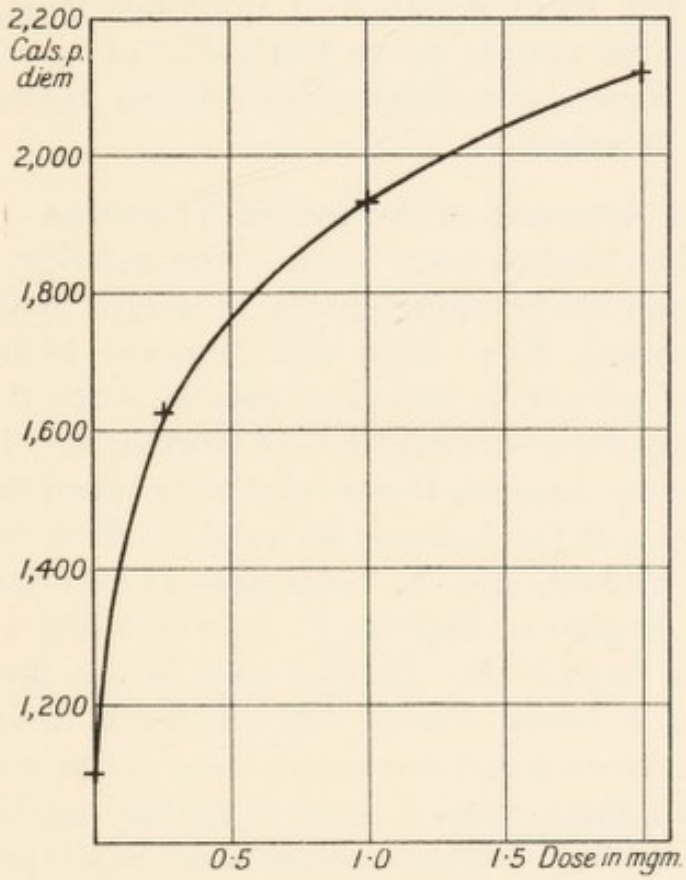

Fig. 53.—The action of thyroxin on the metabolism of a myxœdematous patient.

Abscissa: dose of thyroxin (in mgm.) injected daily intravenously. Ordinate: metabolism in calories per diem.

The curve is drawn to the formula: $(\text{Cals.} - 1100) = 800 \times \sqrt[3]{\text{dose.}}$ (Boothby, Sandiford et al. 1926.)

than that needed to raise the B.M.R. to a normal level (30–40 per cent. increase). Larger doses of thyroxin produce little further increase. These results are inconsistent with the relation action varies as the logarithm of the dose, but approximate to the relation obtained when the dosage-action curve follows a hyperbola. Boothby and Sandiford concluded that

the increase in metabolism varied as the cube root of the dosage of thyroxin.

Finally, it is only fair to mention that some experienced workers actually deny that there is any accurate relationship between dosage and effect of thyroxin. For example, Kunde (1927) concluded as a result of extensive experiments on normal and thyroidless dogs that " a quantitative relationship between the amount of thyroid substance ingested and the increase in the basal metabolism does not exist." Such a conclusion raises doubts as to the value of any attempt to establish an accurate mathematical relation between thyroid dosage and its effect.

Dosage and Amount of Action of Vitamins

Quantitative studies have been made relating the dosage of vitamins and the resulting change of weight of experimental animals. Coward, Kay, Dyer and Morgan (1930) measured the change of weight in groups of rats kept for 5 weeks on a diet containing variable quantities of vitamin A. Their results (Fig. 54) show an accurate linear relation between the logarithm of the dosage and the increase in weight over a 30-fold range of dosage. Pilcher and T. Sollmann (1926) made similar experiments on pigeons kept for 21 days on a diet with varying quantities of vitamin B. In this case also a linear relation was obtained between dosage and change in weight. Semi-quantitative experiments have also been made with vitamins C and D. In these cases a scale of effects has been worked out and the relation between dosage and effect produced has thus been estimated. Key and Elphick (1931) studied the action of vitamin C on guinea-pigs and concluded that there was a linear relation between the dosage of orange juice and the amount of protection afforded. Bourdillon and his co-workers (1931) found that the action of vitamin D was proportional to the logarithm of the dose. These workers recognized 12 divisions in the curative effect of vitamin D on rickets in rats. They found a logarithmic relation between dosage and effect. If one unit produced an improvement of 1 division, 4 units would be needed to produce an improvement of 3 divisions, and 8 units an improvement of 4 divisions, etc.

In these cases the fundamental action of the vitamin is presumably to alter the rate of growth and division of certain

cells. The effect measured is the change of weight after a certain interval. There is, however, a logarithmic relation between rate of growth and increase of weight after a given interval, and hence it is difficult to calculate the probable relation between dosage and fundamental action. Animals

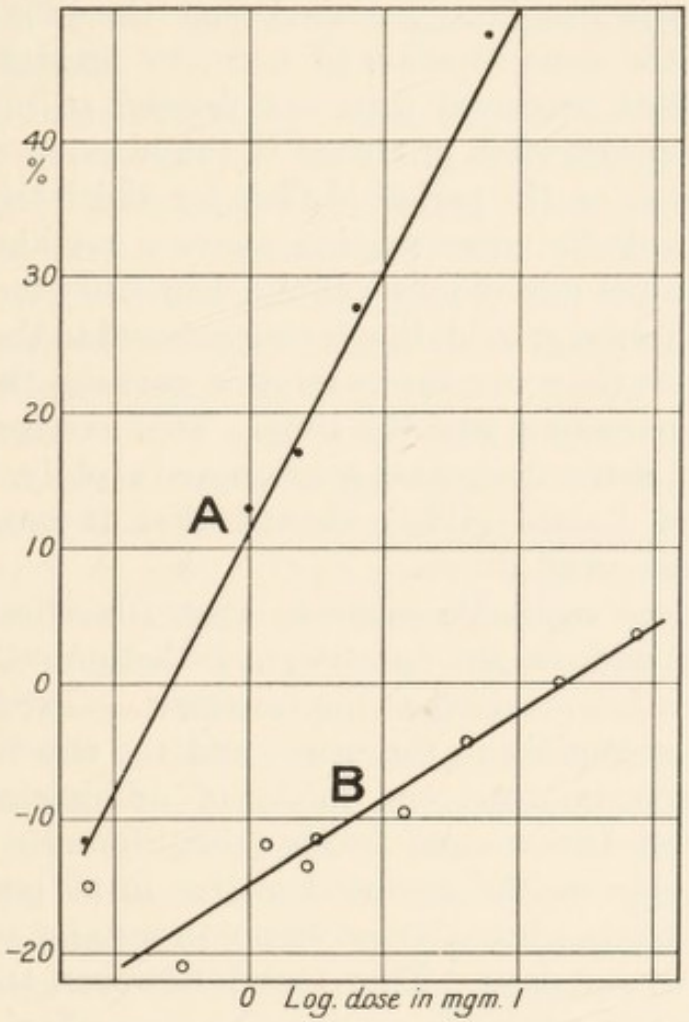

Fig. 54.—The relation between vitamin dosage and growth.

Abscissa : logarithm of dose in mg. Ordinate : the per cent. increase of the original body weight.

Curve A. Effect of cod-liver oil fed for five weeks to rats. (Coward, Kay et al. 1930.)

Curve B. Effect of yeast extract fed for three weeks to pigeons. (Pilcher and Sollmann. 1926.)

have a considerable power of storing vitamins and hence it is probable that most of the vitamin given to an animal on a vitamin deficient diet will be utilized. It is, however, difficult to estimate whether the amount of growth measures the intensity of effect produced or the amount of effect produced by the vitamin. In general there appear to be too many

unknown factors to make it profitable to attempt any explanation of the fact that the effect produced varies as the logarithm of the dosage.

Duration and Amount of Action of All-or-none Effects

In the discussion on the relation between dosage and action of hormones it has been assumed that the effect produced depends on the concentration of hormone present. In some cases the effect produced does not depend so much on the maximum concentration produced in the blood-stream by any given dosage as on the period of time for which the dose given has maintained the concentration above a certain minimum. In most cases the rate of removal of a drug from the circulation by either excretion or oxidation is proportional to the concentration and hence there is a linear relation between the logarithm of the drug remaining and the time. This has been observed in the case of many drugs that are excreted rapidly. Similarly, Boothby and Baldes (1925) showed that thyroxin removal followed a die-away curve.

The evidence available suggests that the effect produced by œstrin depends on the duration of its action rather than on the concentration attained at any moment. Œstrin is known to be excreted rapidly in the urine, and the effect by a given quantity depends upon the mode of administration. For instance, Allan, Dickens and Dodds (1930) found a given dose of water-soluble œstrin produced œstrus in 50 per cent. of a population of rats when divided into 6 doses and administered at intervals over 3 days. They found, however, that 40 times this quantity was needed to produce the same effect when given in a single injection. On the other hand, when absorption of the œstrin was delayed by giving it in an oil-water emulsion the quantity needed was only 8 units.

These results can easily be explained on the assumption that the action of œstrin depends on the duration of its action and that the rate of excretion varies as the concentration. Under these circumstances the duration of action will vary as the logarithm of the dose. If 6 divided doses each produce an action for 1 unit of time, the quantity needed for a single dose to act for 6 units of time will be about 100 times the amount of each divided dose.

It is not possible to measure quantitatively the relation

between dosage and intensity of action produced by œstrin, but qualitative observations indicate a remarkably wide range of dosage. Marrian and Parkes (1930) found that the quantity of œstrin needed to produce uterine changes and copulation in mice was 200 times the quantity needed to produce the vaginal signs of œstrus. If the action produced depends on the duration of the action of a dose this range of dosage is easily intelligible.

Œstrin is standardized by estimating the occurrence or non-occurrence of œstrus, i.e. an all-or-none effect, and various workers have estimated the relation between dosage and incidence of effects. Coward and Burn (1927) standardized oil-soluble œstrin in rats and D'Amour and Gustavson (1930) injected water-soluble œstrin into rats, using the single dose method. Marrian and Parkes (1929) tested water-soluble œstrin in mice ; they used 4 injections at 12-hour intervals, and their standard curve is the average of about 500 tests. Allan, Dickens and Dodds (1930) measured the action of water-soluble œstrin on rats. They gave 6 injections spread over 3 days. Their standard curve is based on 658 rat experiments. The results obtained by these workers show asymmetrical sigmoid curves in which the probable variation is about 40 per cent. of the median. A wide scatter of this type is to be expected because the intensity of action of œstrin appears to depend chiefly on the length of time for which an effective concentration is maintained in the blood-stream, and this will be influenced profoundly by any variations either in the rate of absorption or in the rate of excretion.

Summary

The examples studied in this chapter prove chiefly the extreme difficulty there is in obtaining reliable data relating dosage and effect. In the case of hormones such as acetyl choline and adrenaline which produce a simple mechanical response in isolated tissues it seems possible that the results obtained may express some definite physico-chemical process. Even in these favourable cases when the hormones are tested on intact animals the relations between dosage and action become much more obscure.

In the case of such hormones as insulin and thyroxin the relation between dosage and action appears simple until it is

studied critically. There is a general tendency for a linear relation to be found between the logarithm of the dose and the intensity or the amount of the effect. This relation obtains with the greatest accuracy in the case of the action of thyroxin on normal animals, but unfortunately in this case it is obviously necessary to make some allowance for the endogenous thyroxin and any such allowance destroys the linear relation.

Another important fact is that two methods may give similar sigmoid characteristic curves, expressing a similar distribution of variation, although it can be shown that one method includes more variable factors than does the other.

The chief fact that emerges from these results is that the only studies in quantitative pharmacology that are likely to yield results of theoretical interest are those made on the very simplest systems.

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CHAPTER XI

DRUG ANTAGONISMS

General

The action of any drug on a cell population is likely to be influenced by the action of any other drug that has a well-marked effect, hence there are innumerable examples of drugs that potentiate or inhibit each other's actions. The analysis of the action of a single drug is so difficult that in most cases it is of little interest to try to analyse the effects produced by two drugs acting simultaneously and hence the subject of drug antagonism as a whole has only a limited interest. In certain cases, however, one drug inhibits the action of another in a remarkable and selective manner and such cases deserve consideration because they throw a certain amount of light on the subject of drug action. The following are some of the chief types of drug antagonism.

(1) *Chemical antagonism.* The antagonists react *in vitro* to form a harmless product, e.g. acids and alkalies: calcium salts and citrates: toxins and antitoxins.

(2) *Physiological antagonism.* The substances produce opposite but independent effects on cells, e.g. potassium and calcium salts, atropine and morphine.

(3) *Specific cellular inhibitions*: (*a*) The substances compete for common receptors in the cells, e.g. oxygen and carbon monoxide. (*b*) The substances act at different points in a chain process, e.g. cyanides and oxygen. (*c*) Specific antagonisms of unknown nature, e.g. atropine and acetyl choline, ergotamine and adrenaline, etc.

This classification indicates some of the chief types of antagonism. The class of special interest is that mentioned last, but it is necessary to consider the characteristics of the different classes.

Inorganic Models of Drug Antagonism

The literature of contact catalysis provides some remarkable examples of highly selective drug antagonism. Bredig

showed that the catalytic action of colloidal metallic sols was inhibited by traces of such substances as sulphuretted hydrogen and hydrocyanic acid. Freundlich (1926, p. 499) quotes McBain as finding that the activity of platinum sol was reduced 50 p.c. by 0·0005 millimols of hydrocyanic acid per litre.

It was the study of the action of poisons on catalysts that led to the conception of catalysis being due to the presence of specific active patches on contact catalysts. The inhibition of reactions in contact catalysts provides remarkable analogies with drug antagonism, and the analogy suggests that the normal functions of the cell may depend on the presence of certain active patches on the cell surface and that drugs act and antagonize each other by occupying these patches.

Chemical Antagonism

There are an indefinitely large number of toxic substances that react *in vitro* to form an inert compound. The complexity of the reactions that occur ranges from the case of acids and alkalies to that of toxins and antitoxins.

The antagonism of calcium salts and citrates deserves mention because it shows how a large number of variables may result in a fairly simple relation. Clark, Percival and Stewart (1928) studied the response of the isolated frog heart to mixtures of calcium chloride and sodium citrate added to Ringer's solution. They concluded that the citrate acted by reducing the concentration of calcium ions. The Ringer's solution contained phosphates and the ions acted on the surface of the heart where some carbonate must have been present ; therefore the equilibrium between the concentrations of citrate and calcium ions must have depended on extremely complex relations.

The following concentrations were, however, found to produce an equal degree of activity in the frog heart.

M. molar conc. $CaCl_2$.	0·6	1	2	4	8	16
M. molar conc. sodium citrate :						
(i) observed . .	0	0·5	2	4·5	12	30
(ii) calculated from formula . .	–	0·49	1·97	5·19	12·3	28·9

If the concentration of calcium needed to produce the action in the absence of citrate (0·6 m.mol.) be subtracted from the figures for calcium, then an accurate linear relation is found between the logarithm of the two sets of figures and the relation can be expressed by the formula

$$\frac{(\text{m.mol. conc. } CaCl_2 - 0{\cdot}6)^{1{\cdot}1}}{\text{m.mol. conc. sodium citrate}} = 0{\cdot}74.$$

The figures in the table show that the agreement between the observed and the calculated figures is as good as can be expected in the case of biological data. Furthermore the formula is of exactly the same type as those which relate the iso-active concentrations when mixtures of such antagonists as atropine and acetyl choline are studied.

In the case of the antagonism between calcium and citrates enough is known of the physical chemistry of the system to make it unlikely that the formula found has any significance, and the agreement is probably an accident. This example indicates the danger of drawing too many conclusions from the fact that a particular set of figures are fitted by a formula.

Physiological Antagonism

Within certain ranges of concentration the antagonism shown between calcium and potassium ions in a sensitive tissue such as the frog's heart appears very complete. If, for example, the concentration of potassium chloride in the Ringer's solution be trebled, the mechanical response of the heart is at once reduced but can be restored completely by trebling the calcium concentration. The antagonism only extends, however, over a limited range, for if the potassium content be increased sixfold, the heart is not restored with calcium (Clark, 1926A). A similar limitation in the antagonism of these ions was noted by Loeb and Wasteneys (1911) in the case of Fundulas.

Detailed analysis of the action of excess of potassium and of calcium indicates that these ions are not exact antagonists. For example, potassium excess and calcium lack both depress the mechanical response of the heart, but potassium excess interferes greatly with the conduction process, whereas calcium lack produces scarcely any effect on the conduction process even when the mechanical response is nearly extinguished.

The action of morphine and atropine on the pupil is an example of two drugs which produce opposite effects on the same organ, but which act through different channels and are not true antagonists.

Specific Cellular Inhibitions

This term implies that one drug produces an action on a population of cells and that this effect can be inhibited by introduction of another drug, without producing any other marked effects on the cells. When two drugs produce actions which are opposed, it is safer to describe them as physiological antagonisms, unless there is definite evidence regarding their mode of action. The term specific cellular inhibitions is reserved for the minority of cases where there is positive evidence that one drug interferes in some manner with the reaction between the other drug and the cell receptors.

Contact catalysts provide a simple example of such actions and they show that the catalytic activity of an apparently uniform surface to be due to the presence on the surface of active patches. Moreover, there is evidence that several types of active patches may be present on one surface. Hence a poison may inhibit one catalytic activity and leave other activities unaffected.

These results can be compared with those obtained by Quastel and his co-workers (1930) on bacteria. They showed that the ferment actions of bacteria were due to active patches on the bacterial surface and by means of selective poisoning they were able to prove the existence of about twelve different varieties of active patches on the surface of *B. coli*.

Respiratory Pigments

The action of poisons on the uptake of oxygen by hæmochromogens has been studied in considerable detail and provides interesting examples of the types of antagonism that may arise. According to Anson and Mirsky (1930) the combination of hæm and a nitrogen compound constitutes a hæmochromogen, and to this class belong hæmoglobin, cytochrome and other unidentified compounds. They believe that the respiratory ferment of cells is not cytochrome but some other unidentified hæmochromogen.

The simplest antagonism is that between oxygen and carbon

monoxide. These two gases both form reversible compounds with hæmochromogens and compete for the same receptors. In the case of a solution of hæmoglobin in water the following equation applies: $Kx = \frac{y}{100 - y}$ or $y = \frac{100Kx}{1 + Kx}$ where $x =$ concentration of gas and $y =$ percentage of hæmoglobin combined. K is a constant which is 240 times greater for carbon monoxide than for oxygen in the case of human hæmoglobin. The amount of human hæmoglobin combined with carbon monoxide is given by the formula $K\frac{\text{Press.CO}}{\text{Press.O}_2} = \frac{\text{p.c. COHb}}{\text{p.c. O}_2\text{Hb}}$ (Douglas and Haldane (1912) and Hartridge and Roughton (1923) where K is a constant with a value of about 200.) Warburg and Negelein (1928) showed that the combination of these gases with the respiratory ferment of yeast followed the same formula, but in this case the value of K was about 10.

The specific action of cyanides in inhibiting oxygen uptake by cells is well known, and it has already been mentioned that they are particularly powerful inhibitors of contact catalysts. According to Anson and Mirsky (1930) the following type of reaction occurs:

Hæmochromogen (hæm + protein) + cyanide
= (hæm + cyanide) + protein.

This is a more complex type of antagonism than that previously considered. The combination between cyanide and respiratory ferment inactivates the latter as an oxygen acceptor, but the oxygen and cyanide combine with different receptors.

The narcotics exert a third type of action, for they do not interfere with the respiratory ferment, but they inhibit the activity of some reducing ferment (Keilin, 1925), hence the respiratory ferment remains oxidized, but the cell cannot obtain the oxygen.

The three best-known classes of drugs which inhibit oxygen uptake act therefore in three different ways on the cellular respiratory mechanism.

Antagonism of Narcotics and Cyanides

Warburg (1921) concluded that the aliphatic narcotics produced a purely physical action on cells. They were adsorbed

on the cell surfaces at which biological reactions such as oxygen uptake occurred and the adsorbed layer acted as a blanket and prevented access of other substances. More recent work suggests that the action of narcotics is more specific since they inhibit reduction of the respiratory ferment, but do not prevent its oxidation (Keilin, 1925).

The end effect of narcotics and of cyanides is to decrease cell respiration, but yet the presence of narcotics does not increase but rather inhibits the action of cyanide. This was shown by Warburg (1911) in the case of sea-urchin eggs. Lipschitz and Gottschalk (1921) have shown the same to be true for the action of narcotics and cyanides on the power of minced frog's muscles to reduce di-nitro-benzole. Two narcotics added together gave a purely additive action, but combination of narcotics and cyanides produced no additive effect, and sometimes the combined action was less than the action of either alone. On the other hand, Cook, Haldane and Mapson (1931) found that phenyl urethane alone produced only a slight reduction in the oxygen uptake of *B. coli*, but it potentiated the action produced by cyanide. For example, the figures these authors found show the following reduction, in the oxygen uptake of *B. coli* in the presence of lactate KCN 10^{-3} Mol. caused a 40 per cent. reduction ; and phenyl urethane (about 1/6 saturated) a 22 per cent. reduction ; but the two drugs combined gave a reduction of about 85 per cent. This is a most remarkable contrast to the results obtained by the authors who worked with vertebrate tissues. Warburg's theory of cyanide and narcotic action implied that the two drugs competed for the same receptor but that cyanide fixation produced a much more intense depression of respiration. This theory appears to be untenable and it would appear that cyanide and narcotics act at two separate points on a chain process. This fact would imply that the additive effect would be feeble, but the antagonism observed can only be explained by supposing that narcotics in addition to inhibiting dehydrogenation also antagonize the fixation of cyanides by the cells. Our present knowledge appears to be inadequate to suggest any probable explanation of the potentiation effects observed. The probable complexity of the respiratory system of cells is indicated by the fact that Cook, Haldane and

Mapson believe that there are at least three different kinds of oxygenase present in *B. coli*.

The action of drugs on cell respiration is of interest because it has been studied in considerable detail. The general result has been to reveal an increasing complexity of the system studied. The antagonism between oxygen and carbon monoxide appears to be one of exceptional simplicity and most other examples are much more complex. The antagonism of other drugs appears in many cases to be simpler than most of the cases described, but the reason for this is probably that few systems have been studied with such care as have the respiratory ferments.

Atropine and Acetyl Choline

Cushny (1915) measured the antagonism of atropine and pilocarpine on the salivary secretion of dogs and found that the ratio of pilocarpine to atropine necessary to oppose its action remained the same, one part of atropine to 10 of pilocarpine, to reduce the effect of the latter by more than 90 per cent. Le Heux, van Leeuwen and Brocke (1920) confirmed Cushny's conclusion on the isolated rabbit-gut. Their results agree with the formula Conc. pilocarpine/(conc. atropine)^n = constant. In one experiment the value of n was 1·4. The amount of atropine needed to antagonize pilocarpine varied greatly in different specimens of gut, but well-marked antagonism was noted with a ratio of 1 to 1000. These workers showed that Jeude (1918) was wrong in his conclusion that the amount of action produced by these drugs on the isolated rabbit-gut depended on the quantity of drug, for they found that the action depended wholly on the concentrations.

The author (1926B) investigated the antagonism of atropine to acetyl choline as regards the action of the latter drug in depressing the isolated frog heart and producing contracture of the isolated frog *rectus abdominis*. Waller (1908) had described the latter effect in the case of atropine and muscarine. With both preparations the author (1926B) found that the effects produced by acetyl choline over a 50,000-fold range of concentration could be inhibited completely by atropine. Fig. 55 shows the type of result obtained. The general formula that fits the results is as follows:

Let C_{AC1} and C_{AC2} be the respective concentrations of acetyl choline that produce an equal effect respectively alone and in the presence of a concentration of atropine C_{AT}.

$$\text{Then } \frac{C_{AC2} - C_{AC1}}{(C_{AT})^n} = \text{constant.}$$

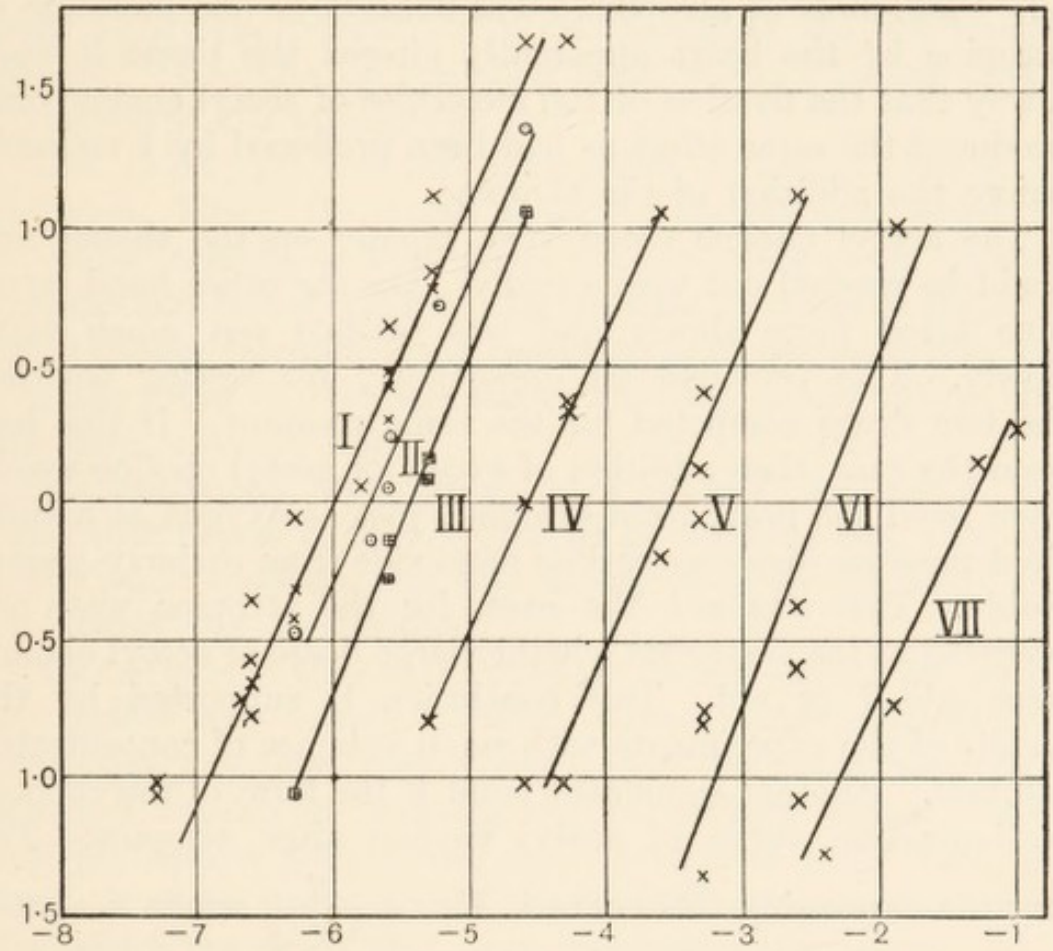

Fig. 55.—The antagonism by atropine of the action produced by acetyl choline on the frog's isolated heart.

Abscissa : logarithm of molar concentration of acetyl choline (x). Ordinate : logarithm of $\frac{y}{100 - y}$ (y = per cent. reduction of isochoric response of heart).

The curves show results with the following molar concentrations of atropine : I, no atropine ; II, 10^{-8} ; III, 10^{-7} ; IV, 10^{-6} ; V, 10^{-5} ; VI, 10^{-4} ; VII, 10^{-3}. (Clark. 1926b.)

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In the case of the frog heart $n = 1{\cdot}1$, and in the case of the *rectus abdominis* $n = 1{\cdot}5$. In these experiments the volume of fluid was large in comparison with the volume of the tissue and the action depended wholly on the concentra-

tions. When minute quantities of concentrated solutions were used similar results were obtained. In these experiments it was found that the response of a 15 mgm. heart strip was reduced 50 per cent. by the addition of 10^{-10} gm.-mols of acetyl choline and that the same effect was produced by about 10^{-8} gm.-mols of acetyl choline in the presence of 10^{-10} gm.-mols of atropine. The fixation of one molecule of atropine by the heart apparently altered the tissue in such a way that the fixation of 100 molecules of acetyl choline only produced the same effect as had been produced by 1 molecule before the addition of the atropine.

The acetyl choline acted very rapidly on the tissues and could be washed out very rapidly. On the other hand, atropine acted more slowly and was washed out much more slowly. This provided an opportunity for testing whether the two drugs competed for the same receptor. If this had been the case, then addition of excess of acetyl choline would have freed the heart from atropine, just as oxygen at a sufficient pressure displaces carbon monoxide from carboxy-hæmoglobin. This was not the case, for the atropine wash-out occurred at the same rate whether large doses of acetyl choline were added or not. This conclusion is supported by the results of the experiments with small volumes of concentrated solution. Another significant point is the form of the concentration-action curve of acetyl choline after atropine. The formula expressing this curve is $Kx = \frac{y}{A + y}$ where $x =$ concentration, $A =$ maximum action observed with highest concentrations and $y =$ action produced. In the frog's heart there is an individual variation regarding the value of A, for in some hearts this is 100 per cent. whilst in others it may be as low as 80 per cent. inhibition. The addition of atropine does not alter the value of A, but merely alters the value of K, so that a series of parallel curves are obtained (cf. Fig. 55).

The action of acetyl choline is affected by changes in the ionic content of the Ringer's fluid and in particular is antagonized by increased alkalinity (Clark, 1927). In this case, however, the chief change is a reduction in the value of A, as is shown by the following figures:

Reaction of Ringer's fluid *p*H . . .	7·5	9·0
Maximum per cent. inhibition produced by high concentrations of acetyl choline . .	90	40
Molar concentration of acetyl choline that produces half maximum action. . .	2×10^{-8}	6×10^{-8}

Alkalinity therefore appears to antagonize acetyl choline by rendering a portion of the cell receptors irresponsive to the drug, whilst the remainder of the receptors are not greatly affected. Atropine, on the other hand, reduces the sensitivity of the whole cell population.

Cook (1926) studied the antagonism of acetyl choline by methylene blue. He found that the results were fitted by the same formula as that given for the antagonism between atropine and acetyl choline. In the case of atropine the ratio acetyl choline/atropine that results in a 50 per cent. response is of the order of 100, whereas in the case of methylene blue this ratio is nearly unity.

The methylene blue action is of interest because it was found that it was rapidly reversible by washing out, although at the same time the dye was slowly fixed by the heart and this action was irreversible.

The methylene blue could produce its full antagonist action long before any visible staining of the heart occurred and alternatively a heart deeply dyed could be made susceptible to acetyl choline by washing out with clean Ringer's fluid. This provides a simple and fairly complete proof that the action of these drugs occurs on the cell surfaces and is unaffected by the drugs that have penetrated the cells.

Adrenaline-Ergotamine Antagonism

Quantitative measurements of this antagonism on the rabbit's uterus were made by Mendez (1928), who found that the concentrations of drugs which resulted in an equal response could be expressed by the formula :

$$\frac{C_{AD2} - C_{AD1}}{(C_{ET})^n} = K$$

where C_{AD1} and C_{AD2} are the concentrations of adrenaline that produce the response alone and in the presence of ergotamine at a concentration of C_{ET}. Fig. 56 shows the concentrations of adrenaline and ergotamine which when mixed produce equal effects both in the rabbit's uterus and in various other

tissues. In the case of the rabbit uterus $n = 1$ and $K = 40$, but the value of unity obtained for n in this case has no general significance, for in the case of the vas deferens of the guinea-pig n equals 0·5. In many tissues n is approximately unity, but there is a very large variation in the value of K.

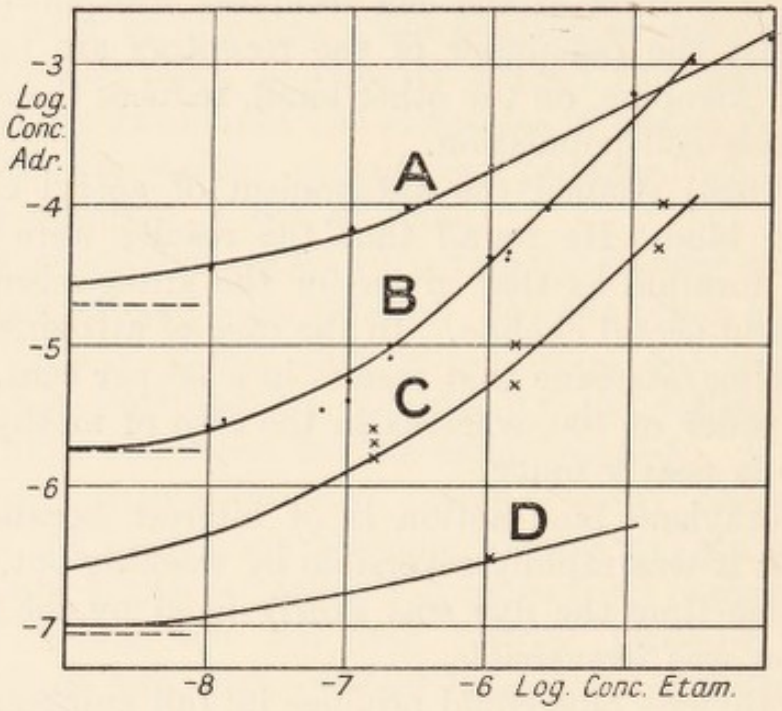

Fig. 56.—The antagonism by ergotamine of effects produced by adrenaline on various isolated tissues.

ABSCISSA : logarithm of molar concentration of ergotamine (C_E). ORDINATE : logarithm of molar concentration of adrenaline required to produce a certain selected effect.

The curves are drawn to the general formula : $\frac{C_{A1} - C_{A2}}{C_E} = k$. C_{A1} and C_{A2} are the concentrations of adrenaline needed to produce an equal effect in the presence of and in the absence of ergotamine respectively. The dotted horizontal lines indicate values for C_{A2}. The values of C_{A2} and of k are different in each curve.

The curves measure the following responses. A. Contraction of vas deferens of guinea-pig. (Mendez. 1928.) B. Contraction of rabbit's uterus. (Mendez. 1928.) C. Inhibition of rabbit's ileum. (Nanda. 1930.) D. Inhibition of rabbit's colon. (Nanda. 1930.)

The following values for K have been obtained in rabbit's tissues :

	K
Contraction of uterus (Mendez, 1928)	40
Inhibition of pendulum movements of ileum (Nanda, 1930) .	7
Inhibition of tonus of duodenum (Nanda, 1930) . . .	2
Inhibition of tonus of colon (Nanda, 1930)	0·02

These figures show that in the case of the uterus there is a physiological balance between adrenaline and ergotamine when the ratio of concentration is 40 to 1, whereas in the colon this ratio is about 1 to 50. This great variation in the intensity of antagonism implies that this cannot be due to any simple chemical reaction between the drugs and some cell receptors.

Summary

The examples of drug antagonism discussed in this chapter serve chiefly to illustrate the extreme complexity of the reactions that occur between drugs and cells. The development of knowledge regarding the action of drugs upon the oxygen uptake of cells is of particular interest. This process was at first thought to be simple. Warburg postulated a single form of oxygen receptor, namely an iron compound, and put forward an attractive and simple hypothesis, founded on quantitative measurements, of the manner in which oxygen, carbon monoxide, cyanides and narcotics competed for this type of receptor. More detailed analysis on simpler systems has shown that the oxygen uptake of cells can be effected by a variety of processes and that each of these involves a chain of catalytic reactions. Poisons may act selectively on any or all of these processes, and when two poisons act on a single process they may inhibit it at different points in the chain of reactions.

There is no reason to assume that other forms of cellular activity are simpler than the oxygen uptake. Imperfect knowledge appears therefore to be the most probable reason for any apparent simplicity in processes of drug antagonism.

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CHAPTER XII

QUANTITATIVE MEASUREMENTS OF THE ACTION OF RADIATIONS

General

The mode of action of radiations, or of other physical processes such as heat, upon living tissues may be represented as follows :

A. Radiation.
B. Primary chemical change.
C. Secondary chemical change.
D. Biological response.

The probable complexity of the whole process is indicated by the fact that the detailed nature of the photo-chemical changes produced by light in a silver halide emulsion is not yet known exactly, and it seems reasonable to assume that the response of a living cell is far more complex than that of a particle of silver bromide.

The absorption of a quantum of radiation activates atoms, and this activation inaugurates a chain of physico-chemical processes. The activation is a temporary process, whilst the secondary processes may be either reversible or irreversible. This latter distinction is of considerable importance in the case of biological reactions, because quantitative measurements are much easier when irreversible changes occur. For example, the changes induced by visible light in the retina are rapidly reversible, and hence although the amount of change produced at any moment can be measured accurately, yet the total amount of change produced in a given period cannot be measured directly. Measurements of the latter type can, however, be made in the case of light acting on chlorophyll because in this case it is easy to measure the total amount of carbon dioxide assimilated. The destructive effects produced on tissues by ultra-violet light, X-rays, beta rays and

gamma rays are to a large extent irreversible and hence are convenient for the measurement of the relation between the amount of irradiation and the amount of response.

The quantitative relations between the amount of irradiation and the action produced upon living cells show a close parallel to those found in the case of drugs. Not only are similar relations found in the two cases, but similar difficulties are met with in regard to their interpretation. The mode of action of radiations upon living tissues is too large a subject to be treated as a whole in this chapter, but certain problems will be considered in order to determine how far they can be explained by the hypotheses adopted in the case of the actions produced by drugs.

In the first place it is necessary to consider certain general problems such as the applicability to living cells of the Bunsen-Roscoe law and the possibility of the occurrence of graded effects.

The Application of the Bunsen-Roscoe Law to Biological Systems

This law states that if a given intensity of irradiation (I) acting for a certain time (t) produces a certain effect (E), then the same effect will be produced, however much I and t are varied, provided that the product It remains constant. The law does not state that the effect varies directly as the product It, and indeed in many cases the effect is known to vary approximately as the logarithm of It.

Even in the case of a simple inorganic system such as the photographic plate, the Bunsen-Roscoe law has been found to hold only over a certain range of intensity of illumination, and not to hold with very low intensities. Trivelli and Loveland (1930) explained this fact by the assumption that much of the primary light effect was wasted with weak illumination on account of the occurrence of some reverse reaction.

The effects produced by radiations on living cells may be divided into two main classes, namely physiological and injurious actions. The action of light on the retina and the action of X-rays on cells are examples of these two classes. The Bunsen-Roscoe law may hold over a very wide range in the case of physiological effects, but in the case of injurious effects it does not hold below a certain intensity because of the occurrence of repair processes in the living cells. As in

the case of drugs, so in the case of irradiation there is a minimum concentration or intensity needed to produce any response, and as this figure is approached the product It increases. The action of radiations on living cells is to a certain extent an all-or-none process in that cells show a certain power of recovery to minor injuries but cannot recover when the injury exceeds a certain degree. The action of radiations is therefore not accurately cumulative, and the true relation between amount of irradiation and effect is more likely to be found with short exposures to intense irradiation, than with long exposures to feeble irradiation.

Bovie and Hughes (1918) measured the rate of recovery of *Paramoecium caudatum* from the effects of ultra-violet light. Irradiation for 8 seconds caused cytolysis of 57 p.c. of the population, but irradiation for 2 periods of 4 seconds each at an hour's interval caused cytolysis of only 6 p.c. Shorter intervals resulted in figures intermediate between 57 and 6 per cent., but a measurable amount of recovery occurred within 10 minutes.

In the case of X-rays, Crowther (1926) found that in order to kill *Colpidium* it was necessary to give the lethal dose (80,000 r.) within 20 minutes. If the same dose were given over a longer period, the cells appeared moribund but recovered after a few hours. Moreover irradiation with sub-lethal doses on successive days produced no permanent injury. Packard (1927A) found the same to be true for the sensitive *Drosophila* eggs in which the median lethal dose was 180 r.

Strangeways and Fell (1927) irradiated young chick embryos (20–25 hours' incubation) with X-rays and found that temporary injury followed by partial recovery occurred. In the case of later embryos there was no recovery because the irradiation destroyed the circulation, and the embryos died of asphyxia.

The apparent effect of X-rays on cells also can be affected by the temperature at which the cells are kept after exposure. Packard (1930) found that if *Drosophila* eggs after irradiation were divided into two batches, which were kept at 28° C. and 17° C. respectively, the latter showed a much higher survival rate. Strangeways and Fell (1927) noted a similar effect with tissue cultures.

Canti and Spear (1927) studied the action of gamma rays of radium on tissue cultures and found that at a distance of 1·0 mm. a 50 p.c. reduction in mitosis was produced in 12 minutes, but that 600 minutes' exposure was needed to produce the same effect at 5 mm. This result represents only a moderate deviation from the Bunsen-Roscoe law, but they also showed (1929) that a small amount of irradiation caused an initial reduction in mitosis that was followed by a compensatory increase, whereas a larger dose caused a decrease without a compensatory after-effect.

Braun and Holthusen (1929) concluded that the action of X-rays on ascaris eggs was influenced by the oxygen supply. In anoxybiosis there was a full cumulative effect, i.e. the same amount of radiation produced the same effect when the intensity was low and the time long as when the intensity was high and the time short. When oxygen was present this relation was not maintained owing presumably to repair processes occurring in the cells.

These results all agree in demonstrating that the Bunsen-Roscoe law is only approximately true in regard to the action of radiations on living cells, because these possess powers of repair and, unless the irradiation is of sufficient intensity, the repair process can partly or even completely balance the destructive effect.

Two other fundamental laws of radiant action are that the effect produced is due only to radiations that are arrested and that the intensity of irradiation varies inversely as the square of the distance from the source of irradiation. Both these laws appear to be applicable to the case of radiations acting on living tissues. In the case of the first law some doubt arises as to the effect produced by radiations that are not arrested but only scattered, but there is no quantitative evidence on this point. A number of workers have shown that the effects produced by radiations is inversely proportional to the square of the distance of the source, provided that the intensity of irradiation is not reduced to a level at which the Bunsen-Roscoe law ceases to hold good.

Packard (1927A) showed in the case of *Drosophila* eggs that the dose needed to kill 50 p.c. of the population, namely 180 r., remained the same when the intensity was varied by

altering the distance of the cells from the X-ray tube, or by altering the amperage, or by introducing filters. Canti and Spear (1927) studied the action of gamma radiation on cell division in tissue cultures and their results show that $t \times \frac{1}{d^2}$ is approximately constant except for the longest distances (t = duration exposure and d = distance of cells from radium).

Gradations of Effects Produced

The destructive action of radiations on cell populations is usually measured as an all-or-none effect, i.e. the proportion of cells which have sustained a certain injury is counted. This is a somewhat artificial system of measurement and obscures the fact that the irradiation can produce gradations of injury. Holthusen and Zweifel (1932) analysed the action of radiations on ascaris eggs. They measured in the first place the relation between the amount of irradiation and the per cent. of the population which showed abnormal development, and secondly counted the proportions of the population that were arrested in various stages of development (1–8 cell stage, many cell stage, morulæ, abnormal development in post morula stage).

Their results show that the relation found between the amount of irradiation and the effect produced depends upon what end-point is chosen.

For example, the following figures were obtained for the incidence of impaired development with various amounts of irradiation.

Irradiation in r.	1,700	15,000	44,000
Per cent. population in which the development was arrested at or before the morula stage	29	69	98

In this case the effect produced varies approximately as the cube root of the amount of irradiation. A further qualitative analysis showed, however, that with 15,000 r. dosage only 2 p.c. of the eggs were arrested before the morula stage and the remainder were arrested in that stage. On the other hand a dose of 44,000 r. arrested 86 p.c. before the morula stage. Hence if the relation between the amount of irradiation and the incidence of failure to arrive at the morula stage be measured the result suggests an all-or-none effect.

A comparison of the effects produced by X-rays with those produced by ultra-violet light shows that in this case also the ratio of activity found between these two forms of irradiation depends entirely on the end-point chosen. The effects of 1700 r. and 3 min. U-V irradiation are very similar, and about 20 times these doses cause 100 p.c. injury in both cases, but a qualitative analysis of the results produced by these larger doses shows that the ultra-violet light produces 96 p.c. of the severest type of injury as compared with the 1 p.c. produced by the X-rays.

Holthusen and Zweifel found that there was a general resemblance in the qualitative effects produced by soft and hard X-rays and by beta and gamma rays of radium, whereas the effects produced by ultra-violet light were similar to those produced by heat. The work of Redfield and Bright (1918) provides another example of a graded effect produced by short radiations. These authors studied the effects produced by beta rays of radium upon *Nereis* eggs, and showed that a thickening of the membrane was produced that could be measured quantitatively. These results are discussed on p. 260.

Stimulant Action of Irradiation

The question as to whether sub-lethal exposures to X-rays or radium stimulate cells is a matter of great practical importance. The evidence, which is very conflicting, has been summarized by Packard (1931), who concluded that: "The primary effect of radiations was always to injure cells. The cells might recover from this injury completely and the degeneration products produced by the injury might temporarily accelerate certain processes such as mitosis." Canti and Spear (1929) found that the effective doses of gamma rays caused a primary depression of mitosis, but that over a certain range this primary effect was followed by a temporary increase in the rate of division. A diphasic effect such as this might easily be mistaken for true stimulation.

Kinetics of the Effects produced by Radiations

The primary photochemical or radiochemical effects produced by radiations in living tissues are followed by a chain of secondary chemical processes which result in biological effects. Moreover the primary biological effects produced may cause

secondary effects. For example, Strangeways and Fell (1927) found that the effects produced by irradiation on the chick embryo more than 2 days old were very largely due to the irradiation destroying the circulation and thereby causing asphyxia. The relation between initial dosage of radiation and the time before a given biological effect appears is therefore complex in all cases and may be extremely complex. The duration of the latent period can be studied most conveniently in cases where the duration of irradiation is short compared with the latent period. Rahn (1931) quotes figures obtained by Woertz for the relation between ultra-violet irradiation of chick's red blood-corpuscles and the appearance of hæmolysis. The results, which are given below, show that the product of the exposure and the duration of the latent period is approximately constant.

Quantity of U-V irradiation (I) (duration in minutes)	4	8	16
Time in hours at which 50 per cent. hæmolysis appeared (t)	24	11·2	5·5
It	96	90	88

The relations found by Waller (1931) between U-V irradiation of the skin and time before erythema appears are more complex.

Time of exposure in minutes . .	3	6	9	12	15
Latent period in hours . . .	2·6–3·5	2·3–2·5	1·75–2	2·5–1·5	1·3

In this case there appears to be a minimum latent period of about 1 hour.

When the duration of exposure and the duration of the latent period are of similar lengths, the results are more difficult to interpret. For example, Martin and Westbrook (1930) studied the production of the browning of leaves by ultra-violet light. They obtained the following figures :

A. Time of irradiation by U-V light in minutes . .	12	10	8	6	4	3	2	1	$\angle 1$
B. Latent period in minutes after termination of irradiation	0·5	3	6	12	19	27	42	68	∞

In this case the effect obviously began as soon as the irradiation started. If as a first approximation the latent period

is taken as $\frac{1}{2}A + B$, then It, i.e. $A(\frac{1}{2}A + B)$, gives an approximately constant product.

The temperature coefficient $Q/10$ of the latent period of effects produced by irradiation lies usually between 2 and 3 (Martin and Westbrook, 1930). This is natural because the length of the latent period depends on the rate at which secondary chemical processes proceed.

The latent period of visual processes resembles the case of the browning of leaves by ultra-violet light in that there may be no great difference in the lengths of the latent period and of the exposure. For example, Hecht (1931) obtained the following times in the case of *Ciona* :

Duration of exposure (in seconds) . . .	0·16	0·29	0·39	0·49	0·58
Latent period (in seconds) after exposure . .	3·15	2·15	1·69	1·42	1·26
Reciprocal of latent period . . .	0·318	0·465	0·59	0·704	0·793

Hecht concluded that there was a linear relation between the duration of the exposure and the reciprocal of the latent period. He also found a linear relation between the logarithm of the intensity of the illumination and the reciprocal of the latent period.

Adrian and Matthews (1929) found in the eel's eye a linear relation between the quantity of light and the reciprocal of the reaction time. Hecht and Adrian and Matthews agree that the temperature coefficient of the latent period is high ($Q/10 > 2$).

In the case of the time relations of drug actions it was shown that usually the time measured was a mixture of the time taken for the drug to penetrate the tissues, the time taken by a chain of chemical processes and the latent period of the tissue. As has been shown, there is a similar confusion in some cases of physical excitation, but in those cases where the duration of exposure is small in comparison with the duration of the latent period the two factors can be distinguished clearly. The relation between duration of latent period and intensity of stimulus found in most cases is that the product of the two is approximately constant. Moreover, the temperature coefficient of the latent period has usually

a value characteristic of chemical rather than physical processes. These facts are consistent with the hypothesis that the latent periods of the forms of physical excitation considered measure the duration of a series of secondary chemical processes.

Individual Variation in Response to Radiations

Radiations show a remarkable selective action on tissues. The doses needed to kill different cells vary enormously. For example, 50 p.c. of *Drosophila* eggs are killed by 180 r. (Packard, 1931), whereas 1500 r. are needed to produce the same effect on ascaris eggs (Holthusen and Zweifel, 1932), and 80,000 r. to produce this action on *Colpidium* (Crowther, 1926). Bergonie and Tribondeau (1906) concluded that the sensitivity of cells to radiations varied directly with the reproductive capacity of the cells and inversely with their degree of differentiation. This principle will not explain the contrasts mentioned above, but is true in many cases. For example, embryonic heart-tissue is fairly sensitive to radiations, whereas adult heart-tissue is extremely insensitive.

There is a very marked individual variation in all cell populations on which the action of radiations has been measured. There is indeed a great practical difficulty in obtaining material of adequate uniformity. For example, Holthusen (1927) noted that with ascaris eggs it was necessary to compare results obtained with eggs from a single individual because the eggs of one worm might be twice as sensitive as those of another worm. Packard (1927B) found that the resistance of *Drosophila* eggs increased rapidly with age and that in order to obtain consistent results it was necessary to use eggs of exactly similar age.

Gates (1929) studied the action of ultra-violet light on *S. aureus* and found that the dose needed to kill 28-hour cultures was nearly twice that which sufficed to kill 4-hour cultures. Individual variation was shown in Chapter VI to be one of the main difficulties attending the biological measurement of the potency of drugs, and it appears to be an equally important factor in regard to the response of cells to radiations.

Influence of Wave-length on Biological Action of X-rays

Differences in wave-length and consequent differences in

penetrative power are believed to influence greatly the action produced by X-rays in clinical work. It was expected that the same differences would be found in quantitative measurements of X-ray action made on populations of cells. A number of workers concluded that soft X-rays produced a more intense action than hard X-rays, but the most recent and extensive work has failed to confirm this opinion. Wood (1924, 1925) studied the action of radiations on finely-minced tumours and found that equal lethal effects were produced by equal doses of X-rays with wave-lengths of 0·68 and 0·22 Å.

Packard (1929) worked with *Drosophila* eggs and found that 50 p.c. of the population was killed by 180 r. whether the rays were filtered gamma rays from radium (0·02 Å), or by X-rays of the following types: very hard (0·15 Å), hard (0·22 Å), soft (0·68 Å), or very soft (1·70 Å). Other workers who have confirmed the biological equivalence of X-rays of varying length are Braun and Holthusen (1929; ascaris eggs) and Glocker, Hayer and Jüngling (1929; beans).

The Relation between Amount of Irradiation and Graded Effects

Very few of the effects produced by ultra-violet light and X-rays on biological material are suitable for the measurement of the relation between quantity of irradiation and the amount of action produced, but some of the effects produced by visible light are suitable for this purpose. The information required is the relation between the amount of irradiation and the amount of chemical change produced in some material.

The Action of Light on Photosensitive Systems

The relations between intensity of illumination and sensation produced in the eye are of particular interest because the retina is a photosensitive tissue capable of responding to very feeble stimuli and also capable of distinguishing gradations of illumination over an extremely wide range of intensity. The theoretical significance of the relations found between the intensity of illumination and the effect produced are however uncertain, and therefore it is worth while considering first the relations found in a simpler system, namely the photographic plate.

Harter and Driffield in 1890 showed that the sensitivity of

a photographic plate could be expressed by plotting the logarithm of the exposure against the amount of chemical change produced. The characteristic curve thus obtained is of a sigmoid shape, with a middle portion that is almost linear between 20 and 80 per cent. of the maximum action. (Sheppard and Mees, 1907, p. 282.)

This curve closely resembles in general shape the curves relating the concentration and action of acetyl choline (Fig. 33) and adrenaline (Fig. 35), and also the curves relating the concentration and the incidence of effects of these drugs (Figs. 34 and 37).

The general theory developed by Sheppard and his co-workers is given as follows by Trivelli and Loveland (1930):

" On the surface of the grains of a ripened photographic emulsion are scattered specks of a sensitizing material containing silver sulphide, as has been shown by S. E. Sheppard (1925), and which serve during exposure as nuclei for silver-silver sulphide centres of sufficient size to cause the grains to become developable."

The grains of a photographic emulsion vary in size and the distribution is usually of a highly skew form with a great majority of small grains. The larger grains have the larger specks and it requires more energy from light to convert the smaller specks into centres of sufficient size to produce developability. The developability of any grain is an all-or-none character. Wightman, Trivelli and Sheppard (1923–4) in a series of papers showed that there was a close correlation between the size of grains in emulsions and their sensitivity.

This theory therefore explains the characteristic curve of an emulsion as an expression of the skew distribution of size, and in consequence of sensitivity, in the grains composing the emulsion. The action of light on the individual grain is regarded as a gradual production of a photo-chemical change, whilst developability is an all-or-none effect which results when a certain amount of photo-chemical change has been produced. Sheppard, Trivelli and Loveland (1925) concluded that between 500 and 800 quanta of light were needed to produce developability in a single grain of $1\mu^2$ surface area.

There is general agreement that the developability of halide emulsions depends on light activating certain specks in the

grains. There are, however, two theories regarding the mode of action of the specks. The topochemical theory outlined above regards the specks as centres around which collect the products of a primary photo-chemical action. The alternative theory is that the specks are activated by a single quantum and then produce some form of catalytic action on the grain. This quantum theory implies that the area of the speck is about 1/100,000 of the area of the grain, and that in consequence only a very small proportion of the quanta of light absorbed by the emulsion produces any photo-chemical effect.

The existence of these two rival theories of photo-chemical action is of considerable general interest, because there is a somewhat similar division of opinion regarding the mode of action of radiations on living cells. The topo-chemical hypothesis put forward by Sheppard appears to have the greater amount of experimental evidence in its support and therefore may be accepted for the purposes of the present discussion. This theory implies that the shape of the characteristic curve relating exposure to light and photo-chemical change in a silver halide emulsion is due to skew distribution in grain size, and to the sensitivity of the grains depending on their size.

The characteristic curves of ultra-violet light and of X-rays are probably similar to those of visible light, but the evidence for this is much less complete. Figures given by Clark (1924) show that when ultra-violet light acts on lithophone paint, the relation between duration of exposure and amount of darkening shows an approximate linear relation between effect and logarithmic duration. This relation would agree with the middle portion of the characteristic curve obtained in the case of visible light.

Action of Light on the Retina

Henri and Larguier des Bancels (1912) pointed out the general relation that the logarithm of the intensity of the illumination plotted against the effect produced gave a sigmoid curve with a linear middle portion, and that this was true for the results obtained by the following authors: Koenig and Brodhun, who studied visual discrimination; Haas (1903), who measured the retinal current; Henri and Henri (1912), who measured the phototropic effect of ultra-violet light on cyclops.

There has been a general agreement amongst subsequent workers that the characteristic curve relating the logarithm of the intensity of illumination and the amount of sensation produced follows a sigmoid course. The middle portion of such a curve shows a linear relation between log. I and sensation and therefore is in accordance with the Weber-Fechner law, but there is a general agreement that this law does not express the general form of the curve.

Two explanations have been advanced to explain the curve as a whole, and these are as follows :

(*a*) That the relation depends upon the fact that the light produces a primary photo-chemical change that is rapidly reversible, and the sensation depends upon the amount of this substance present in a changed form at any moment.

(*b*) That the relation expresses a skew distribution of variation in the sensitivity of the receptors.

Hecht (1931) made exhaustive experiments on the response to light by various invertebrates (Mya arenaria, Ciona, bees) and found in these cases the same variation in $\frac{\Delta I}{I}$ that had been noted by previous workers in the human eye. The variation in this ratio as determined by Koenig and Brodhun is shown in Fig. 45. It will be seen that the ratio remains approximately constant while the intensity varies about 1000-fold (i.e. from 50 to 50,000 in the units used in Fig. 45). It was this constancy that led to the belief that the action varied as the logarithmic intensity. When, however, the intensity is reduced below 50, the ratio $\frac{\Delta I}{I}$ rises steadily with falling intensity, and when the intensity rises over 50,000 the ratio also begins to rise.

This U-shaped variation in $\frac{\Delta I}{I}$ proves conclusively that there is no linear relation between the effect and the logarithm of the intensity. It is, however, the variation that would be observed if the relation between intensity and effect followed a rectangular hyperbola. Hecht's explanation is as follows : Light acts on a photo-sensitive substance (S), splitting it into P and A. $S \rightleftharpoons P + A$. The rate of this action varies as intensity I and the amount of S present which equals $(a - y)$,

where a is the amount present in the resting eye and y is the amount transformed. The action is reversible and the reverse process $P + A \rightarrow S$ varies as y^2. Hence $KI = \frac{y^2}{a - y}$.

This formula was found to give an approximate fit to the variations observed in $\frac{\Delta I}{I}$ in the various eyes studied. Adrian and Matthews (1929) studied the electrical response produced in the optic nerve of the conger eel by irradiation of the retina. They considered that their results agreed with Hecht's in indicating that the primary effect produced by light in the retina was a reversible photo-chemical change. There is therefore reasonable evidence for the assumption that the action of light on the eye is due to light acting on a photo-sensitive substance and converting it into a compound which persists only a short time, and that the physiological stimulus (measured either by sensation or by electric variation in the nerve) varies directly as the quantity of compound produced. This relation between stimulus and effect follows a hyperbola, and hence action varies as the logarithm of the concentration over a wide range of intensity.

The relations between illumination and discrimination obtained by Koenig (1897) are shown in Fig. 57. His interpretation of his results is shown in the figure. He concluded that with low intensities of illumination only the rods were stimulated and that there was a linear relation between the logarithm of the illumination and the power of discrimination. Higher intensities of illumination stimulated the cones, and with these also there was a linear relation between the logarithm of the illumination and the power of discrimination, but the slope was much steeper.

Hecht (1931) concluded that the relation between illumination and discrimination really followed a hyperbola. Fig. 58 shows two curves. Curve A was obtained by Hecht from the averages of Koenig's figures; it follows approximately the formula $kI = \frac{y^2}{(100 - y)^2}$. Curve B expresses the results obtained by Hecht in experiments of the visual acuity of bees. This curve follows approximately the formula $kI^2 = \frac{y}{100 - y}$.

Both curves have been reduced to percentages of maximum acuity.

Hecht explained these relations between intensity and effect

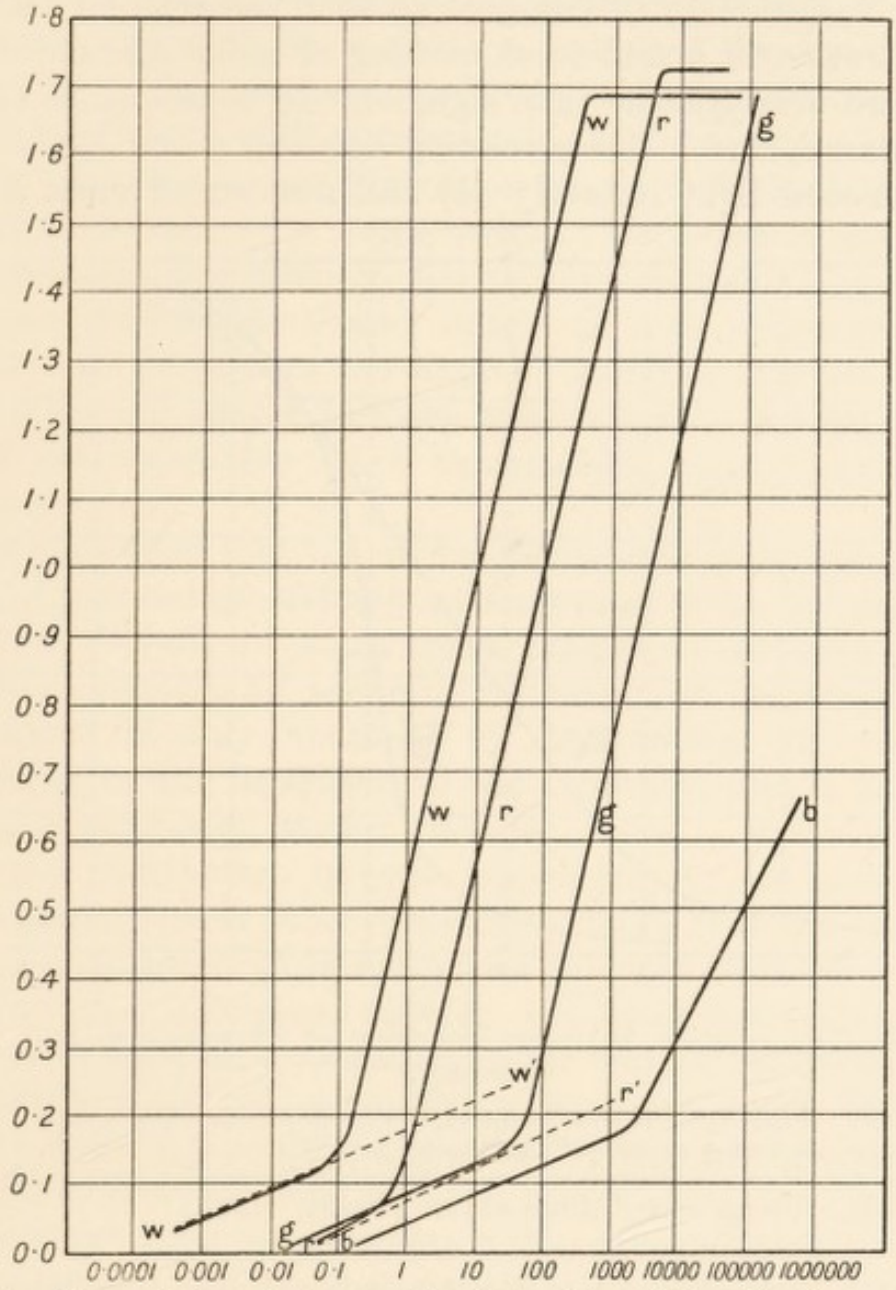

Fig. 57.—The relation between the intensity of illumination and the power of discrimination.

Abscissa: illumination on logarithmic scale. Ordinate: power of discrimination in arbitrary units.

The curves show the results obtained with white, red, green and blue light. (Koenig. 1897.)

as follows. He assumed that the relation between light intensity (I) and amount of chemical change produced at any moment (y) followed the formula $kI = \frac{y^2}{100 - y}$, and that

the units of the visual apparatus (e.g. rods and cones or ommatidia) varied as regards the amount of y that was required to produce stimulation. He assumed that there was a wide variation in this respect so that the relation between concentration of y and total number of units stimulated (E) was more nearly linear than sigmoid. In consequence of this approximation to a linear relation between y and E the relation between light intensity (I) and number of units stimu-

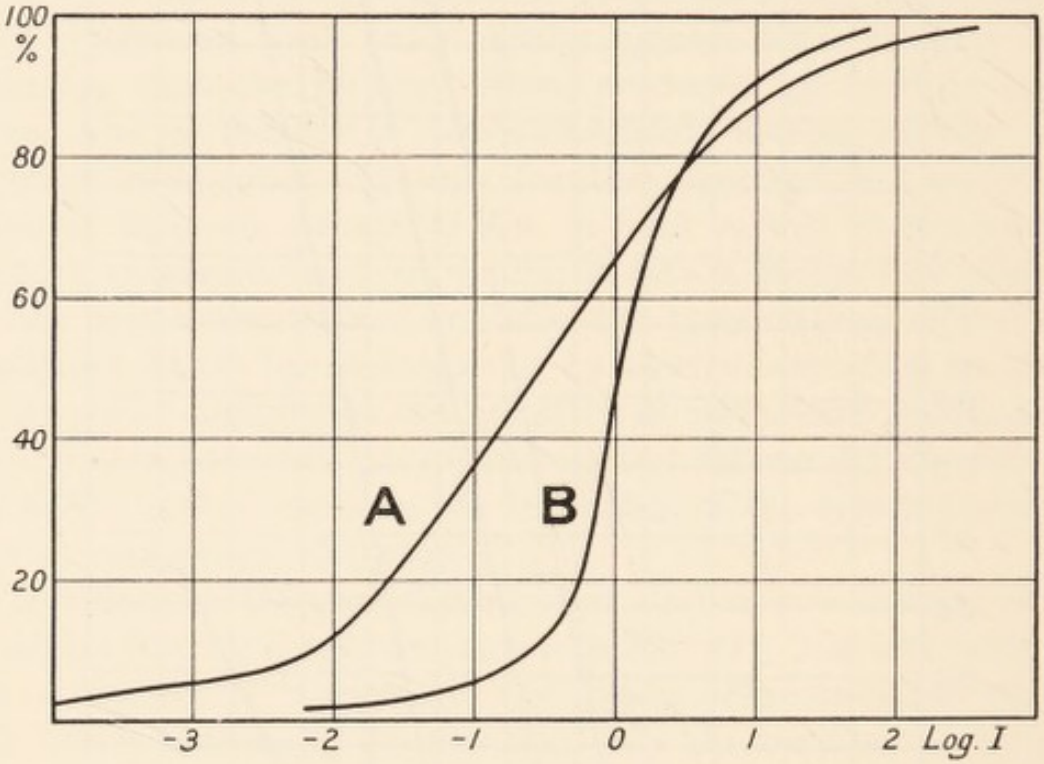

Fig. 58.—The relation between intensity of illumination and visual acuity.

Abscissa : logarithm of the intensity of illumination. Ordinate : visual acuity expressed as per cent. of maximum.
Curve A. Hecht's interpretation of Koenig's figures for human vision.
Curve B. Visual acuity of bee's eye. (Hecht. 1931.)

lated (E) approximated to the formula relating light intensity (I) and chemical change (y). This explanation is of the same type as that adopted by the writer to explain the skew characteristic curves found with insecticides and various other drugs (cf. Chapter VI).

Houston (1932) put forward the alternative hypothesis that all the characteristic curves obtained that related illumination and sensation expressed individual variation of the receptors. He pointed out that the ratio $\Delta I/I$ did not

measure the power of discrimination but that this was expressed by the reciprocal $I/\Delta I$, a value that was greatest when the power of discrimination was greatest. He showed that if $I/\Delta I$ were plotted against log. I, the figures fitted the Gaussian curve of error that is expressed by the formula $y = e^{-x^2/2}$. He therefore interpreted the sigmoid curves relating log. I both with discrimination of illumination and with acuity of vision as an expression of an individual variation amongst the receptors in the retina. The resemblance of the curves under discussion to the curves expressing the relation between concentration and action of such drugs as acetyl choline and adrenaline (cf. Figs. 33 to 36) is obvious. Since the curves are almost identical it is only natural that in both cases the same two rival explanations should have been advanced.

The statement that the curves can be interpreted as integrations of a variation distributed according to the normal curve of error sounds an attractively simple explanation. It is, however, scarcely an accurate statement of the facts. This agreement is only obtained by distributing the variation according to the logarithm of the intensity, and if it is distributed according to the intensity, then a highly skew or J-shaped distribution becomes apparent. In the case of the photographic plate there is direct evidence that the variation of one characteristic of the grains, namely their size, is distributed in this unusual manner, but there is no evidence in support of such an assumption in the case of the retinal cells. Furthermore, it has been shown by Adrian and Matthews (1927) that the stimulation of a limited area of the eel's retina spreads excitation over a much wider area, and this fact seems to preclude the assumption that the individual variation of the retinai units is measured by the sensation produced.

Action of Light on Chlorophyll

This is a biological effect in which it is possible to measure accurately the amount of radiation and the total amount of chemical action induced. Warburg (1919) measured the relation between intensity of illumination and the rate of carbon dioxide assimilation by *Chlorella*, while Emerson (1929) on the same preparation measured the relation between lighting intensity and oxygen production. Their results,

which are shown in Fig. 59, suggest the general relationship $KI^n = \frac{y}{100 - y}$ where y is the amount of action expressed as per cent. of the maximum. The curves show values for n intermediate between 1 and 2.

The relation between exposure and chemical response can be interpreted perfectly simply in this case as the expression of a reversible primary photo-chemical process that is followed

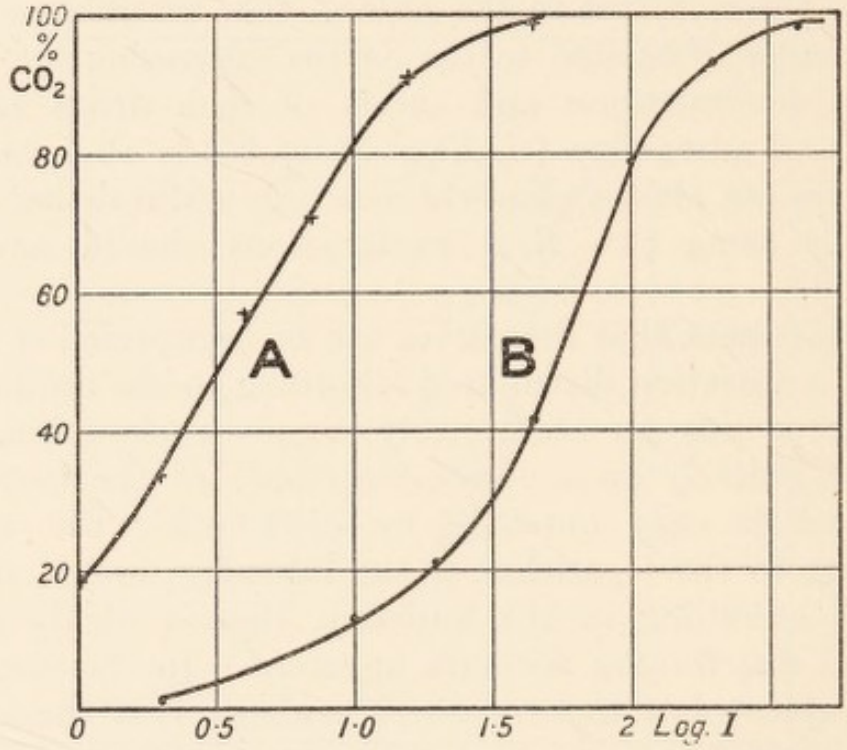

Fig. 59.—The relation between exposure to light and the CO_2 assimilation of the green alga *Chlorella*.

ABSCISSA : logarithm of the exposure to light. ORDINATE : rate of assimilation of CO_2 expressed as per cent. of maximum.
CURVE A. Warburg (1919).
CURVE B. Emerson (1929).

by irreversible secondary processes. It seems to be a straining of probabilities to attempt to interpret a chemical action of this type as the expression of an unusual and unproven form of distribution of variation amongst some hypothetical receptors.

The relations between the intensity of illumination and the effect produced on *Chlorella*, on the retina and on the photographic plate show a resemblance that is striking. The characteristic curve in the case of *Chlorella* appears almost certainly to be an expression of a reversible photo-chemical

process. The evidence available regarding the characteristic curve of silver halide emulsions is in favour of the view that this expresses the individual variation in the size and sensitivity of the grains in the emulsion. In the case of the retina the evidence is inconclusive.

The writer favours the opinion that all the curves under discussion obtained with biological material are expressions of a reversible photo-chemical change. This hypothesis provides a rational physico-chemical basis for the effects observed, and does not involve the unproven assumption of an unusual form of distribution of variation in the receptors.

In Chapter VII it was shown that the curves relating the concentration and action of acetyl choline and certain other drugs probably expressed a reversible chemical reaction, but that they might express some form of individual variation. Curves of a very similar type are found to relate the intensity of illumination and the effect produced on a variety of living and non-living systems. Unfortunately this resemblance does not help in the least to solve the problem as to the nature of the curves obtained with drugs, for the characteristic curves obtained with irradiations appear to express individual variation in the case of light acting on the photographic plate and to express the progress of a reversible reaction in the case of light acting on chlorophyll.

Action of Radiations on Enzymes

Many enzymes are destroyed by radiations and their rate of destruction has been measured quantitatively. Hussey and Thompson measured the action of beta radiation on trypsin (1923), on pepsin (1924) and on invertase (1926A). Clark and Northrop (1926) measured the action of soft X-rays on trypsin. Hussey and Thompson also measured the action of ultra-violet light on pepsin (1926B) and amylase (1932). All these workers agree that the rate of destruction of ferments follows the course of a monomolecular reaction, i.e. a constant proportion of the ferment present is destroyed in unit time. The same relation is observed with heat inactivation of ferments. There is therefore a linear relation between the logarithm of the quantity of ferment present and the duration of exposure, and there is an exponential relation between the exposure and amount of action.

Graded Effects produced by Short Radiations

Unfortunately the evidence regarding the graded effects produced by short radiations is scanty. Indeed it is so scanty that some writers have assumed that these radiations can only produce all-or-none effects. This assumption already has been shown to be untrue, and there are some measurements of graded effects sufficiently extensive to indicate the nature of the relation between exposure and effect.

Redfield and Bright (1918) measured the quantitative effect of irradiation on *Nereis* eggs. Such treatment causes thickening of the membrane of the egg, and the average amount of thickening was measured. The eggs were exposed to the beta and gamma rays of radium emanation, and the effect was measured 60 minutes after radiation. Their results are puzzling in that they do not agree with the Bunsen-Roscoe law, for the authors found that the same total amount of radiation produced a greater effect with long exposures at low intensity than with short exposures at high intensity. The general relation found was that the effect varied as the logarithm of the intensity, but with low intensities the effect became more nearly proportional to the intensity. The results suggest that the relation between amount of irradiation and amount of action follows a similar curve in biological material as it does in the photographic plate.

The General Shape of Characteristic Curves obtained with Radiations

There is a general agreement that photo-chemical processes are of a highly complex nature, and that the nature of the processes involved can only be analysed in the simplest systems. The theory of the subject in relation to biological processes has been discussed by Risse (1930) and Gudden (1930). It seems quite certain that in all biological systems radiations must produce a complex chain of reactions. The examples quoted in the previous pages show that in many cases the relation between amount of radiation and effect produced approximates to the type of formula $Kx = \frac{y}{100 - y}$. In the case of enzymes their destruction follows a monomolecular course. In all cases, however, the relation between amount of irradiation (It) and effect produced (E) does not follow the

simple relation E varies as It, but more nearly approximates to the relation E varies as log. It.

It seems probable that a wide variety of relations will obtain according to the nature of the secondary processes in the chain of reactions set up by the primary photo-chemical change. There is, however, no reason for assuming any direct linear relation between amount of irradiation and effect produced. Moreover, there appears to be a fair probability for the occurrence of a sigmoid relation between the logarithm of the amount of irradiation and the effect. This curve implies the following approximate relations between effect (E) and amount of irradiation (It) :

Range of effect as p.c. of maximum possible	Relation between E and It
0 to 20	E varies as It
20 to 80	E varies as log. It
80 to 95	E varies as $(It)^{\frac{1}{2}}$ to $(It)^{\frac{1}{4}}$

Characteristic Curves of Deaths produced by Irradiation

The rate of destruction of cells by chemical and physical agents has been discussed in Chapter VIII. The relation between exposure to a chemical agent and percentage of death in the population must be determined by the rate of entry of the drug. The rate of death due to exposure to dry air is presumably determined by a chemical process, namely, loss of water. The rate of death due to heat in the case of a large organism might measure the time taken for the temperature to change, but in the case of a micro-organism the temperature change must be instantaneous and the rate of death must be determined by the rate of the chemical changes induced by the rise of temperature.

In the case of deaths produced by irradiation there are two variables, namely intensity and duration, but as the Roscoe-Bunsen law is approximately true in the case of the biological effects produced by irradiation it is simplest to relate the amount of irradiation (It) with the amount of effect, and to regard the curves obtained as concentration-action curves rather than as time-action curves.

The shapes of the characteristic curves obtained with radiations show, however, a remarkable resemblance to the time-action curves obtained with drugs. In consequence their

interpretation has evoked a controversy parallel to that concerning the significance of time-action curves. Fortunately there is a fair agreement about the shape of the characteristic curves obtained with irradiation. Fig. 60 shows typical results obtained by Packard (1926) with X-rays acting on *Drosophila* eggs. Irradiation of a feeble intensity resulted in a curve that was sigmoid in shape, whilst the more intense irradiation produced a curve that approximated to a logarithmic curve

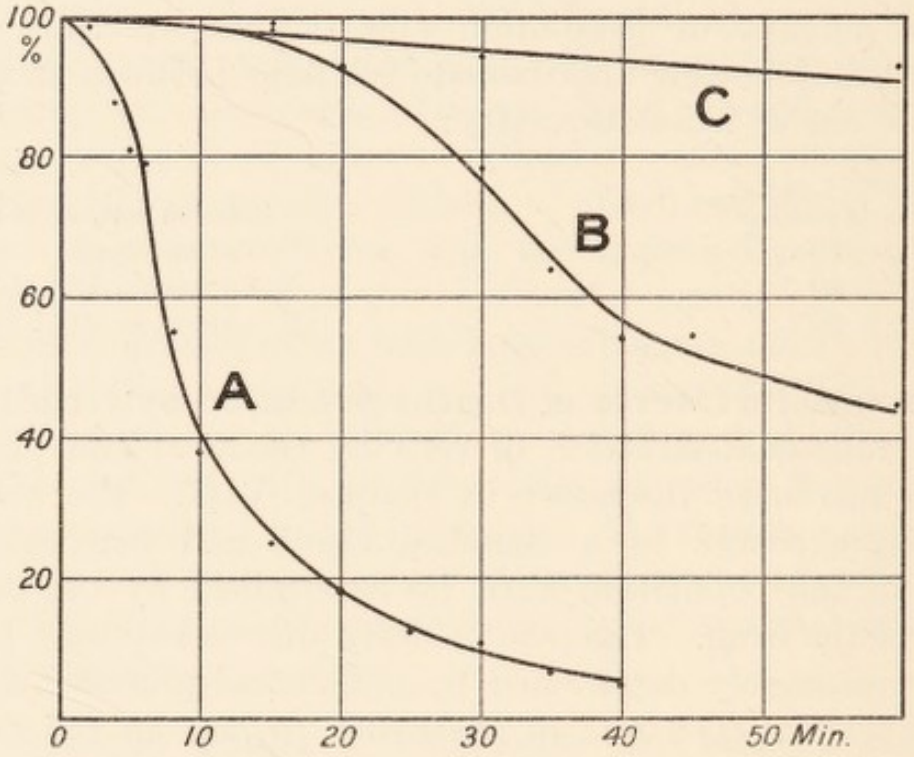

Fig. 60.—The relation between exposure to X-rays and the incidence of death in *Drosophila* eggs.

Abscissa : duration of exposure in minutes. Ordinate : per cent. mortality.

Curves A, B, and C show the effects of irradiation at respective distances of 25 cm., 56 cm., and 79 cm. (Packard. 1926.)

(i.e. a period of lag followed by a linear relation between the logarithm of the survivors and the dosage).

Condon and Terrill (1927) concluded that some of Packard's curves were truly logarithmic, but the latter author (1929) states that these results were probably inaccurate and that probably all these curves are sigmoid in type. This doubt about the shape of the curves is not very important, however, because other observers have obtained curves that were accurately logarithmic. For example, such curves have been

obtained for the destruction of bacteria by cathode rays (Wyckoff and Rivers, 1930), by X-rays (Wyckoff, 1930), and by ultra-violet light (Wyckoff, 1930).

It seems probable therefore that these characteristic curves may vary from the sigmoid to the logarithmic form, and that such variations may occur in a single population when the intensity of irradiation is varied. The controversy does not concern the shape of the curves but the manner of their interpretation. Blau and Altenburger (1922) first suggested that death was produced by the absorption of a limited number of quanta. This would result in a curve showing a period of lag followed by a linear relation between log. p.c. survivors and amount of irradiation. The number of quanta absorbed needed to kill the cell can, according to this theory, be calculated from the shapes of the curves. The number of quanta calculated to kill a cell by the upholders of the quantum theory varies. In the case of bacteria Wyckoff (1930) found the number to be one. P. Curie (1929) and Lacassagne (1929) and Holweek and Lacassagne (1930) found 4 and 9 quanta for varied wave-lengths, whilst with colpidia, Crowther (1926) found this number to be about 40. In actual fact the agreement regarding the shape of the curves obtained is not too good. For example, in the case of ultra-violet light several observers have obtained logarithmic curves. Clark and Gage (1903) action of sunlight in killing *B. coli communis*; Lee and Gilbert (1918) destruction of *B. coli communis* by ultra-violet light; Wyckoff (1932) destruction of bacteria by ultra-violet light. There is, however, no unanimity on this point, for the following authors have obtained sigmoid curves with the same system: Gates (1929) destruction of *S. aureus*; Baker and Nanavatty (1929) destruction of *B. coli*. The writers who have made the most extensive observations on the action of X-rays on biological material appear to agree that the characteristic curves are sigmoid in shape.

A summary of the evidence up to 1930 has been made by Packard (1931). Tests have been made on a wide variety of cell populations: Packard (1926–9) *Drosophila* eggs; Wyckoff (1930) and Holweek (1929) bacteria; Crowther (1926) *Colpidium colpoda*; Glocker, Hayer and Jüngling (1929) and Björling (1930) horse beans; Braun and Hol-

thusen (1929) ascaris eggs; Langendorff (1931) sea-urchin eggs.

Inspection of any of the curves under dispute will show that the distinction between a sigmoid curve and a logarithmic curve preceded by a lag depends almost entirely on the rate of death of the first 10 p.c. of the population. There are, however, very few populations that give 100 p.c. survivors in the controls, and hence the figures for the commencement of destruction are the least certain of any in the curves.

The writer's general belief is that in any system containing living cells there are nearly always too many variables present to allow of absolute proof of the nature of the processes causing quantitative relations, and hence controversies such as the one described must be decided on general probabilities.

The most obvious method of approach to a problem of this nature is to consider the relations that obtain in simpler systems, and silver halide emulsions are the most important of such systems because the action of light on the photographic plate has been studied very carefully. The evidence that has been considered shows that the nature of primary effects produced by light on the photographic plate is still uncertain, and that although the relation between exposure and effect in this system is very similar to that observed when light acts on photo-sensitive living tissues, yet this fact does not afford much help because there is a similar uncertainty regarding the significance of the relation obtained in non-living as in living systems. The mere fact that there is still an uncertainty regarding the nature of the effect produced by light on the photographic plate shows, however, that it is absurd to expect any conclusive proof regarding the nature of the effects produced by light on living systems. The writer considers, however, that the hypothesis that cells are killed by a few quanta of light implies postulates that are so improbable that they verge on the impossible.

The quantum theory postulates that the cells do not vary significantly in their resistance to radiations, that the action of radiations is a non-cumulative process, and that the cells possess no power of repairing the damage done.

There is direct evidence that all these assumptions are untrue. Unless the greatest care is taken to obtain uniform

populations it is impossible to get accurate quantitative measurements of the action of radiations. The evidence in general indicates that cells are extremely variable in their response to radiations. Experiments such as those of Redfield and Bright (1918) show that radiations can produce graded actions on cells. Finally, all observers agree that unless the irradiation attains a certain intensity it produces no effect on cells, and that in consequence the Bunsen-Roscoe law does not hold for irradiations of low intensity.

The explanation the writer advances is that the curves express individual variation. If all the cells were absolutely uniform they would all die after the same dose of irradiation. The cells vary extensively and the curves express cell variation distorted by various factors. In the first place the cells possess powers of repair, and hence with irradiations of low intensity there is a "tail" of resistant individuals in whom injury and repair are nearly balanced. The irradiation produces some mechanical effect in the cell and the relation between amount of irradiation and amount of chemical effect is not linear in any biological system for which it has been measured. As a first approximation we may assume that amount of effect varies as the logarithm of the amount of irradiation, a relation that has been shown to occur in the case of enzymes. If, now, the cells show a sigmoid variation in respect to the amount of effect (chemical change) needed to kill them, then the relation between amount of irradiation and the per cent. of cells killed will be highly skew. This is the same explanation as was advanced to explain the skew frequency curves observed in the case of drugs.

This hypothesis regards the action of radiations as a cumulative process, and assumes that the cell possesses a limited power of repair in respect to the injuries produced. The exposure-action curves may either express the individual variation in the power of repair (sigmoid curves) or the rate of accumulation of the toxic effect (logarithmic curves). This hypothesis is supported by the fact that a similar range of curves are found in the case of the destruction of bacteria by heat (Fig. 40), and in this case it is impossible to suppose that death is due to the absorption of a few quanta. Moreover, a similar range of curves can be obtained in the case of simple

systems in which it is fairly certain that the change measured expresses two factors, namely, the action of an injurious agent and the variation in the resistance of the cell. The exosmosis of salts from poisoned trouts' eggs (Fig. 61) provides a good example of such a process.

Formal proof of the action of radiations on living cells is impossible to obtain as our present knowledge is far too imperfect; this, however, is no reason for accepting any hypo-

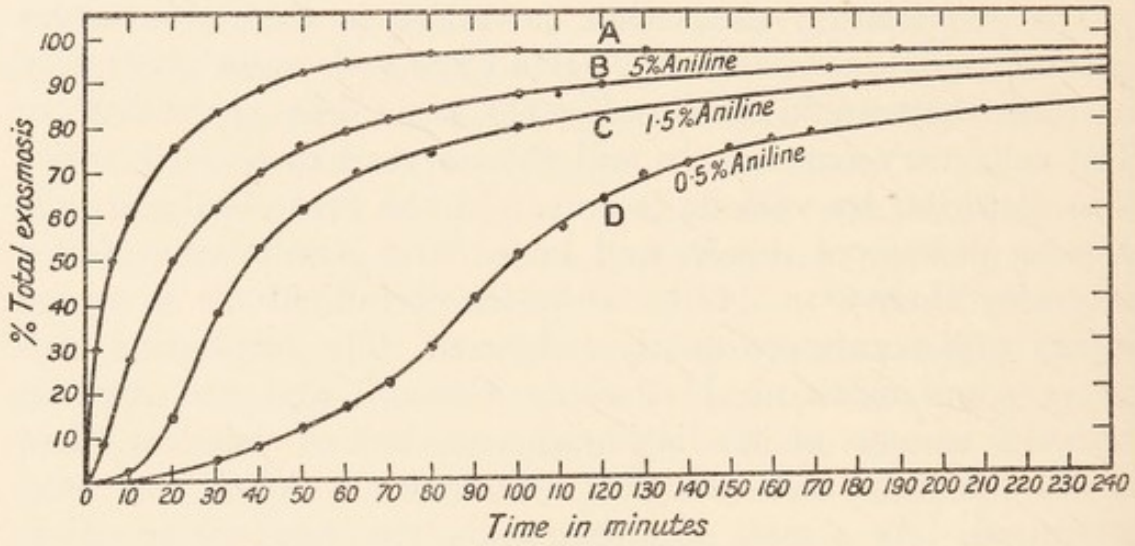

Fig. 61.—Graphs showing rate of exosmosis from dead (A) and living trout eggs (B, C, D).

In A a dead egg was saturated with 0·125 M CaCl; the graph shows the course of exosmosis into 3 c.c. of distilled water. In B a living egg was exposed to 3 c.c. of 5 per cent. aniline in distilled water; in C an egg was exposed to 1·5 per cent. aniline in distilled water, and in D an egg was exposed to 0·5 per cent. aniline in distilled water. In all cases there was no latent period owing to the powerful action of the aniline solution combined with the mechanical agitation experienced by the egg in the conductivity cell; latent periods appear when either of these factors is reduced in intensity. (Gray. 1932.)

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thesis that gives an approximate interpretation of the phenomena regardless of its inherent improbability.

The safest guide in these matters is the principle of William of Occam that forbids the assumption of unnecessary hypotheses, and this rules out the theory that the destruction of living cells by radiations is produced by the absorption of a few quanta, because the evidence available can be interpreted in a simpler manner.

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CHAPTER XIII

EFFECT OF TEMPERATURE CHANGE ON DRUG ACTIONS

General

The effects produced by changes of temperature on the action of drugs ought to provide evidence regarding the nature of the reaction between drugs and cells for two reasons.

In the first place a rise of temperature greatly increases the velocity with which a chemical system proceeds towards equilibrium. Usually the velocity is increased two- or threefold by a rise of 10° C. On the other hand, the velocity of physical processes usually is increased less than 1·5-fold by a rise of 10° C. Hence the effect of temperature change on the kinetics of drug action ought to provide evidence whether this action depends on a chemical or a physical process.

In the second place, a rise of temperature causes a displacement of chemical equilibrium in the direction in which the reaction takes place with absorption of heat. The effect of temperature changes on the extent of the action produced by a drug at equilibrium ought therefore to provide evidence as to whether the action of the drug is endothermic or exothermic.

In actual practice it is extremely difficult to draw any conclusions from the effects produced by temperature change on drug action. The first obvious difficulty is that the action produced by drugs is estimated by measuring some function of living tissues, and no conclusions regarding drug action can be drawn until we know exactly the effect of temperature change on the functions measured in the absence of the drug.

A second fundamental difficulty is that the rate of any chain reaction is set by the rate of the slowest reaction in the chain. Most chemical processes involving living tissues are

chain reactions and hence the effect of temperature change only measures the effect produced on the rate at which a single step occurs. This fact which appears almost self-evident has been termed the law of Blackman (1905) and Pütter.

The general theory of the action of temperature on chemical reactions has been expressed by Nernst (1916, p. 719) as follows :

" Since chemical equilibrium is established aperiodically, we are concerned with a process of the same kind as the movement of a particle under great friction, the displacement of ions in a solvent, or the diffusion of dissolved substances. In all these cases the velocity of the process is at each instant directly proportional to the driving forces, and inversely to the frictional resistance ; so that we conclude that an equation of the form Velocity of reaction $= \frac{\text{chemical force}}{\text{chemical resistance}}$ similar to Ohm's law must hold. The ' chemical force ' at any moment may be derived from the change in free energy ; of the chemical resistance we know little. . . . According to all experience the chemical resistance increases rapidly with fall of temperature, and becomes infinitely great at the absolute zero."

Van't Hoff (1884) expressed the relation between the rate of a chemical reaction and temperature by the formula

$$\frac{V_1}{V_0} = e^{c(T_1 - T_0)} \text{ or } \log_e V_1 \log_e V_0 = C(T_1 - T_0)$$

where V_0 and V_1 are the velocities of the reaction at temperatures T_0 and T_1.

This formula implies that the rate of increase of a reaction caused by a rise of temperature is constant for all temperatures. This rate of increase is usually calculated over a range of 10° C. and expressed as

$$\frac{\text{rate at } t + 10}{\text{rate at } t} = Q/10.$$

This formula was found to be inaccurate, because the values obtained for Q/10 nearly always fell as the temperature rose, and Arrhenius (1889) put forward an improved formula, which expressed accurately the influence of temperature on the rate

of reactions in homogeneous systems. This had the form $\frac{V_1}{V_0} = \varepsilon^{\frac{\mu}{2}\left(\frac{T_1-T_0}{T_0T_1}\right)}$. T_1 and T_0 are expressed as absolute temperatures.

The relation between Q/10 and μ has been plotted as a nomogram by Buchanan and Fulmer (1930) and a few figures are given in Table XVI. The divergences observed in the

TABLE XVI

The values of Q/10 that correspond to various values of μ in the biological range of temperature

Values of μ.	5,000	10,000	15,000	20,000	25,000
Temperature					
5° C.	1·37	1·93	2·65	3·65	5·00
15° C.	1·34	1·84	2·50	3·30	4·55
25° C.	1·32	1·76	2·35	3·05	4·15
35° C.	1·28	1·68	2·20	2·85	3·75

two sets of figures within the biological range of temperature are worth noting because the constant Q/10 is easier to determine than the constant μ, and these figures indicate the extent of the variations in Q/10 that can be corrected by the use of Arrhenius's formula.

It is rarely possible to study living cells over a range of more than 30° C. A comparison of Q/10 at 5° C. with that at 35° C. shows that when $\mu = 5{,}000$ Q/10 falls only 6 p.c. in this range of temperature; when $\mu = 15{,}000$ the change in Q/10 is 16 p.c. and when $\mu = 25{,}000$ the change is 25 p.c.

If, therefore, a series of biological observations shows a variation of Q/10 of more than 25 p.c. it is extremely unlikely that the employment of Arrhenius' formula will give a constant value for μ. In most cases the substitution of the Arrhenius for the Van't Hoff formula will only correct variations of about 10 p.c.

The measurements of temperature coefficients of processes occurring in homogeneous solutions has been found of general value in distinguishing between chemical and physical processes. Even in such cases abnormal results may be obtained. Hoeber (1924) mentions that in many reactions in watery

solutions the Q/10 is not constant but has a maximum value at about 20° C. Moreover some chemical processes have abnormally low temperature coefficients. For example the saponification of many esters has a Q/10 of less than 1·6.

Temperature Coefficients in Heterogeneous Systems

Since living cells are complex heterogeneous systems it is unwise to assume that they will follow kinetic laws based on the chances of collisions between molecules moving freely in homogeneous systems. Certainly it is advisable to examine the evidence provided by simpler non-living heterogeneous systems before making such an assumption. Even in homogeneous solutions exceptions are found to the rule that physical processes have temperature coefficients lower than those of chemical processes, and in heterogeneous systems the distinction is very uncertain.

The sorption of gases by charcoal is a simple physical process, but this has a Q/10 of 2 (Francis and Burt, 1927). Similarly the rate of catalysis by colloidal metals or by enzymes is believed to be regulated by diffusion and it might therefore be expected to have a low temperature coefficient, but actually the Q/10 in such cases is usually higher than 2 (Lewis, 1926, p. 458). The combination of carbon monoxide with hæmoglobin represents another extreme, for its rate is not influenced by temperature change (Hartridge and Roughton, 1925).

In some cases abnormally high temperature coefficients are found in relatively simple systems. For example, Kassel (1929) found a Q/10 as high as 12 for the dehydration of calcium carbonate hexahydrate in the presence of water. This abnormal value he explained as being due to the reaction proceeding as a chain process. Bell (1928) found a Q/10 of 13 for the oxidation of benzoyl o-toluidine in benzene by an aqueous solution of potassium permanganate.

These examples suffice to show that temperature changes may produce very curious changes in the rate at which reactions proceed in heterogeneous systems.

A study of the temperature coefficients of actions of enzymes suggests that in these processes the Q/10 is not constant but falls as the temperature rises; moreover the change observed is too great to be corrected by the use of Arrhenius's formula.

For example, Harris and Creighton (1915) found that the Q/10 of tissue reductase rose from zero at 0° C. to 2 at 10° C. and remained constant at this figure between 10° C. and 40° C., but fell when the temperature rose above 40° C. Van Slyke and Cullen (1914) found with urease a constant Q/10 between 10° C. and 50° C., but the Q/10 rose when the temperature fell below 10° C. Kuhn (1925) has tabulated the temperature coefficients for about 30 ferments and in some cases these cover a range of 20° C. These latter figures give the following average values for μ.

	10–20° C.	20–30° C.	30–40° C.
Average of 4 cases	9,100	6,800	—
Average of 6 cases	—	16,000	11,700

There is therefore a fairly general agreement that the temperature coefficient of enzymatic processes falls as the temperature rises.

In the case of lyophobic sols Freundlich (1926, p. 466) mentions that the value of μ for the velocity of coagulation is 4500, but in the case of reversal of adsorption $\mu = 13{,}925$, which is a value very high for simple chemical processes, but common in biological systems.

Very high temperature coefficients are found in some cases of denaturation of proteins, and Freundlich (1926, p. 612) quotes v. Halban's suggestion that when a reaction involves the rearrangement of small molecules such as water inside a large molecule then the value for μ usually lies between 10,000 and 30,000.

The temperature coefficient of protein denaturation is, however, far higher than the values mentioned above, for values of Q/10 from 100 to 600 have been found in the case of hæmoglobin and egg albumen (Anson and Mirsky, 1925). On the other hand, the temperature coefficient of the rate of syneresis of silica gels (Q/10 = 1·8) is of the same order as that of an ordinary chemical reaction (Ferguson and Appleby, 1930), and the temperature coefficient (Q/10) of the rate of precipitation of egg albumen by phenol is about 2·0 (Cooper and Haines, 1928).

Changes in temperature often produce very striking discontinuous changes in heterogeneous systems, and in particular

in the viscosity of such systems. Gasser (1931) investigated examples of this and showed that the viscosity and also the electrical resistance of certain mixtures might rise almost vertically at certain critical temperatures. He pointed out that "Chemical reactions in heterogeneous systems can have very low temperature coefficients; and physical qualities can be cited which have temperature coefficients covering the whole range possible for chemical reactions and extending beyond it."

In the case of heterogeneous systems the influence of temperature change both upon the rate of chemical reactions or changes of physical state and also upon the equilibria attained is extremely complex. This suggests that great caution is needed in attempting to interpret the effect of temperature changes on biological processes.

The influence of temperature on equilibria in heterogeneous systems is very irregular. As a general rule the amount of substance adsorbed has a negative temperature coefficient, and this is nearly always true for the sorption of gases. On the other hand, the sorption of substances from solution often has a positive temperature coefficient (McBain, 1932, p. 11) and therefore no generalizations are possible.

The Temperature Coefficient of Biological Processes

The extensive literature on this subject has been summarized by various writers (Kanitz (1915); Przibram (1923); Janisch (1927); Cameron (1930)). Arrhenius (1915) emphasized the approximate correspondence of the temperature coefficients of living processes with those of chemical reactions. Crozier and his co-workers (1924–9) have sought to identify the temperature coefficients of living processes with those of specific chemical reactions.

Doubts have been raised in recent years regarding the value of temperature coefficients in interpreting the nature of biological processes by various authors (Heilbrunn (1925); Belehradek (1926, 1928); Gasser (1931)). Some of the outstanding points of this dispute are as follows.

Warburg (1914) pointed out that the temperature coefficient of very simple biological processes was not constant but fell steadily as the temperature rose. Some of his results are shown in Table XVII. A number of authors have measured

TABLE XVII

Oxygen uptake of sea-urchin eggs. (Usui. Quoted from Warburg. 1914.)		Rate of fermentation of living yeast cells. (Slator, 1906.)	
Temperature range.	Q/10	Temperature.	Q/10
—	—	5° C.	5·6
0–16·4	5·0	10° C.	3·8
16·4–28	3·2	20° C.	2·2
28–38	2·4	30° C.	1·4

various simple forms of protoplasmic movement and have found that Q/10 falls steadily as the temperature rises. A few typical figures are shown in Table XVIII. The results

TABLE XVIII

Temperature.	Velocity of amœbæ (Pantin, 1924). Q/10	Movement of cilia of Mytilus (Gray, 1923). Q/10	Oxygen usage of narcotized frog (Krogh, 1914). Q/10	Frequency of isolated frog heart (Clark, 1920). Q/10
0–5	16·9	3·52	—	4
5–10	3·19	3·00	3·19	2·78
10–15	2·31	2·37	2·47	2·55
15–20	2·04	2·25	2·41	2·43
20–25	—	—	2·35	1·96
25–30	—	—	—	1·66

show a remarkable uniformity and the changes in the value of Q/10 are far too great to be corrected by the use of Arrhenius's formula. The application of this formula shows an average fall in the value of μ from about 18,000 at 5–10° C. to 14,000 at 15–20° C. In the case of the frog's heart the value of μ at different temperatures is as follows: 5° C. $\mu = 17{,}000$, 15° C. $\mu = 13{,}000$, 25° C. $\mu = 10{,}000$. Figures similar to this have been obtained with a very large number of rhythmically contractile vertebrate and invertebrate tissues, both in intact animals and with isolated tissues.

Crozier and his co-workers have interpreted this effect by assuming that the process that sets the pace of the reaction

changes at different ranges of temperature. It is of course always possible to interpret a shallow curve as being composed of a series of linear relations. The chief objection to this theory is that the variations in μ observed with complex systems such as hearts and nervous systems are remarkably similar to those found in unicellular organisms and even in

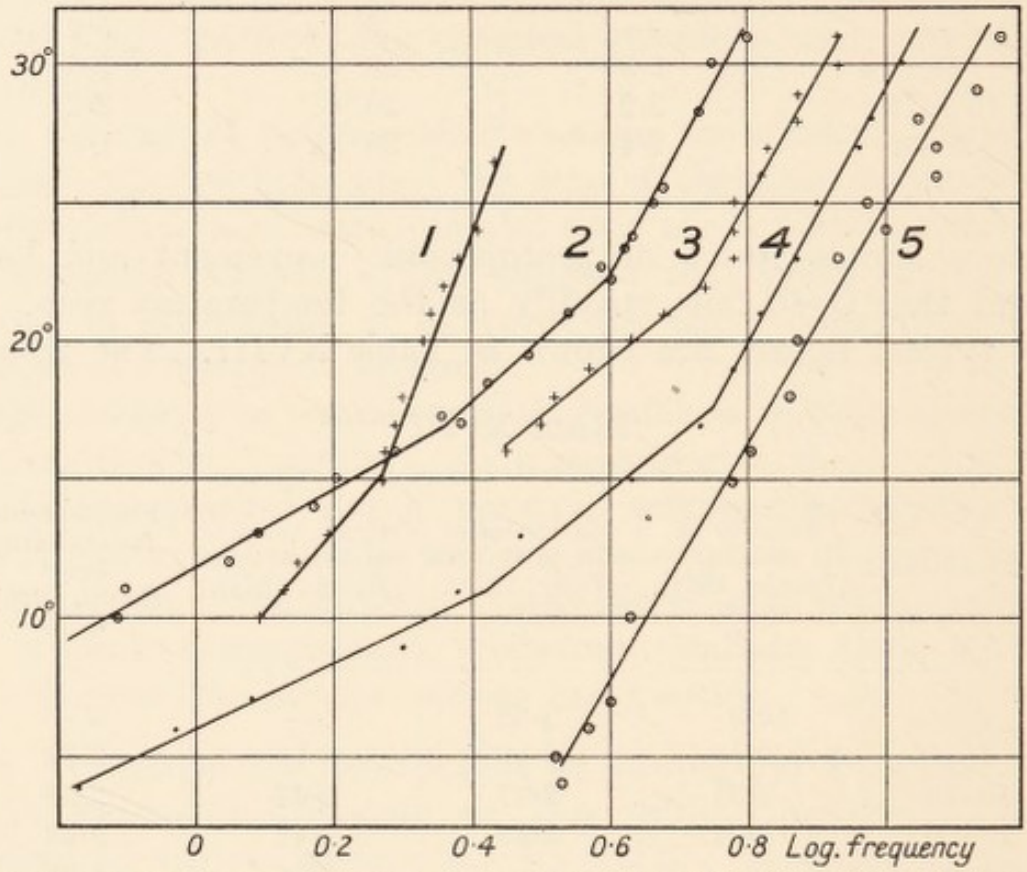

Fig. 62.—The influence of temperature on the frequency of the pulsation of the vacuoles of ciliated protozoa.

Abscissa: logarithm of frequency per minute. Ordinate: temperature in degrees centigrade.

(1) *Euplotes charon* (Rossbach, 1872). (2) *Paramœcium caudatum* (Cole, 1925). (3) *Paramœcium* (Khainsky, 1911). (4) *Glaucoma colpidium* (Degen, 1905). (5) *Stylonychia pustulata* (Rossbach, 1872). (Figures collected by Metzner, 1927.)

ferments. In the case of ferments it seems improbable that the pace-maker of the rate of reaction changes as the temperature changes, although this may not be altogether impossible.

Fig. 62 shows the effect of temperature change on the frequency of contraction of the pulsating vacuoles of various protozoa. This figure shows the relation between the logarithm of the frequency and the temperature. Any deviation from

the linear indicates a change in the Q/10. The figure shows a constant Q/10 in one case, but in the remaining four cases the Q/10 decreases as the temperature rises. The curves have been expressed in the figure as discontinuous linear relations, but they could have been drawn as shallow curves without deviating markedly from the experimental results. The changes in temperature coefficient shown are far too great to be corrected by the use of Arrhenius's formula. The values calculated for μ in the four curves in Fig. 62 which extend from 10° C. to 30° C. are as follows :

Curve	1	2	4	5
Value of :				
(*a*) From 25° C. to 30° C.	5,800	9,300	9,300	9,900
(*b*) From 10° C. to 15° C.	13,500	27,800	21,000	9,000

Cole (1925) concluded in the case of *Paramœcium caudatum* (Fig. 62, curve 2) that since two clear changes occurred in the value of μ there must be at least three underlying reactions responsible for pulsation. Since these changes of μ can be obtained in unicellular organisms it is clear that similar changes in this value in larger organisms do not prove the occurrence of any change in the site of the pace-maker of the activity that is being measured. Since, however, very similar changes in the value of μ are observed with ferments it seems simplest to accept the outstanding fact that Arrhenius's formula does not interpret the changes produced by temperature changes in the activities of the ferments of protoplasm.

Belehradek (1930) concluded from a comparison of the evidence provided by ferments and living cells : " One cannot expect that a chemical law, which does not hold good in enzymatic processes, should yield better results in processes taking place inside the living cell. It may be said that the Van't Hoff-Arrhenius law has no real validity in biology."

A possible explanation of this fact is that a change of temperature in a heterogeneous system containing lyophil colloids causes a number of more or less independent changes. Snyder (1911) and Filon (1911) both observed the constant fall in temperature coefficients with rise of temperature in biological systems and the former worker suggested that it was due to a fall in the viscosity of the protoplasm. Pütter

(1914) made the same suggestion. Heilbrunn (1929) measured the effect of temperature change on the viscosity of the protoplasm of various organisms, e.g. amœbæ, eggs of clam, etc. He found that a change of temperature from 18° C. to 12° C. increased the viscosity of amœba protoplasm sevenfold. The viscosity was at a minimum at 18° C. and a change from 18° C. to 22° C. caused a threefold increase.

Belehradek (1930) supported the view that changes in viscosity influenced the rate of diffusion and in consequence caused changes in the temperature coefficient, but Stiles (1930) showed that in non-living systems changes of viscosity did not affect the rate of diffusion as much as might have been expected and that the coefficient of diffusion was not inversely proportional to the viscosity of the medium.

The influence of viscosity on diffusion is therefore too uncertain to permit the calculation of the probable extent of its influence on the rate of biological processes.

There is, however, no doubt that biological processes as a class do not show a constant value for μ when extensive changes of temperature occur, and this fact naturally raises doubts as to the real validity of the application of Arrhenius's formula to such processes. Belehradek (1926, 1930) argued that since most of the biological processes on which the effect of temperature change had been measured were arrested at about zero centigrade, therefore this and not absolute zero ought to be taken as the biological zero, and that there was little theoretical justification for the application of Arrhenius's formula to biological processes. He put forward the empirical formula $y = \frac{a}{x^b}$, where x is the temperature measured in degrees centigrade, y is the time taken by the process (i.e. the reciprocal of the velocity), and a and b are constants. He showed that this formula fitted most biological data quite well. Root (1932) has recently discussed this problem and has shown that Belehradek's formula fits the effects produced by change of temperature on the production of light by *Vibrio phosphorescens*.

It has already been mentioned that temperature coefficients cannot be relied upon to distinguish chemical and physical processes in heterogeneous solutions. There is direct evidence

for this conclusion in the case of living cells. McCutcheon and Lücke (1926 and 1927) found that a value of $Q/10 = 2$ to 3 for the swelling of *Arbacia* eggs in hypotonic saline. Irwin (1927) found that a $Q/10$ of more than 4 for the diffusion of brilliant cresyl blue with *Nitella*. These processes appear to be of a physical nature, i.e. diffusion, and there is no evidence of any chemical change and yet the temperature coefficients are those typical of chemical processes.

The Influence of Temperature on the Kinetics of Drug Action

The significance of time-concentration curves was discussed in Chapter V, where it was pointed out that the time measured might be composed of several different factors such as time spent in fixation of drug, in diffusion into cell interior, in production of slow colloidal changes, and might express chiefly the lag between the production of the chemical change and the appearance of the biological response.

It has been shown that the significance of temperature coefficients in heterogeneous systems is very doubtful, and therefore the study of the influence of temperature change on the rate of drug action involves at least two unknown variables. There is, however, a chance of some information being obtained because the temperature coefficient of a complex chain reaction expresses only the coefficient of the slowest process in the chain.

There are a certain number of drug actions in which the influence of temperature has been studied fairly fully and these are the most favourable cases for consideration.

Kinetics of Disinfection

The estimation of the rate of disinfection is relatively easy because when strong concentrations of disinfectant are used the time-action curves approximate to the logarithmic form, and the proportion of organisms killed in unit time is constant over considerable periods. In such cases the velocity constants can be compared. In other cases the time-action curves are sigmoid, and in this case it is necessary to compare the times required to kill a certain proportion of the population. In theory it is best to measure the time at which 50 p.c. destruction occurs, but this is often a very short period and it

is more convenient to measure to 90 or 99 p.c. destruction. The easiest end-point to measure is total destruction, as this makes it unnecessary to count the survivors, but theoretically this is a poor end-point in the case of a population with variable resistance.

Chick (1908) studied the temperature coefficients of the rate of action of various disinfectants upon *B. paratyphosus* She found a Q/10 of about 3 for metallic salts, but found that in the case of phenol the Q/10 varied from 10 to 2 according to the age and number of the bacteria used.

Chick (1930, Table V) has published a summary of the results obtained by a number of workers who have measured the temperature coefficient of disinfection. In the case of acids the figures for Q/10 vary from 1·5 to 3·4, whilst in the case of metallic salts the value usually lies between 2 and 3. The figures for the Q/10 of phenol and of coal-tar disinfectants vary, however, from 5·5 to 15. The last-mentioned figures prove that the temperature coefficient of phenol is exceptionally high, and that it can be altered by a variety of changes in the experimental conditions. There appears to be little profit in speculating on the significance of such a variable result.

Temperature Coefficients of Hæmolysis

Arrhenius (1915, p. 65) quotes observations by Madsen, Walbum and Noguchi on the temperature coefficient of the hæmolysis produced by ammonia, and calculated from them a value for μ of 26,760. This, he inferred, was evidence for a monomolecular reaction between the drug and the corpuscles. It is, however, well known (Christophers, 1929) that the chemical reaction between a base or acid and corpuscles occurs very rapidly and is followed by a slow swelling of the hæmoglobin which finally bursts the cell. The time measured in the experiments quoted by Arrhenius therefore was not the time required for ammonia to react with the cells, but the time required for the altered hæmoglobin to imbibe sufficient water to burst the cell-membrane. The temperature coefficient in this case gives no information regarding the primary reaction between the drug and the cells.

Ponder and Yeager (1930) have examined carefully the influence of change of temperature on saponin hæmolysis. They conclude that the effects produced on the hæmolytic

process by a change of temperature are too complex to be adequately described by the Arrhenius equation. They point out that many investigations made on systems much more complex than red blood-corpuscles have yielded results which are apparently simpler. They suggest that the reason for this is as follows. It is difficult to analyse the effects produced by even two variables and very difficult to interpret the results when three variables are present. If, however, a large number of variables are acting the result is quite likely to be a smooth curve or even a simple linear relation.

The most profitable line of investigation regarding the influence of temperature on drug action appears to the writer to be of the qualitative description. For example, Arrhenius noted that the rate of hæmolysis by ammonia had a temperature coefficient (Q/10) of about 4·8, whereas with sodium oleate hæmolysis the Q/10 was about 1·3, and the hæmolysis produced by cobra venom had a Q/10 of about 1·2. The investigation of differences so gross as these might give some hint as to the processes involved.

Kinetics of Cardiac Glucosides

Weizsäcker (1913) measured the influence of temperature on the time taken by strophanthin to arrest the isolated frog heart. He took the precaution of maintaining a constant heart-rate and obtained the following figures :

Temperature range . . .	7–17	17–29	16·5–31·5
Q/10	3·8	2·1	1·9

Sollmann, Mendenhall and Stingel (1914) measured the influence of temperature on the rate at which ouabain produced systolic arrest in the intact frog and in the isolated heart in which the frequency was allowed to vary with the change of temperature. They obtained the following results for the intact frog :

Temperature range . . .	5–15	10–20	15–25	20–30
Q/10	16·1	9·4	3·6	2·1

In the case of the isolated heart the ratios varied greatly but the average values were about 10.

The frequency of the frog's heart and the oxygen consumption both increase two- or three-fold when temperature rises

from 10 to 20° C. The simplest explanation of the figures of Sollmann et al. are that the rise of temperature directly increases the rate of action of strophanthin, as was shown by Weizsäcker, and indirectly increases the rate of action of the drug by increasing the heart frequency and oxygen consumption, and that the Q/10 observed is the multiple of these two effects (e.g. $3 \times 3 = 9$).

This is a fairly obvious example of the complexity of the results that are likely to be produced by temperature change, and illustrates Pütter's (1914) contention that temperature change must always produce an action not only on the drug but also on the animal.

A further point that requires consideration is that the delay in the production of systolic arrest of the isolated frog's heart by digitalis and its allies is due to two separate factors, namely, the time occupied by the fixation of the drug and the time occupied by the lag in the biological response after fixation has occurred. Gander (1932) tried to estimate these two factors separately, but he found that in both cases the Q/10 lay between 2·7 and 3·0 in the temperature range of 14° C. to 28° C.

Various Drug Actions

Veley and Waller (1909, 1910) found that the temperature coefficients of the rate of action of narcotics on the isolated frog's sartorius were as follows : Alcohol and ether Q/10 = 2 ; chloroform Q/10 = 1·6. Ing and Wright (1931) found that the temperature coefficient of the rate of paralysis of the isolated frog sartorius by quaternary ammonium salts was Q/10 = 1·5 both for 3·5° to 13·5° C. and for 13·5° to 23·5° C. In the case of narcotics the rate measured is likely to be a diffusion rate, but in the case of the quaternary ammonium salts the delay due to diffusion could be excluded, and the lowness of the temperature coefficient is therefore of interest. Hill (1909) measured the rate of action of nicotine in isolated frog muscles at temperatures from 6° C. to 20° C. and found values of μ varying from 11,500 to 23,000 with a mean value of 17,000.

Hartmann (1918) investigated the influence of temperature on the rate of poisoning of *Cladocera* by about 40 different poisons. The chief conclusion that the author drew from his

results was that the temperature coefficient of toxicity varied markedly according to the substance used, the organism studied and probably also according to its physiological condition. Moreover, he found that it differed in different temperature regions and, finally, according to the concentration of the suspension fluid used. Inspection of these results shows that the Q/10 of acids and alkalies varies around 2·0. The Q/10 of neutral salts and heavy metals is distinctly lower and varies around 1·5. The Q/10 of ethyl alcohol and of chloral hydrate is about 2·0.

Huxley and Fulton (1924) found that the time taken by insulin to produce convulsions in frogs over a temperature range from 7° C. to 30° C. showed a Q/10 of 2·4 with the lower temperatures and of more than 3·0 with the higher temperatures.

The Influence of Temperature on Equilibrium between Drugs and Cells

Measurements have been made in a number of cases to determine the effect of alteration of temperature on the action produced by drugs when time is allowed for equilibrium to occur. The usual method is to determine the concentrations of drug required to produce a given action at varying temperatures. Van't Hoff formulated the general principle that a rise of temperature favours systems formed with heat formation and a fall of temperature favours systems formed with heat loss. If it is certain that the action of a drug on a cell is due to a simple chemical reaction between the drug (D) and some substance (S) in the cell, ($D + S \rightleftharpoons DS$), then the heat of formation of DS can be calculated by measuring the change produced by alterations of temperature in the dissociation constant of this reaction. Such calculations have been made in the case of the combination of hæmoglobin with oxygen, carbon monoxide, etc. Unfortunately in most cases of drug action it is fairly certain that the reaction between the drug and the cell is a chain of physical and chemical processes (e.g. adsorption on cell surface, distribution between solution and cellular substance determined by differential solubility, reactions between drug and cellular constituents resulting in change in the colloidal state of the latter, etc.). In such cases the influence of temperature on the equilibrium is

unlikely to do more than indicate roughly the nature of the process that proceeds at the slowest rate.

Influence of Temperature on Narcotic Uptake

This problem has been studied in detail in the hope of proving or disproving the theory that narcotic uptake is regulated by the differential oil/water solubility of narcotics. Winterstein (1926, pp. 135 ff.) gives a full account of the controversy and the following are some of the chief facts. Meyer (1901) showed that a rise of temperature decreased the oil/water distribution coefficient of salicylamide, benzamide and monacetin and increased that of most narcotics (e.g. ethyl alcohol, chloral hydrate and acetone). He claimed that the minimum narcotic concentration for tadpoles varied as the change in the distribution coefficient. Moral (1918) confirmed this conclusion on nerve-muscle preparations. Unger (1918), Bierich (1919) and Hoeber (1919) found the activity of all narcotics increased by a rise of temperature. Hoeber concluded that the effect of temperature bore no relation to distribution coefficients. Zehl (1908) estimated the concentrations necessary to arrest growth of algæ and found that a rise of temperature decreased the action of chloroform, ether, ethyl urethane and benzamide, but increased that of alcohol and chloral hydrate. In most cases the Q/10 for the alteration of effective concentration was + or — 1·3 to 1·5. Hartmann's (1918) figures indicated that a rise of temperature increased the action of alcohol and chloral hydrate on *Cladocera* (i.e. reduced the minimum effective concentration). Collett (1922) found that temperature change affected the action of narcotics on tadpoles in a highly irregular manner. Redonnet (1919) concluded that temperature change acted by altering the rate of diffusion and Dennecke (1920) showed that this was not the case. Issekutz (1924) showed by analyses that a cold frog was narcotized by a lower concentration of narcotic in the brain than was a warm frog. Montuori (1915) found that sea animals when warmed showed a higher resistance to most narcotics. Finally, van Leeuwen and van der Made (1916) found that decerebrate cats were most resistant to chloroform at 38° C. and that raising or lowering the temperature decreased the action of the drug.

The above account indicates the extreme complexity of the

results obtained. Every system studied appears to provide a different result. This really is not surprising since the results must represent the sum of two completely independent variables, namely, the effect of change of temperature on the distribution of narcotic between tissues and solution and, secondly, the effect of temperature change on the organism and in consequence its effect on the response of the organism to the drug that it fixes. The results suggest that the latter is the more important variable.

Temperature Change and Lethal Agents

Cooper and Haines (1928) measured the effect of temperature change on minimum lethal concentrations of disinfectants on *B. coli*, and found that the drugs fell into three classes as regards the effects noted.

1. Temperature change produced no effect.
2. The temperature coefficient (Q/10) was 2 to 3.
3. In the case of phenol the temperature coefficient (Q/10) was 8 to 20.

Walker (1927) measured the effect of temperature change (9·5 to 27° C.) on the minimum concentration of various drugs needed to kill *Colpidium* in 3 minutes. Such results express partly disinfectant rate and partly disinfectant power. The following values for Q/10 were obtained : *p*-chlorophenol 1·0, formaldehyde 1·0, mercuric chloride 1·15 and nickel chloride 1·3.

Temperature Change and Isolated Organs

The effect of temperature changes on the rate of response in isolated organs usually gives temperature coefficients which might be correlated with some physico-chemical alteration. The effect of temperature changes on the extent of the effect produced by drugs on isolated organs is however extremely erratic.

Brand and Lipschitz (1930) studied the response of the isolated rabbit-gut to pilocarpine and adrenaline and found that a change from 37° to 17° C. made little difference, but that reduction below 17° C. rendered the tissue insensitive to the drug. Githens (1913), van Leeuwen and le Heux (1919) found that reduction in temperature made the guinea-pig's isolated uterus more sensitive to histamine. The action of

veratrin on the isolated frog muscle is greatly reduced by cooling to 2° C. The Q/10 is about 2·2 (Lamm, 1901). Mansfeld and Horn (1928) measured the effect of temperature on the minimum concentration of ouabain needed to arrest the isolated sinus of the frog in an hour. They found that between 15° C. and 25° C. the Q/10 was 2·0, whilst between 20° C. and 30° C. it was 1·5. Gander (1932) found a Q/10 of 1·2 between 18° C. and 28° C. for the minimum concentration of gitalin that produced systolic arrest of the isolated frog's heart.

The Effect of Temperature Change on Minimal Lethal Doses

The effect of temperature on the minimal lethal dose has been studied in detail in the case of the action of digitalis and strophanthin on frogs. Baker (1912) found that the toxicity of strophanthus and ouabain was increased by a rise of temperature, and this was confirmed by Sollmann, Mendenhall and Stingel (1915). Baker concluded that temperature changes did not affect the digitalis action. Smith and McClosky (1925) found that the effect of temperature was exactly the same on digitalis and on ouabain. The results obtained were as follows:

	Q/10 10–20° C.	Q/10 15–25° C.	Q/10 20–30° C.
Ouabain (Baker, 1912)	—	2·2	2·2
,, (Sollmann et al., 1915)	—	3·8	1·9
Digitalis tincture (Smith and McClosky, 1925)	—	2·4	1·6
Digitalin (Roth, 1916)	2·0	—	1·3
Digitoxin ,, ,,	1·45	—	1·1

Increase of temperature therefore increases the susceptibility of the frog's heart to cardiac glucosides both in the isolated condition and in the intact animal. This fact is of practical importance in regard to the biological standardization of these drugs, but it does not appear to give much information as regards the nature of the reaction that occurs in this case between the drug and the tissues.

The power of rats and mice to maintain a regular body temperature is limited, and hence changes in external temperature in these animals may alter profoundly their response to drugs. This effect has been found to be a very serious source

of error in drug standardization. Trevan and Boock (1926) measured the convulsive dose of insulin in mice at 29° C. and at 38° C. and found a Q/10 of 2·6. Cassidy, Dworkin and Finney (1925A) found that insulin convulsions were inhibited in dogs and cats when the body temperature was lowered to 25° C., but they also found (1925B) that this alteration in body temperature produced very little change in the blood-sugar curves following insulin administration. The effect of the cooling must therefore be to make the central nervous system insensitive to reduction in the blood-sugar.

Temperature Coefficient of Thermal Destruction

Thermal destruction of living organisms is in most cases a fairly sharp all-or-none effect. Very frequently the organisms can live indefinitely at a certain temperature, but a rise of two or three degrees causes rapid death. Since a small variation in temperature changes the rate of destruction from nil to a high figure the temperature coefficient is necessarily very high.

A similar phenomenon is seen in the heat coagulation of proteins. The process of destruction of living cells by heat shows many resemblances to the heat coagulation of proteins. It is quite possible that heat actually produces cell death by destroying the proteins and therefore it is worth while considering the simpler system first. Chick and Martin (1910, 1911, 1912) showed that the heat coagulation of hæmoglobin and egg albumen proceeded in two stages, namely a chemical process of denaturation which was followed by a physical process of agglutination. The second process proceeded much quicker than the first : Lepeschkin (1922) showed that agglutination proceeded 200 times as quickly as denaturation. The denaturation involves a combination with water, for dry proteins can be heated without being denaturated. The process is affected by the presence of salts and by the reaction of the solution. Chick and Martin (*loc. cit.*) and Hartridge (1911) showed that the process of denaturation followed accurately the course of a monomolecular reaction.

The temperature coefficient of the process is, however, far greater than that of any ordinary chemical reaction. The increase of rate per 1° C. rise of temperature is in the case of egg albumen 1·3 to 1·9 (Chick and Martin), and in the case

of methæmoglobin 1·93 (Hartridge). The value for HbO_2 was 1·3 and for HbCO 1·18, but in these last two cases the rate measured was probably the rate of conversion of HbO_2 and HbCO to methæmoglobin (Anson and Mirsky, 1925).

These extreme changes over a moderate range of temperature show a certain resemblance to such changes in physical state as the melting of solids.

In most cases the heat destruction of living cells proceeds as follows. Up to a certain temperature very few cells are killed and the survivors live and reproduce in a normal fashion : over a narrow range of temperature there is a very rapid destruction and above a slightly higher temperature the destruction is almost instantaneous. Brown and Crozier (1927) found that *Daphnia pulex* lived and reproduced at 30° C. but died at 32° C. and that *Mœna macropa* lived and reproduced at 39° C., lived a few days at 40° C., but died in a few minutes at 41° C.

These authors have collected the figures for the temperature coefficients of the destruction for a large variety of living organisms. Their table shows the value of μ in 28 cases, in 18 cases this value lay between 60,000 and 120,000, it was above these limits in 4 cases and below this in 6 cases. Calculation of these results by Van't Hoff's formula shows a Q/1 between 1·5 and 1·7, or a Q/10 between 50 and 300.

There appears to be some close connection between the heat denaturation of proteins and the heat destruction of living cells, but it is difficult to compare either process with the effects of heat on any ordinary chemical reaction.

The Influence of Temperature upon the Biological Effects produced by Radiations

The action of radiations on cells was shown in Chapter XII to consist of a primary radio-chemical effect, which probably was of a reversible nature in most cases, and that this was followed by a series of secondary chemical changes which were probably irreversible in many cases.

The primary effect must be of a physical nature, and will therefore have a low temperature coefficient, whilst the secondary chemical changes may be expected to have a temperature coefficient above 2. The temperature coefficient (Q/10) of the biological effects produced by radiations may

therefore be near unity or above 2, according to whether the rate of action is regulated by the rate of formation of the activated molecules or by the rate at which the secondary chemical processes occur.

Blackman in 1905 showed that a low temperature coefficient was obtained when light of feeble intensity acted upon chlorophyll, but that it rose to a value resembling that of a chemical reaction when the light was intense. This result caused him to formulate the law that the temperature coefficient is a measure of the change of rate in the slowest process in any chain of processes. Hecht (1931) found that the temperature coefficient of the duration of the latent period in the eye of *Ciona* was about 3 and that the speed of dark adaptation showed the same coefficient. On the other hand, the temperature coefficient of the intensity of action of light was between 1·04 and 1·07 and the same value was found by Adrian and Matthews (1927) for the action of light on the retina of the eel.

Martin and Westbrook (1930) measured the rate at which ultra-violet light produced browning of leaves and found that the Q/10 for the latent period varied between 1·3 and 3·0, but that the Q/10 for the duration of irradiation needed to produce a given effect was less than 1·5. Gates (1929) found a Q/10 of 1·1 for the duration of ultra-violet light irradiation needed to kill *Staph. aureus*, but Hill and Eidenow (1923) found a value of 4 for the Q/10 in the case of ultra-violet light acting on hay infusoria. These examples indicate the complexity of the temperature coefficients of actions produced by radiations on living cells.

Irradiation with visible light can cause immediate response, but other radiations are characterized by the long latent period that intervenes between exposure and the appearance of the response. The production of X-ray erythema is an extreme example of this. The commonest injurious effect produced by irradiations appears to be that it renders cells incapable of further division rather than killing them outright. Alterations of temperature alter the rate of all biological processes occurring in cells and therefore are likely to alter these slow biological responses to irradiation.

Packard (1930) found that *Drosophila* eggs were killed more

readily at 28° C. than at 17° C. by X-rays. The same effect was observed by Strangeways and Fell (1927) in the case of tissue cultures. Holthusen (1921) found that the amounts of exposure to X-rays needed to kill ascaris eggs at 0° C. and 23° C. showed a Q/10 of 1·6.

Discussion

The influence of temperature on the rate and extent of biological reactions to drugs and irradiations appears at first sight to be a promising method of analysis. Unfortunately biological systems are so extremely complex that the study of temperature coefficients seldom provides clear evidence as to whether a process is of a chemical or a physical nature. The best result that can be hoped for is information as to the nature of the slowest process in a chain of processes.

In most cases where it is possible to measure the temperature coefficient of a biological response over a wide range of temperature, it is found to fall as the temperature rises. This phenomenon is observed with ferments, unicellular organisms and complex structures such as isolated hearts of invertebrates or vertebrates. Crozier and his school believe that these alterations are discontinuous and indicate changes in the " pace maker " of the reaction studied. Others believe that these changes are continuous and indicate that Arrhenius's formula does not hold for living tissues. This question can only be settled when further information is available concerning the influence of temperature on complex colloidal systems.

The study of the effect of temperature changes upon the reactions between drugs and tissues is a more complex problem than is the study of their effects upon the rate of biological processes. The rate at which a drug produces its effects depends in most cases upon two factors, namely the rate of combination between the drug and the tissue and secondly upon the rate at which the biological response follows the chemical stimulus. Change in temperature is certain to affect both factors and therefore the temperature coefficient of the rate of action of a drug does not necessarily indicate the rate at which any primary chemical change is produced, unless it happens to be possible to exclude changes in the rate of the biological response.

The measurement of the effects produced by temperature

change on the minimum effective concentration or dose of a drug is subject to a similar error, for the temperature change affects not only the reaction between the drug and the tissue but also may change the response of the tissue. For example, a fall of temperature may decrease the ease with which convulsions can be produced in an animal, but this effect probably depends upon a diminution in the excitability of the brain and not upon any change in the reaction between the drug and the central nervous system.

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