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MONOGRAPHS ON EXPERIMENTAL BIOLOGY

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THE CHEMICAL BASIS OF GROWTH AND SENESCENCE

BY

T. BRAILSFORD ROBERTSON, PH.D., D.SC.

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MONOGRAPHS ON EXPERIMENTAL BIOLOGY

THE CHEMICAL BASIS OF GROWTH AND SENESCENCE

BY

T. BRAILSFORD ROBERTSON, PH.D., D.Sc.

PROFESSOR OF PHYSIOLOGY AND BIOCHEMISTRY, UNIVERSITY OF ADELAIDE, SOUTH AUSTRALIA.

"Is there not a wonderful division of labour, a marvellous solidarity among the parts of an organism, perfect order in infinite complexity? . . . . . .It implies at once the multiplicity of elements and the interpenetration of all by all." BERGSON, "*Creative Evolution.*"



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PREFACE

THE brilliant philosopher whose sagacious words have been quoted upon the title-page of this monograph has embodied them in a volume which is devoted to the exposition of his belief that the scientific method can never ascertain a solution of the riddle of development.

This assertion affords but one among very many contemporary instances of waning faith in the power of intellect; a reaction, perhaps, against its overwhelming successes, which nevertheless continue without ceasing, and augment. But how curious is this confidence in intellectual bankruptcy! For the period during which the collective intellect of mankind has been consciously applied to the task of unravelling the complexities of our environment, is but a day in the epoch of humanity. Purposeful intellectual endeavor, which is science, is, as a geologist has recently told us: "but the last chapter in the last volume of a series which would fill a library." So little effort, and we are now exhausted! So brief a period, and we abandon the conflict! We declare ourselves impotent, because in a day we have not comprehended the ultimate significance of life!

There is danger in this prophecy of failure, for it will some day be overtaken by the nemesis of discovery. While it is uncertain whether the **power** of human understanding itself has undergone any evolutionary development within the period of history, yet it is certain that its **content**, which is knowledge, does most evidently increase. M. Bergson has himself eloquently and convincingly dwelt upon the vast import of machinery in the enlargement of our understanding, and the argument which he advances is applicable no less to the more intangible advances of theoretical knowledge. Each successive acquirement en-

larges our power to grasp more, and the progress of human knowledge is, in fact, autocatalyzed.

Thus, if at the present moment we are not in a position to interpret fully the phenomenon of life, that is ground for hope rather than despair. We can see fairly clearly what type of physical and chemical knowledge will be requisite for this interpretation, and we realize that the merest rudiments of this essential knowledge have been so recently acquired that they are yet unfamiliar tools, of which the consequences are barely apprehended.

In this volume, a preliminary essay has been made towards that interpretation of development which M. Bergson declares to be intellectually impossible. An hypothesis of this kind must necessarily, "in this, our infant state of knowledge," abound in imperfections which will subsequently become manifest. Yet it too, may evolve by adaptation until it comes to a fruition which will truly comprehend the phenomena, and if it should ever achieve this, then it will have accomplished the task for the performance of which it has been contrived.

T. B. R.

Adelaide,
South Australia.
January, 1923.

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THE CHEMICAL BASIS OF GROWTH AND SENESCENCE

CHAPTER I

INTRODUCTION

1. **The Self-accelerated Character of the Growth-process.**—Regarded from the chemical point of view, the growth of plants or animals consists, essentially, in the transformation of simple, unorganized foodstuffs, such as inorganic salts, fats, carbohydrates, amino-acids, and so forth, into new chemical entities which, regarded collectively, form the organized protoplasm of the plant or animal tissues. Growth, therefore, involves the synthesis of a variety of chemical compounds in due proportion and succession to one another.

This process obviously does not take place with uniform velocity throughout life. It is not at all unusual, for example, for an infant to grow at the rate of two pounds per month during the first months succeeding birth. Were this rate of growth maintained, then at twenty-six years of age we would weigh something in the neighborhood of five hundred pounds. Nevertheless, the process of growth is not one which undergoes a uniform retardation, diminishing in velocity by a similar proportion per annum. On the contrary, the growth of children, as also that of animals or plants, takes place in spurts, separated more or less distinctly from one another by periods of relatively languid growth. Thus, the rate of growth *in utero* during the first half of gestation is so slow that the weight of the human fetus is inappreciable in comparison with that of the mother during this period.[534]

This interval of slow growth is succeeded by the extraordinarily rapid accretion of tissue which characterizes development during the months immediately prior to and succeeding delivery.

Towards the end of the first year of extra-uterine life, however, there occurs a definite slackening of growth which is not an artefact, dependent upon weaning, since it occurs just as strikingly in artificially fed infants as in infants which are suckled. This resting period or "plateau" in the curve of growth is succeeded by the relatively rapid growth of the third, fourth and fifth years of life. Another pause or slackening of growth succeeds this, to be followed by the energetic growth which accompanies adolescence.

The growth of man, therefore, consists of periods of rapid and slow growth which alternate with one another, so that if we plot the growth in any dimension, for instance weight, on "coördinate paper," measuring weights vertically and ages horizontally, we obtain a diagrammatic picture of the growth-process which is not a straight line, nor even a single curvilinear sweep, like the outline of a parabola, or of the logarithmic curve which represents the progress of the ordinary type of chemical reaction. On the contrary, our diagram reveals distinct waves, or large oscillations in the growth-process, and resembles, as a matter of fact, the diagram which may be obtained by superimposing three S-shaped curves upon one another in such a manner that their adjacent extremities merge into one another.

Similar phenomena are exhibited in the growth of every animal which has been carefully studied from this point of view. Thus Minot[304] and Read[384] have described them in considerable detail in the guinea-pig, and Donaldson[99] in the rat. Ostwald[362] and Robertson[402] have observed three successive periods of rapid growth alternating with periods of slow growth in the mouse, and Brody has described the same phenomena in the develop-

ment of the fowl.[46] Portions of the total growth-curve, revealing the same peculiarities, have been described in a variety of animals.[362,389] Similar phenomena have been described in the growth of annual plants.[385,390]

These oscillations in the curve of growth are not accidental. They are readily distinguishable from the minor fluctuations which occur at frequent and irregular intervals in the growth of every individual. These irregular fluctuations, due to minor ailments, to temporary hyper- or hypo-activity of the muscular or endocrine systems or, in the case of plants especially, to climatic variations, are as frequently positive, *i.e.*, supernormal, as they are negative or subnormal, and hence, in the average curve supplied by a sufficient number of individuals, they cancel out and disappear. The larger fluctuations, or "Cycles of Growth," as they have been termed,[389,402] appear always at the same place in the total growth-curve of any group of animals of the same sex and species, and hence, so far from being smoothed out in the average curve from a large number of animals, they are actually emphasized. Furthermore, although the positions and relative magnitudes of the cycles differ in different species, the few species of mammals and birds which have as yet been studied throughout the duration of their development agree in displaying three successive cycles of normal growth.[a]

These growth-cycles are all alike in that they consist each of a period of slow growth, followed by a period of relatively rapid growth, which, in turn, is succeeded by a period of slow growth, culminating in the attainment of the maximum dimensions attributable to the cycle. Usually the curve expressing any one cycle is symmetrical,

[a] The rat may possibly constitute an exception to this rule. In the curves given by Donaldson,[99] while the ante-natal cycle is well defined, there appears to be but one post-natal cycle. On the other hand, in the mouse the two post-natal cycles, although distinguishable, are nevertheless partially fused,[402] and it is probable that in the rat two post-natal cycles actually exist but are still more completely fused. (See Chapter III.)

that is, the latter half of the cycle, during which the rate of growth is decreasing, and which we may term the "autostatic phase," reproduces inversely the first half of the cycle, during which the rate of growth is progressively increasing, and which we may term the "autokinetic phase."[b] Thus if we imagine the S-curve to be turned about its centre through an angle of 180°, the resultant curve would be indistinguishable from the original curve. The number of instances in which a cycle of growth has been defined with quantitive exactitude is, however, not as yet sufficient to enable us to affirm that this symmetry is an invariable characteristic. In certain instances, for example, in the third growth-cycle of the Rodentia, a decided asymmetry of the growth-process is evident, but in these animals it is difficult to decide whether this really indicates asymmetry of a single growth-cycle, or merely fusion of two or more cycles of differing velocity, for the cycles succeed one another so rapidly that the resultant growth at any moment is to some extent contributed by each of the post-natal cycles, and the proportion of the total effect which is due to any individual cycle has not, as yet, proved possible to determine.

Thus in the growth of an organism we have a process which takes place at first slowly, later with greatly accelerated velocity, and later still as slowly as it began. In seeking to interpret the meaning of this characteristic of the growth-process we are immediately led to inquire whether any chemical processes of this particular type are known to occur elsewhere than in the tissues of a growing organism.

As a matter of fact a number of processes of this character are familiar to the chemist. As examples we may cite the decomposition of cane-sugar by boiling (neutral) water,[221] the decomposition of castor-oil in the pulverized seeds of the castor-oil bean,[75] the decomposition of methyl

[b] The terms "autostatic" and "autokinetic" have formerly been employed in a somewhat different sense to that in which they are used here.[395]

acetate by initially neutral water,[361] the oxidation or "tarnishing" of most metals, and the oxidation of a variety of organic materials.[298] All these diverse processes have this feature in common, namely, that one of the products of the chemical change which is going on has the property of accelerating or "catalyzing" the further

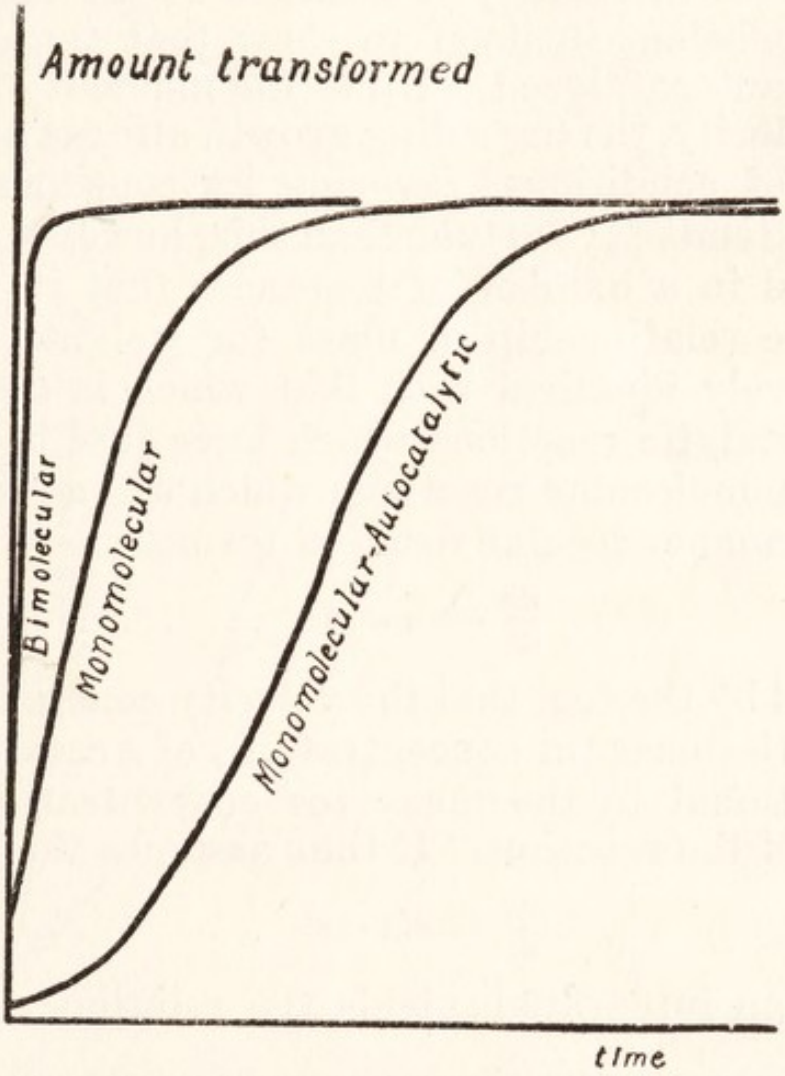


FIG. 1.—Comparison of the relationship of extent of transformation to time in monomolecular, bimolecular, and autocatalyzed monomolecular reactions.

progress of the change. Thus, in the hydrolysis of cane-sugar by neutral water, a certain proportion of mucic acid is formed, which accelerates the conversion of cane-sugar into invert sugar. In the decomposition of methyl acetate, acetic acid, itself one of the products of the decomposition, accelerates the hydrolysis of the methyl acetate. In the oxidation of the metals, the peroxides which are formed act as "carriers" of oxygen to the yet

unoxidized portions of the metal. All of these processes are, in a word, "autocatalyzed" or self-accelerated. The fact that the reaction has occurred is, up to a certain point, a favoring condition for its further occurrence.

The fact that each growth-cycle begins slowly, and progressively increases in velocity until the moment of maximal growth-velocity is attained at the centre of the cycle, is sufficient in itself to show that the process of growth is autocatalyzed. Up to the moment of maximal growth-velocity, the preceding growth affords a condition, or series of conditions, favoring its continuance. The analogy extends far further than this, however, for it has been found in a number of instances that in a cycle of growth the relationship of mass (or weight) to time is quantitatively identical with that which is displayed in the autocatalytic reactions which have just been cited.[c]

In monomolecular reactions which are autocatalyzed, the usual monomolecular reaction-formula:—

$$\frac{dx}{dt} = k(a - x) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

is modified by the fact that the velocity-constant k is multiplied by the mass (or concentration) of a catalyzer which is proportional to the mass (or concentration) of the products of the reaction. It thus assumes the form:—

$$\frac{dx}{dt} = k_1 x(a - x) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

which, when integrated, yields the relation:—

$$\log \frac{x}{a - x} = K(t - t_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

where $K = k_1 a$ and t_1 is the time at which the reaction is half completed, that is when $x = \frac{1}{2}a$. In the accompanying diagram (Fig. 1) the relationship of the extent of the transformation to the time which has elapsed since the initiation of the reaction in an ordinary monomolecular or bimolecular reaction is compared with that which obtains in an autocatalyzed monomolecular reaction. The similar-

[c] See Chapters II and III.

ity of the latter to the curve of growth (Fig. 2) is very evident.

The formula embodied in Equation 3 has been applied to the infantile growth-cycle of man and yields expected weights which, as we shall subsequently see (Chapter II), are in extraordinarily close agreement with the actual

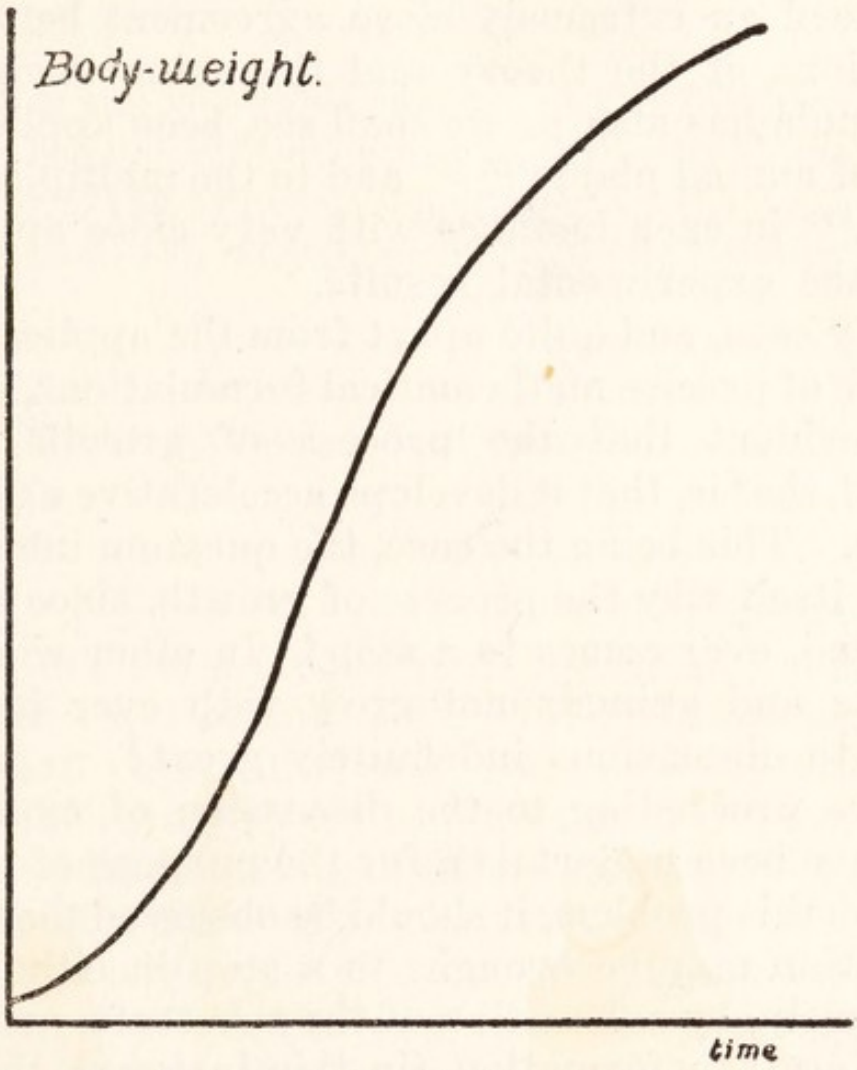


FIG. 2.—Relationship of body weight to age in the white rat (constructed from the measurements of Donaldson).

figures. Comparison with the formula is in this case very much facilitated by the comparative isolation of the infantile growth-cycle in the total growth-curve of man. It is so extremely rapid in comparison with the next succeeding cycle, which is also delayed in its onset, that little disturbance of the autocatalytic time-relations, due to the succeeding cycle, is evident during the first post-natal year of life. In other animals, or the remaining cycles

of human growth, comparison with the formula is rendered difficult by the partial blending of the cycles at their extremities, or even during a considerable proportion of their total duration. Brody and Ragsdale, however, have carried out the comparison in the case of the extra-uterine growth of the cow, and their results similarly reveal an extremely close agreement between the expectations of the theory and the actual weighings.[47] The formula has also, as we shall see, been applied to the growth of annual plants[385,390] and to the multiplication of bacteria,[295] in each instance with very close approximation to the experimental results.

In any case, and quite apart from the applicability or otherwise of precise mathematical formulations, it is sufficiently evident that the process of growth is autocatalyzed, that is, that it develops accelerative agents as it proceeds. This being the case, the question immediately presents itself why the process of growth, since it is self-accelerated, ever comes to a stop? In other words, why do plants and animals not grow with ever increasing velocity to dimensions indefinitely great?

Before proceeding to the discussion of experiments which have been undertaken for the purpose of throwing light upon this problem, it should be observed that a chemical reaction may be brought to a stop in either of two ways, namely, by exhaustion of the substrate, or material undergoing transformation (in this instance, the aggregate of foodstuffs) or, on the other hand, by the accumulation of the products of the reaction, resulting in the acceleration of the reverse reaction to the point of equality of its velocity with that of the forward reaction.

In the derivation of Formulæ 2 and 3, regard was paid only to the forward reaction. It may readily be extended to include the consequences of the reverse reaction in the manner about to be described, assuming that, as usual in catalyzed reactions, the catalyzer accelerates the reverse

reaction to precisely the same degree that it accelerates the forward reaction.

If the reverse reaction also concerns but one species of molecule (to the concentration of which, as a product of the reaction, the catalyzer is proportional) then we have:—

$$\frac{dx}{dt} = k_1 x\,(a - x) - k_2 x^2$$

where k_1 is the velocity-constant of the forward reaction, and k_2 is the velocity-constant of the reverse reaction, the symbols a, x and t having the same meaning as in Formula 2. This equation may be rearranged as follows:—

$$\frac{dx}{dt} = (k_1 + k_2)\, x \left(\frac{k_1}{k_1 + k_2}\, a - x \right) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

which, when integrated, yields:—

$$\log \frac{x}{A - x} = K(t - t_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

where $A = \frac{k_1}{k_1 + k_2}\, a$ and k, as in Equation 3 $= k_1 a$.

Thus the extended formula is indistinguishable in form from the Formula 3 which defines the progress of the forward reaction without reference to the retarding effect of the reverse reaction, the only difference between the two residing in the diminished magnitude of the maximal attainable mass of the product at equilibrium ($=A$) in comparison with its value in the unretarded reaction. The value of A, in the growth of an organism, is the maximum degree of growth attainable in the growth-cycle and is therefore accessible to measurement. The value of a, however, which would be the maximum growth attainable if the reaction of protoplasmic synthesis were not subject to retardation by its simultaneous reversion, is not similarly accessible to measurement. It may be expected to bear a proportion to the abundance of nutrients available to the cells forming the organism (the "nutrient level"), and if any one essential ingredient of protoplasm, such as an amino-acid, chances to be rela-

tively deficient in the dietary, the value of a may be expected to stand in a fixed proportion to the mean available quantity of this limiting factor. But we are as yet not in a position to evaluate these proportionality ratios, and in the absence of such quantitive information the absolute value of a must necessarily remain unknown. Hence, since we cannot ascertain to what extent A in any growth-cycle differs from a, we obviously cannot ascertain in any given case whether or not it differs from a at all. In other words, it is at present impossible, by the mere inspection of the time-relations of a growth-cycle, to ascertain whether the ultimate cessation of growth is due, primarily, to the exhaustion of food-materials, or, on the contrary, to the retarding effect of the accumulation of the products of growth.

The curve which is defined by Equation 5 is also symmetrical about its centre, when $t = t_1$ and $x = \frac{1}{2}A$, and this, as we have seen, corresponds with the facts observed in those growth-cycles which have so far been subjected to quantitative comparison with the autocatalytic formula. If, however, the reverse reaction were bi- or multimolecular instead of monomolecular, the general type of phenomena displayed would be similar, but the curve expressing the relationship of the mass (or weight of the organism) to time would become asymmetrical.

2. **The Principle of the "Master-reaction."**—The extraordinary simplicity of the relationship which actually obtains between growth and time of growth in many instances, constitutes at first sight a very real obstacle to the interpretation of the phenomena. We are only too well aware that the process of protoplasmic synthesis is actually an exceedingly complex one, involving multitudinous parallel, successive and independent reactions. How, then, can it be possible that any simple formula, such as that which characterizes the progress of a monomolecular autocatalyzed reaction, can define the time-

relations which govern such an immense complexity of events?[69]

Two considerations, however, supply a very evident avenue of escape from this dilemma. The first is that in any system of interdependent processes, of no matter what description, the specifically slowest process sets the pace for the rest. In a system of chemical reactions of which each one derives its substrates from the preceding reaction and in its turn supplies raw materials for the reaction which follows it, the specifically slowest reaction in the series will become the "Master Reaction" governing the time-relations of the whole. In the same way, in a factory the slowest of the diverse operations determines the daily output of the finished product. If raw materials are brought up to the factory too quickly, they must accumulate until this slowest operation can perform its part in their transformation. If, on the other hand, the subsequent disposal of the produce of this slowest operation is too rapid, the whole of the machinery involved in this latter part of the process of manufacture must stand idle until the slowest operation has provided fresh material. Thus we may conclude that in the chain of processes which culminate in the production of living matter, there is one which is slower than any of the rest, so that the whole concatenated series of events waits upon this "Master Reaction." We must further infer that this reaction is of such a character that it simulates, in its time-relations, an autocatalyzed monomolecular transformation. We wholly agree with Child that "growth is not a simple chemical reaction and cannot be considered as such: it is a complex physico-chemical process in which changes in the physical character of the substratum as well as chemical conditions are concerned" ([69] p. 453). Nevertheless the facts obviously compel us to conclude that the whole of this great complexity of events waits upon and is set in motion by a process which, taken by itself, is of such a character as to admit of representation by the autocatalytic formula.

In the second place, in the growth of the higher metazoa at all events, the substrates or raw materials for the manufacture of protoplasm do not actually diminish at all, or at any rate to any important degree, while growth proceeds. The medium in which the constituent cells of such an organism are actually immersed is the pericellular fluid which ultimately derives its nutritive constituents from the digestive cavity. The raw materials for the manufacture of protoplasm are constantly renewed in this fluid by the act of feeding. Thus while the composition of the nutrient medium which bathes the cells is subject to short period fluctuations of variable magnitude, corresponding to fluctuations of digestive activity, yet in the long run, if foodstuffs are accessible to the animal, the average flow of nutrient materials to the tissues is maintained by the act of feeding. It is a fact of fundamental significance that the evolution of the higher types of animals has been accompanied by an increasing perfection of various assimilative, circulatory and excretory mechanisms, which are adapted to maintain constancy of the "nutrient level" in the pericellular fluid. In consequence of these mechanisms the medium in which the cells of vertebrate animals live is, under normal dietetic conditions, a medium of almost constant composition and, for the purposes of tissue synthesis, it is inexhaustible since it is continually being renewed.

The substrates, or raw materials for the manufacture of protoplasm, are therefore, as the phenomena of regeneration and repair daily reveal to us, always present in the body fluids of a well-nourished animal in ample quantity to permit the synthesis of living tissue. As a matter of fact the assimilation of food materials, in the Vertebrates at all events, is customarily in excess of the immediate bodily requirements, the surplus nutrients being either stored up in the form of reserves which are gradually discharged during the intervals of assimilation (in the case of the fats and carbohydrates), or else de-

composed and eliminated (in the case of the proteins). The preservation of a relatively constant nutrient level in the pericellular fluids is thus assured. This being the case, the time-relations which govern the growth of an animal, although simulating those which obtain in an autocatalyzed monomolecular reaction, do not exclude the possibility that the forward reaction which governs the rate of growth may actually be multi-molecular rather than monomolecular. For if the substrates ($= a$) are not diminished by the process of protoplasmic synthesis, then if n molecules of various substrates combine or interact to form one molecule of the product, the velocity of the forward reaction will be defined by the equation:—

$$\frac{dx}{dt} = k_1 x a^n$$

while that of the reverse reaction, as before, will be defined by the equation:—

$$-\frac{dx}{dt} = k_2 x^2$$

so that the net effect, or actual growth, would be given by:—

$$\frac{dx}{dt} = k_1 x a^n - k_2 x^2$$

which, rearranging the terms, becomes:—

$$\frac{dx}{dt} = k_2 x \left(\frac{k_1}{k_2} a^n - x\right) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

which, when integrated, becomes:—

$$\log \frac{x}{A - x} = K (t - t_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

where $A = \frac{k_1}{k_2} a^n$ and $K = k_1 a^n$.

The equation thus remains of the monomolecular form, but A, the maximal attainable growth, is no longer directly proportional to the mean concentration of available foodstuffs, but to the n^{th} power of this quantity. Again, because the absolute value of a, in terms of growth,

is not discernible at present, we are unable to define, by the mere observation of the time-relations of a growth-cycle, what relationship the observed value of A actually bears to a. So far as the time-relations of growth are concerned, therefore, in the higher metazoa at all events, we have no present means of ascertaining whether the forward reaction which governs protoplasmic synthesis is actually monomolecular or multi-molecular. In any case the time relations would simulate those displayed by a monomolecular reaction. Obviously, the probability is that the "Master Reaction" of growth is actually multi-molecular.

The monomolecular character of the reverse reaction presents no similar difficulties. Here we are dealing with the decomposition of some doubtless complex material and such decompositions, in the tissues of an organism, are usually either hydrolyses or oxidations. In either case the decomposing agent, water or oxygen, is continuously renewed from the surrounding media, so that only one species of molecule, in this instance the complex product of growth, is actually altering in concentration with time.[d] The symmetry of the curve of growth about its centre is therefore not necessarily indicative of simplicity.

If the reverse reaction of the governing process of protoplasmic synthesis were actually m-molecular instead of monomolecular, then the net velocity of growth would be given by:—

$$\frac{dx}{dt} = k_1 x a^n - k_2 x^{m+1}$$

which, by rearrangement, becomes:—

$$\frac{dx}{dt} = k_2 x \left(\frac{k_1}{k_2} a^n - x^m \right) . . . . . (8)$$

[d] The molecule of the autocatalyst need not necessarily be identical with the molecule of the supposedly complex product which is the objective of the reaction. It may be quite a different substance and a comparatively simple one, provided only that its concentration is proportional to that of the main product.

The integral form of this equation is:—

$$\log \frac{m x}{A - x^m} = K (t - t_1) \ldots \ldots \ldots \ldots \quad (9)$$

where $A = \frac{k_1}{k_2} a^n$ and $K = k_1 m a^n$.

In this case the amount of growth accomplished during the period in which the velocity of growth is increasing is no longer equal the amount of growth accomplished during the second phase, in which the velocity of growth is decreasing, and the curve, turned through 180°, is not identical with the original curve. The maximum velocity of growth is reached when $x^m = \frac{A}{m+1}$ and the maximum attainable value of x is given by $x^m = A$. As the value of m increases in the series 1, 2, 3, etc., the proportion of the total growth of the cycle which is accomplished in the autokinetic phase, or period of increasing growth-velocity, decreases in the series ½, ⅓, ¼, etc. Hence, if the reverse reaction in the governing process were of a higher order than the first, the consequent growth-curves would be asymmetrical, the larger portion of the curve lying to the right of the point of inflection, or moment of maximum growth velocity.

The apparent asymmetry of growth-curves in certain instances, as in the adolescent growth-cycle in the mouse or rat, may possibly indicate that the Master Reaction of protoplasmic breakdown is, in these instances, of a higher order than the first. In many cases, however, the growth-curve for a given cycle is truly symmetrical, or so nearly symmetrical, as for instance in the infantile growth-cycle of man, that the slight departures from symmetry are far less than those which would result if the reverse reaction in the growth-controlling process were of a higher order than the first. In such instances, therefore, the Master Reaction of protoplasmic breakdown must be monomolecular.

While not impossible, it does not appear very likely that a process so fundamental in the life of every cell differs so essentially in different species of mammals as to vary in the number of molecular species involved. Since the apparently asymmetric curves occur in just those animals in which the rapid succession of cycles compels their fusion during a considerable portion of their total extent, it appears more probable that the asymmetry in these instances is due to the partial fusion of a rapid growth-cycle with a specifically slower process of growth.

CHAPTER II

THE PHYSICAL MANIFESTATIONS OF GROWTH IN MAN

1. Pre-natal Growth.—The determination of the time-relations of growth during the early months of pregnancy is beset by the same difficulties which attend the determination of the period at which development begins. The uncertainty attaching to our estimate of the age of the fetus necessarily vitiates the accuracy of our growth-diagram, and that in greater degree the greater proportion the period of uncertainty bears to the period of development which the fetus has undergone. Consequently our estimate of the weight or length of the human fetus at various periods of the intra-uterine life is very inexact during the early months of pregnancy. These estimates, however, become increasingly more accurate as we approach the termination of gestation, so that the error in the estimate of the period of gestation becomes a less important proportion of the age of the fetus.

According to Zangemeister,[534] the average length and weight of the human fetus in the various lunar months of pregnancy are as follow:—

TABLE I

AGE AND DIMENSIONS OF THE HUMAN FETUS

Month	Length cm.	Weight gm.	Monthly increment in weight gm.
1	0.7	1.0	——
2	2.5	2.5	1.5
3	9.0	12.4	9.9
4	13.4	89.8	77.4
5	18.9	252.7	162.9
6	31.6	795.5	542.8
7	34.5	1066.0	270.5
8	39.3	1540.1	474.1
9	45.5	2243.3	703.2
10	49.9	3242.4	999.1

For the reasons indicated, especially in the earlier months, these estimates are possessed of only qualitative value. They indicate, however, that the later portion of the growth in utero is part of a single growth-cycle, being interrupted by no pause or period of slackened growth, and that the velocity of growth (as indicated by the monthly increment in weight) increases continuously, reaching a maximum just before delivery. Regarding the possible existence of cycles of comparatively brief duration in the earlier period of development, these figures naturally fail to supply us with any information. The later portions of the pre-natal curve of growth may be determined with greater precision from the weights of infants born at varying periods prior to the normal date of delivery. The reliability of this method of procedure may be tested by also determining the weights of infants born somewhat later than the normal period.[396] Since this latter curve is identical with the continuation of the normal curve of extra-uterine growth that begins with the time of birth, we have reason to infer that the curve determined by the weights of prematurely delivered, but otherwise normal children, represents the further continuation of the same normal curve of growth backwards into the intra-uterine period.

The data employed for these comparisons were exclusively obtained from "The Queen's Home," a maternity hospital in Adelaide, South Australia, and cover the years 1909–1913. Only those data were employed in which the period of gestation, reckoned from the date of the last menstruation, was definitely known. All infants stated to be suffering at birth from syphilitic infections, deformities, etc., and all infants who died within one week after delivery, were excluded. The infants delivered by mothers who were suffering at the time of delivery or prior thereto from zymotic disease or other pathological conditions of a serious nature, were also excluded.

The following (Table II) are the results obtained for males. All infants born between 275 and 285 days after the onset of the last menstruation are tabulated as having been born at 280 days, all between 285 and 295 days as having been born at 290 days, etc. Those born upon a limiting period separating two classes (*e.g.*, 285 days) are included in both classes (*e.g.*, 280 and 290).

TABLE II. MALES
(UNCORRECTED DATA)

Days pregnancy	Number of infants	Average weight in ounces[a]
190	1	27
200	0	—
210	1	54
220	0	—
230	0	—
240	1	123
250	2	117
260	22	119
270	38	121
280	79	127
290	78	130
300	16	139
310	9	138
320	3	123
330	1	152
340	0	—

A general tendency for increasing weight at birth to accompany increasing length of pregnancy is evident, but this becomes still more striking on correcting the above figures in the following way.

The most probable error in the estimation of the period of pregnancy is that of one month. Hence, it is assumed that all periods of gestation which culminate in the delivery of infants weighing less than the uncorrected average for those born thirty days earlier, or more than the uncorrected average for those born thirty days later, are erroneously estimated. Infants delivered in these

[a] The ounce avoirdupois (1 ounce = 28.34 grams) is used as the unit of weight because, in the first place, the weights of the infants were actually measured in terms of this unit and, in the second place, it is a unit of very convenient dimensions for this purpose.

cases are therefore excluded. In this way we also probably exclude the majority of crypto-syphilitic infants born at the later periods of gestation.

The following (Table III) are the corrected results for males. This table excludes those born before 260 or after 310 days, since they are so few in number and possibly represent either pathological or erroneously estimated gestations.

TABLE III. MALES
(CORRECTED DATA)

Days pregnancy	Number of infants	Average weight in ounces
260	16	111
270	34	117
280	79	127
290	60	137
300	13	145
310	6	146

The following are the corresponding figures for female infants:—

TABLE IV. FEMALES
(UNCORRECTED DATA)

Days pregnancy	Number of infants	Average weight in ounces
190	1	39
200	2	59
210	0	—
220	1	83
230	2	90
240	3	95
250	6	97
260	10	111
270	32	113
280	80	117
290	86	125
300	31	129
310	14	130
320	3	127
330	1	134
340	0	—

The data for the 280-day infants were not corrected for three reasons. Firstly, because the 280-day group is

TABLE V. FEMALES
(CORRECTED DATA)

Days pregnancy	Number of infants	Average weight in ounces
250	6	97
260	8	105
270	27	108
280	80	120
290	70	130
300	25	133
310	11	138

the group nearest the mean, the mean period of gestation for male infants being 282.5 days, **secondly**, because the average weight of the 280-day infants (127 ounces) is the nearest to the average weight of all the infants (127.3 ounces), and **thirdly**, because errors in the estimation of this period would obviously tend to cancel one another.

These figures show perfectly clearly a regular and uninterrupted increase in weight during the last eight weeks of gestation. The average rate of increase throughout the period investigated is 21 ounces (595 grams) per month for the males and 20 ounces (567 grams) per month for the females. There is no evidence of any fluctuation of growth-velocity such as would indicate the participation of more than one growth-cycle. We may therefore infer that the later months of development represent the continuation **in utero** of the first extra-uterine growth-cycle.

2. **The Duration of the Period of Gestation.**—From the above data it is possible to construct a curve displaying the intra-uterine growth of infants from the 250th day of gestation until the 310th day. In order to determine which portion of this curve represents **normally** intra-uterine growth and which portion represents normally extra-uterine growth, however, it is necessary to determine with some exactitude the mean length of the period of gestation for man.

It is customarily assumed[517] that the normal duration of pregnancy is 280 days, reckoned from the first day of

the last menstrual period. Hecker[88] has determined the duration of pregnancy in 109 cases with the following results :—

TABLE VI

Days pregnancy	Percentage of deliveries
258	0.92
265	4.60
272	14.72
279	35.88 : Mode
286	23.00
293	12.88
300	5.52
307, 314, 321	0.92

The Mode, or period of greatest frequency of delivery, obviously coincides very closely with the usual estimate of 280 days.

From a statistical study of 511 normal confinements of South Australian females,[401] of which 247 yielded male infants and 264 female infants, the mean length of periods of gestation yielding males has been computed to be 282.5 days with a "probable error" of 0.55 days, and the mean length of periods of gestation yielding females to be 284.5 days with a "probable error" of 0.57 days. The distribution of the deliveries is shown in the following table :—

TABLE VII

Period of gestation in days	Percentage of 247 male infants born at the designated period	Percentage of 264 female infants born at the designated period
240	0.0	1.1
250	0.8	2.3
260	8.9	3.8
270	15.4	12.1
280	32.0	30.3
290	31.6 : Mode	33.6 : Mode
300	6.5	11.4
310	3.6	5.3
320	1.2	1.1

From these results we may infer that there is only one period, the "normal" period, at which the percentage of infants delivered by normal mothers attains a maximum. The "frequency-curve" which illustrates the distribution of deliveries among the various periods of gestation on either side of the mean is obviously unimodal. In the guinea-pig, on the contrary, it is distinctly bimodal. On tabulating the percentages of deliveries at different periods of gestation in this animal[383,384] two groups of maximum frequency are seen to occur, a smaller "premature" group rather definitely situated, and a larger "normal" group separated from the former by a period in which deliveries are relatively infrequent. This phenomenon is attributed by Read to the fact that intra-uterine growth in the guinea-pig involves the completion of one cycle of growth and the initiation of a second, and that the "premature" group of deliveries occurs at the termination of the first cycle. The unimodal frequency-curve of deliveries in man, therefore, affords confirmatory evidence that there is no junction of growth-cycles in the later months of intra-uterine development, and that in man the greater part of the intra-uterine growth is comprised by the first portions of the "infantile" growth-cycle which is completed during the first year of extra-uterine life.

3. **Bodily Dimensions at Birth.**—The weight of the average infant at birth may be regarded, in view of the comparative invariability of the average duration of gestation, as the weight at a period of approximately 280 days after the initiation of development.

The weight of the infant at birth is very strikingly influenced by a variety of factors, among which may be especially mentioned the nutrition of the mother,[327] the number of previous pregnancies, sex, race, and environment. According to Fourmann[276] each successive pregnancy leads to an average increase of 75 grams (2.65 ounces) in the weight of the child at delivery. The effect

of the first pregnancy is more striking than that of subsequent pregnancies.[177]

The influence of race upon the weight at birth is shown in the following table:—

TABLE VIII

AVERAGE WEIGHT AT BIRTH

Race	Males	Females	Investigator
American (New England)	120.8 ounces	115.7 ounces	Bowditch [45]
English (London)	116.8 "	113.2 "	Pearson [370]
Danish	123.0 "	115.7 "	Heiberg [177]
German (South)	122.1 "	113.7 "	Fourmann [276]
Russian (North)	114.0 "	111.5 "	Tschepourkovsky [404]
Japanese	103.7 "	98.1 "	Miwa [307]

The British Anthropometric Committee[19] reports that the average weight at birth of 451 male infants born in London and Edinburgh is 113.6 ounces, while that of 466 female infants is 110.4 ounces. Through the courtesy of Drs. Smallwood Savage and Elsie M. Humpherson I have been furnished with the weights of one hundred male and one hundred female infants at birth, chosen without selection, from the records of normal full-term deliveries in the Maternity Hospital of Birmingham, England. From these data I find that the average weight of male infants at birth in Birmingham is 114.9 ounces, while that of female infants is 113.5 ounces.[398] In South Australia, however, the average weight at birth of 208 male infants, delivered after normal periods of gestation, was found to be 127.3 ounces, that of 227 female infants being 121.3 ounces.

From these figures emerges the striking fact that South Australian infants weigh from 8 to 10 ounces more at birth than infants born in Great Britain. This fact is remarkable because, according to an article on the "People of Australia" contributed by the Commonwealth Statistician, G. H. Knibbs, to the Federal Handbook on Australia issued in 1914 by the British Association for the Advancement of Science:—

The Australian people, with regard to racial constitution, are virtually British, as the following figures from the last census show, and it may be added that the descendants of other European races disclose but small differentiation from their fellow citizens of British origin. The percentages of the principal races represented are as follows:—

Australian-born 82.90 per cent.; natives of United Kingdom 13.37; of New Zealand 0.72; of Germany 0.75; of China 0.47; of Scandinavia 0.33; of all other places 1.46; that is to say, at the date of the census, 1911, no less than 97 per cent. has been born either in Australasia or in the United Kingdom.

The evolution of the Australian people, therefore, may be regarded as that of the British people under changed climatic, social, and economic conditions.

The results observed cannot be due to any racial selection among the immigrants from the British Isles who have given rise to the population of Australia. In the first place, there is no evidence that such selection has occurred to any greater extent than it has, for example, in London, of which city the very great increase in population during the past century has been contributed by all parts of the British Isles; and in the second place there is no evidence of the existence of a race in the British Isles which is in any noteworthy degree superior in physical dimensions to the average inhabitant of England.[19] We can only infer, therefore, that the superior weight of the Australian infant at birth is attributable to the factors enumerated by the Commonwealth Statistician, namely, the change in climatic, social, and economic conditions. The climate is much less rigorous than that of England. Food is cheaper in proportion to income, or, when equal in price, it is better in quality; and the social and economic conditions are so far an improvement upon those prevailing in England, that whereas women of the laboring and lower artisan classes in England have frequently to supplement the family income by their own exertions, those of the corresponding classes in Australia as a rule confine their physical activities to the management of their households and families. This condition of affairs, quite apart

from the possible direct effects of unsuitable physical labor upon the mother, must have important indirect effects in ameliorating the nutritional and hygienic conditions within the household. These conditions, therefore, taken as a whole, may be conceived as having an appreciable pre-natal effect upon the growth of children. Evidence substantiating this point is disclosed, on the one hand, by the investigations of Pinard, who has shown that the rest and improved nutrition of hospital life during the whole or a part of the period of pregnancy, causes a notable increase in the average weight of infants delivered by working mothers, and on the other hand by those of Prochovnik, who has shown[517] that the size of a child at delivery may be definitely reduced by restricting the diet of the mother.

4. Post-natal Growth.—Data concerning the growth of infants during the first year succeeding birth are exceedingly abundant, but, unfortunately, the data most readily accessible are frequently of very little value. Anyone who looks into the methods of compilation that are commonly employed will readily perceive the numerous sources of error which vitiate a large proportion of observations of this type. In the first place, the sexes are very frequently grouped together, although the above-cited investigations on the influence of sex upon the weight at birth suffice in themselves to demonstrate that this is not permissible. In the second place, the weighings are not infrequently performed carelessly on inaccurate balances by persons to whom accuracy in this connection appears superfluous; the weighings are generally, and necessarily, made at infrequent and irregular intervals. Finally, in constructing a normal standard, the data are frequently rendered still more questionable by the inclusion of sickly, diseased, or abnormal children, or infants who are the fruit of abnormal pregnancies. When we make due allowances for these and other sources of error, the

accessible and reliable data become surprisingly few and fragmentary.

More reliable data are obtainable from the records which have been collected in recent years in England and Australia by the "Schools for Mothers."[396,397,398] These institutions offer instruction to mothers regarding the feeding and care of their infants at a nominal charge. In South Australia the Registrar of Births reports at frequent intervals the births which have occurred in the neighborhood of the "Adelaide School for Mothers Institute." A nurse in the employ of the Institute then calls upon these mothers known to be more or less needy in circumstances, who are likely to have insufficient medical advice, and proffers them the services of the Institute. The infants are voluntarily brought there by the mothers upon certain days when the nurse is in attendance. The infants are weighed without clothing by the nurse, and the weight and date with other important information, such as the infant's food, illness, etc., are noted upon a card bearing the infant's name and sex. The card is then placed on file for record. The children who are brought to the Institute belong for the most part to the laboring and lower artisan classes, and include in their number, a large proportion of sickly infants, because mothers who cannot well afford private medical care for their children are quick to appreciate and take advantage of the benefits bestowed by the Institute.

The following data (Tables IX and X) computed from the records of this Institute, were accumulated in the years 1910-1913. All infants suffering from definite ailments or requiring medical attendance were excluded from these computations. When babies who were otherwise normal, contracted zymotic diseases, such as mumps, measles, etc., the last weights included in the data were those taken immediately preceding or accompanying recognition of the disease. All subsequent weighings were rejected, regardless of whether or not the disease, after its

recognition, appeared to have affected the baby's weight. The weights of infants which had been wholly artificially fed are not included among those averaged in the table, since deprivation of accessory foodstuffs is especially liable to occur in such infants and, as a matter of fact,

TABLE IX

MALES

Age in months of 30 days	Average weight in ounces
1	155
2	187
3	206
4	224
5	254
6	270
7	287
8	300
9	311
10	326
11	333
12	330

TABLE X

FEMALES

Age in months of 30 days	Average weight in ounces
1	153
2	168
3	188
4	209
5	224
6	253
7	263
8	270
9	300
10	315
11	335
12	345

direct comparison showed them to be far inferior in weight to the infants which had been wholly or partially breast-fed.

The various constants in the equation of autocatalysis may now be computed from all of the observations by the method of least squares.[b] In this way we find that for the

[b] For the methods of computation employed and tables for the facilitation of the incidental calculations the reader is referred to the appendix.

first nine months of the extra-uterine growth[c] the equation for the growth-curve for South Australian males is expressed by the formula:—

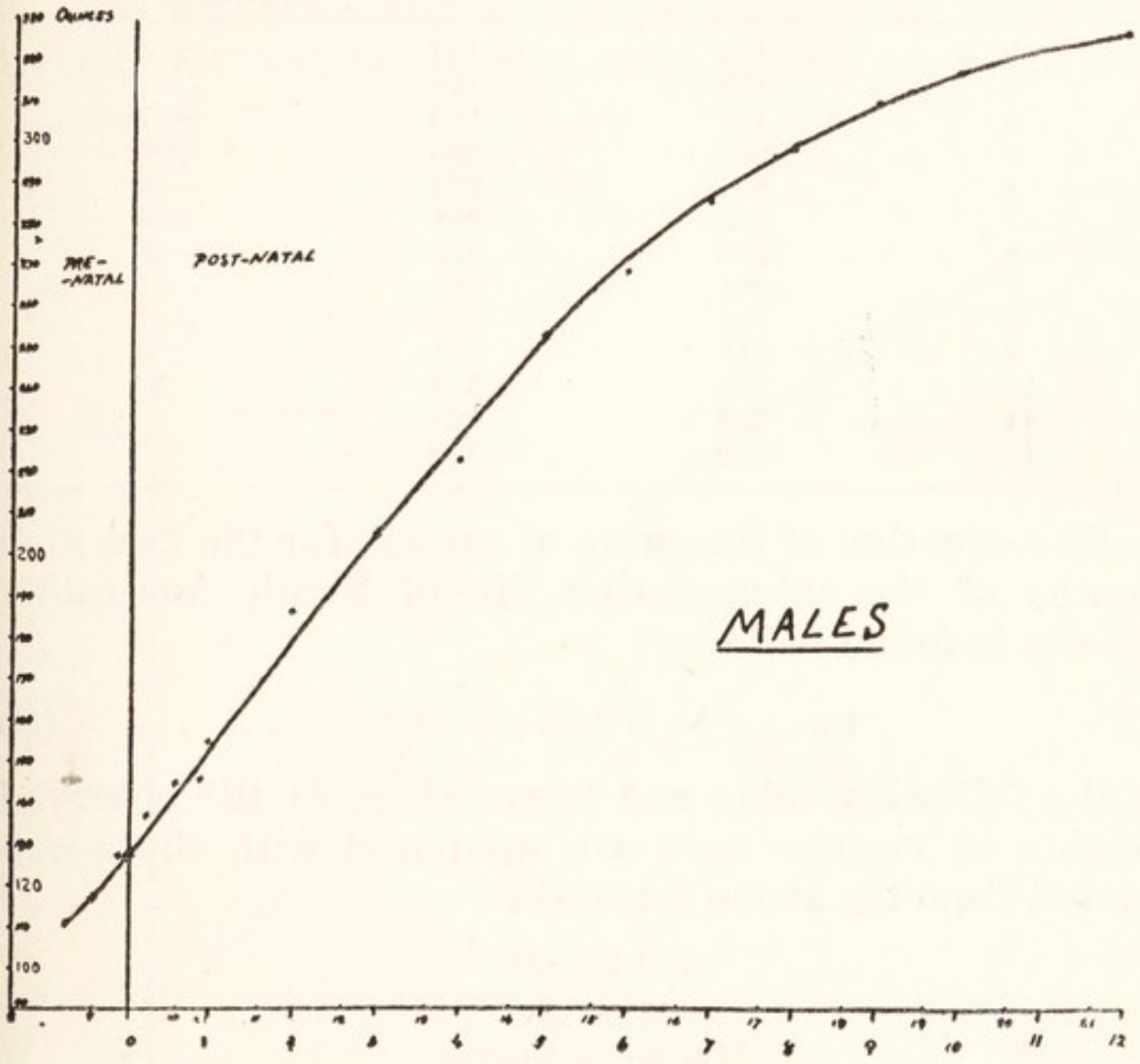


FIG. 3.—Curve of growth of South Australian male infants. The smooth curve is calculated from Equation 10 and the observed weights are indicated by dots.

$$\log_{10} \frac{x}{341.5 - x} = 0.136\,(t - 1.66) \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

t being reckoned in months from birth and x in ounces.

In the following table and figure (Fig. 3) the observed weights at the various ages are compared with those calculated from the above formula:—

[c] Only the data for the first nine months are employed in computing the constants, since in later months the effect of the second growth-cycle becomes more or less apparent.

TABLE XI

MALES

WEIGHT IN OUNCES

Age of infant in months	Observed	Calculated	Deviation from calculated value
0	127	127	± 0
1	155	156	— 1
2	187	180	+ 7
3	206	206	± 0
4	224	230	— 6
5	254	254	± 0
6	270	273	— 3
7	287	288	— 1
8	300	301	— 1
9	311	311	± 0
10	326	319	
11	333	325	
12	330	330	

The equation of the curve of growth for the first nine months of the extra-uterine life of South Australian females is found to be:—

$$\log_{10} \frac{x}{350 - x} = 0.111\,(t - 2.47) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

In the following table and figure (Fig. 4) the observed weights at various ages are compared with those calculated from the above formula:—

TABLE XII

FEMALES

WEIGHT IN OUNCES

Age of infant in months	Observed	Calculated	Deviation from calculated value
0	121	121	± 0
1	153	142	+ 11
2	168	164	+ 4
3	188	187	+ 1
4	209	209	± 0
5	224	230	— 6
6	253	249	+ 4
7	263	267	— 4
8	270	282	— 12
9	300	295	+ 5
10	315	305	
11	335	314	
12	345	321	

In both instances the agreement between theory and observation is extremely good, especially if we consider that a comparatively small number of infants (63 males and 43 females) supplied the observations.

Having thus found an algebraical formula which adequately represents the post-natal growth of South Aus-

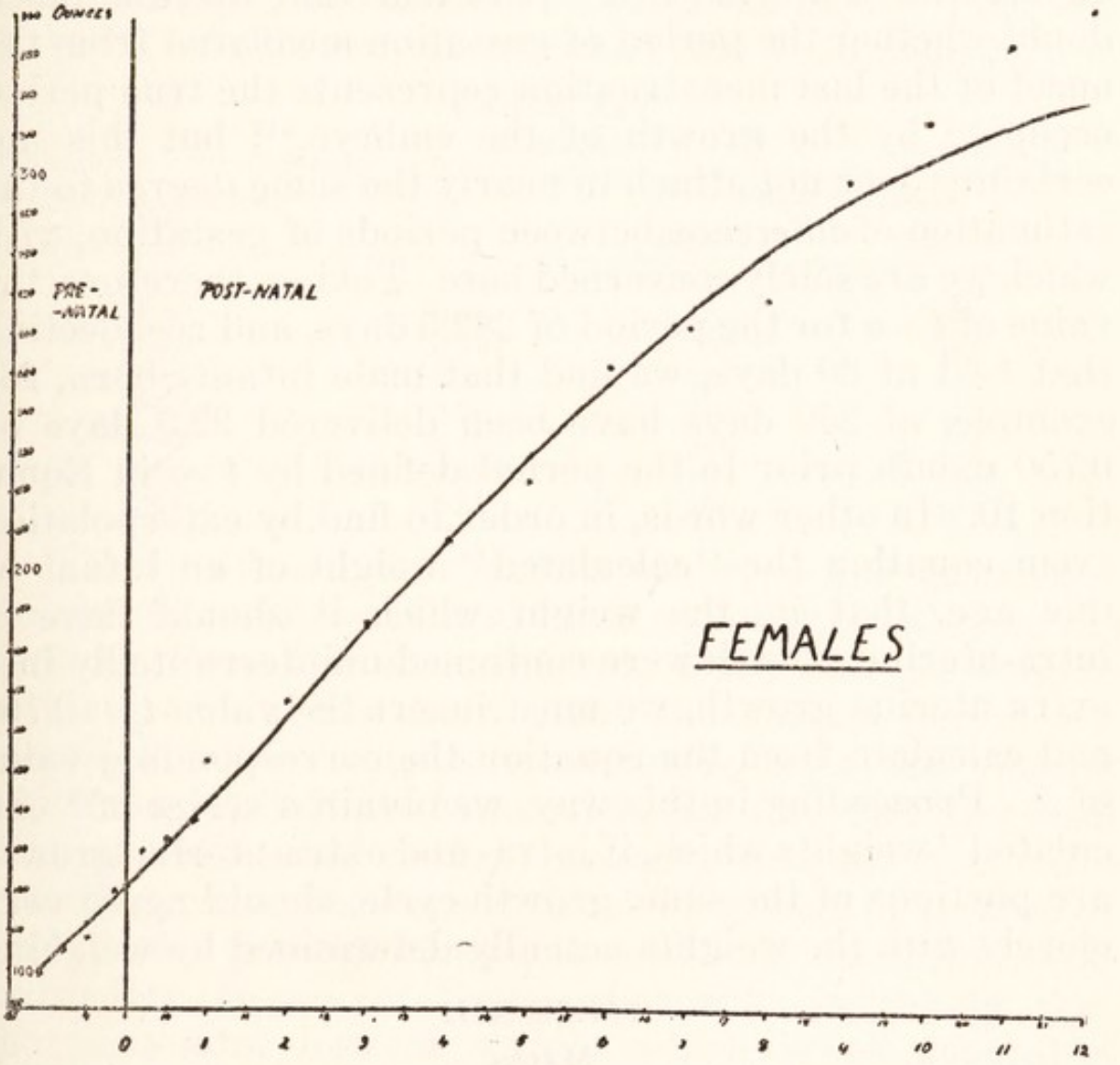


FIG. 4.—Curve of growth of South Australian female infants. The smooth curve is calculated from Equation 11 and the observed weights are indicated by dots.

tralian infants, as determined from direct measurements at varying periods succeeding birth, we can now proceed to exterpolate with the aid of this formula, that is, to continue the observed curve a short distance backward and ascertain to what degree this exterpolated curve agrees with the curve of pre-natal growth determined by the

measurement of the weight of pre- and post-maturely born infants at birth.

The mean period of gestation for South Australian male infants is, as we have seen, 282.5 days. In the Equation 10 therefore, which represents the post-natal growth of males, $t = o$ when the infant has presumably lived 282.5 days of intra-uterine life. It is true that there is much doubt whether the period of gestation measured from the onset of the last menstruation represents the true period occupied by the growth of the embryo,[517] but this uncertainty does not attach in nearly the same degree to the estimation of **differences** between periods of gestation, with which we are solely concerned here. Taking, therefore, the value of $t = o$ for the period of 282.5 days, and recollecting that $t = 1$ at 30 days, we find that male infants born, for example, at 260 days have been delivered 22.5 days or 0.750 month prior to the period defined by $t = o$ in Equation 10. In other words, in order to find by exterpolation from equation the "calculated" weight of an infant of this age, that is, the weight which it should have if intra-uterine growth were continued uninterruptedly into extra-uterine growth, we must insert the value $t = -0.750$ and calculate from the equation the corresponding value of x. Proceeding in this way, we obtain a series of "calculated" weights which, if intra- and extra-uterine growth are portions of the same growth-cycle, should agree very closely with the weights actually determined by weighing

TABLE XIII

MALES

Period of gestation in days	t	Weight in ounces	
		Observed	Calculated
260.0	— 0.75	111	110
270.0	— 0.42	117	117
280.0	— 0.08	127	126
282.5	± 0.00	127	127
290.0	+ 0.25	137	134
300.0	+ 0.58	145	142
310.0	+ 0.92	146	151

infants born at the corresponding periods. A comparison of the "calculated" and "observed" weights of male infants born at varying periods of gestation is given in Table XIII, the "observed" weights being taken from Table III on page 20.

The corresponding computations for females follow (Table XIV). In this case $t = o$ at 284.5 days. The "observed" weights are taken from Table V on page 21.

TABLE XIV

FEMALES

Period of gestation in days	t	Weight in ounces	
		Observed	Calculated
250.0	— 1.13	97	99
260.0	— 0.82	105	106
270.0	— 0.48	108	112
280.0	— 0.15	120	118
284.5	0.00	121	121
290.0	0.18	130	125
300.0	0.52	133	132
310.0	0.85	138	139

The agreement between the observed and calculated weights for both sexes is as close as could possibly be expected. From this we may infer, firstly that the weights at birth of children born after the normal period are identical, within very narrow limits, with the weights which they would have attained at that time had they been born at the normal period; secondly that the post-natal growth-cycle as determined from weighings of infants of from 1 to 9 months of age, is continued smoothly backwards into the pre-natal curve of growth; and thirdly that the post-natal loss of weight which is so general a phenomenon is not a characteristic of the infantile growth-process itself, but an accident attributable to the mechanical and nutritional shock of delivery.

Similar data for children born in England have been obtained[398] from the records of the Pimlico Road and Golden Square branches of the Westminster Health Society and Chelsea Health Society in London, and from the Leeds Babies Welcome. The weights of all in-

fants reported to be suffering from measles, whooping cough, chicken-pox, scarlet fever, diphtheria, pneumonia, bronchitis (unless specifically noted as "slight"), and marasmus were excluded on and succeeding the date upon which the condition was first noted. Data concerning twins and malformed children were likewise excluded. Furthermore, only children stated to be of British parent-

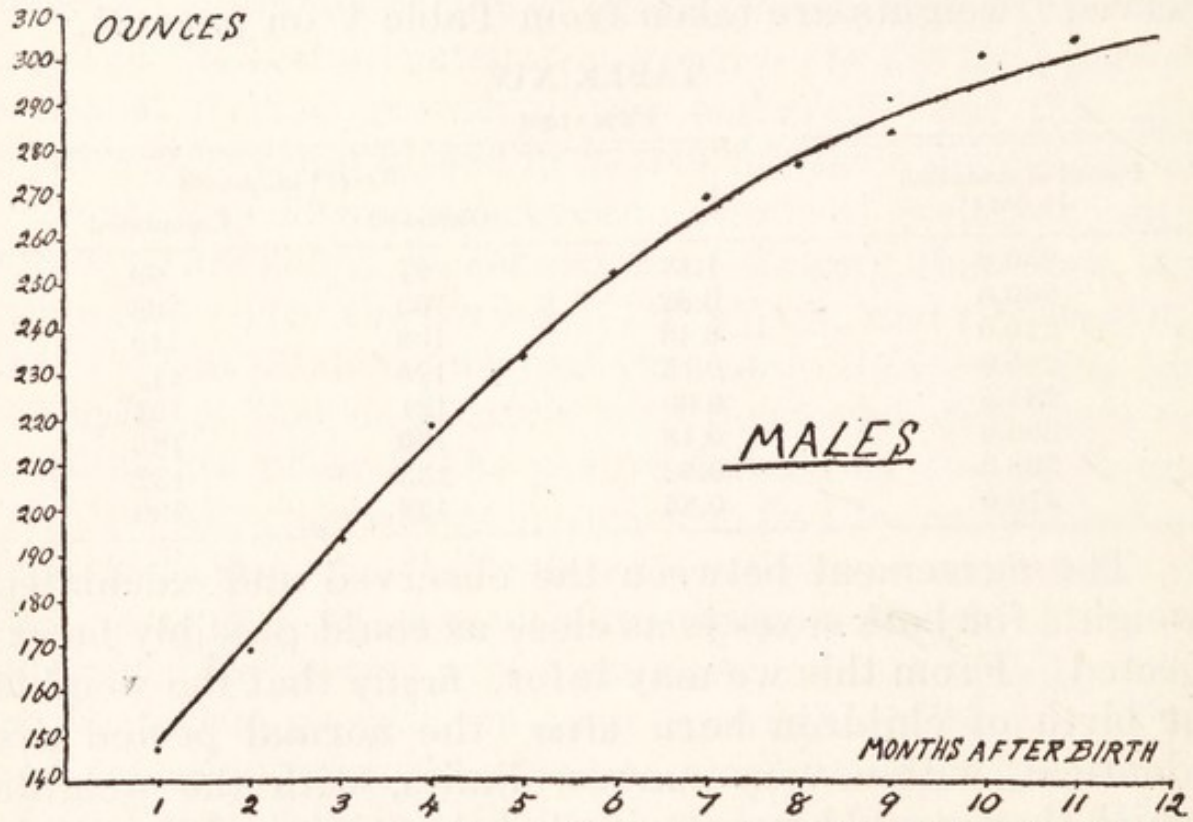


FIG. 5.—Curve of growth of British male infants born in England. The smooth curve is calculated from Equation 12 and the observed weights are indicated by dots.

age on both sides and wholly or partially breast-fed were accepted as sources of data.

The weights reported are exclusively ante-bellum and were for the most part derived from records taken in the years 1911-1914.

The application of the autocatalysis formula to these data showed that the first nine months of the growth-curve of British male infants may be represented by the equation:—

$$\log_{10}\frac{x}{318-x} = 0.127\,(t-1.4) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (12)$$

t, as before, being measured in months of thirty days each

and x in ounces avoirdupois. Inserting the observed value of t in this formula and computing therefrom the corresponding value of x, we obtain a series of "calculated" weights corresponding to the various ages. In the accompanying table and figure (Fig. 5) the observed and calculated values are compared.

TABLE XV

BRITISH MALES

Age in months	Weight in ounces		Newman standard
	Observed	Calculated	
1	147	148	123
2	169	171	142
3	194	194	161
4	219	216	180
5	234	235	204
6	252	252	228
7	269	266	244
8	276	277	255
9	283	287	268
10	300	293	282
11	303	300	295
12	314	304	308

The equation representing the infantile growth of British females proved to be:—

$$\log_{10} \frac{x}{312 - x} = 0.106\,(t - 1.54) \quad . \quad . \quad . \quad . \quad . \quad . \quad (13)$$

The comparison of theory and observation follows (Table XVI and Fig. 6).

TABLE XVI

BRITISH FEMALES

Age in months	Weight in ounces		Newman standard
	Observed	Calculated	
1	143	146	123
2	160	165	142
3	180	184	161
4	202	202	180
5	218	218	204
6	235	233	228
7	253	247	244
8	258	259	255
9	265	269	268
10	273	277	282
11	288	284	295
12	288	290	308

The agreement between the formula and the observations is all that could be desired. Such close agreement between observation and the requirements of a dynamical formula are, in point of fact, rarely obtainable in reactions occurring in laboratory glassware.[298] The probable reasons for this surprisingly exact conformity to formulated values are to be sought in the excellent condi-

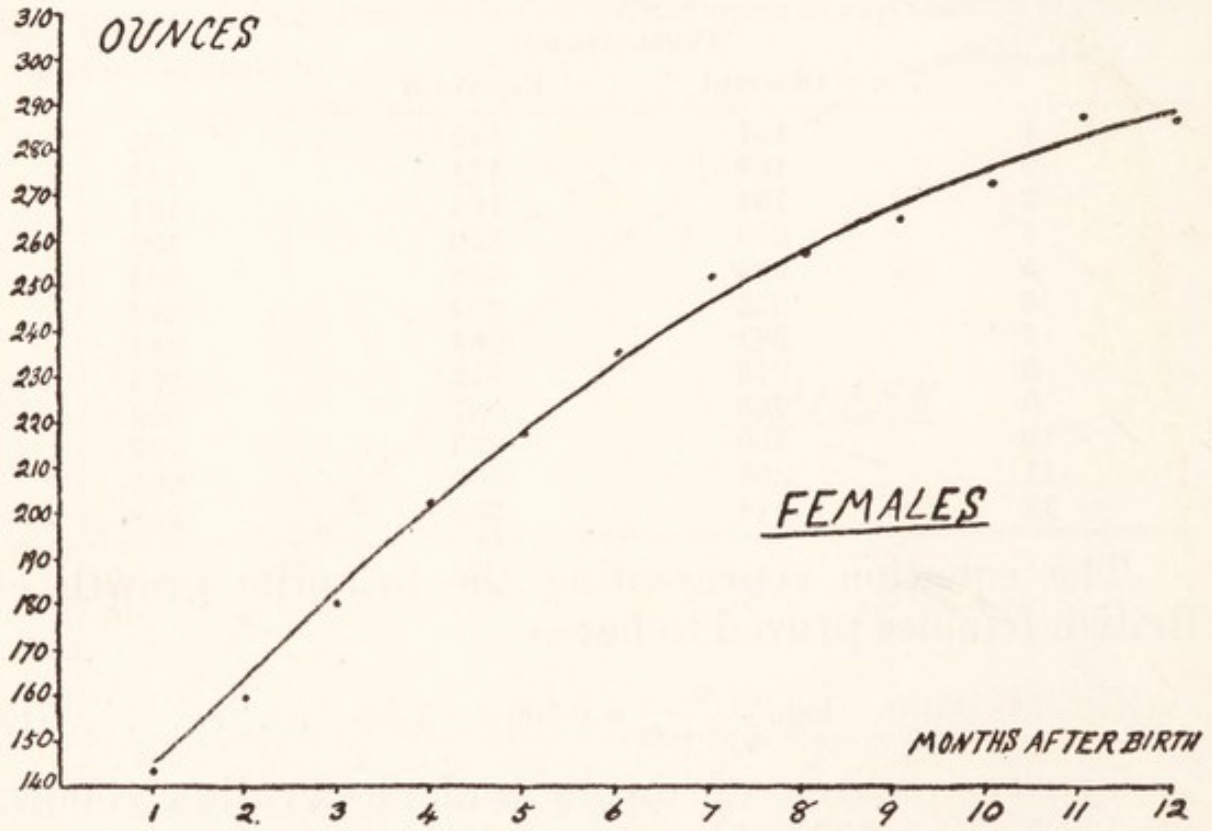


FIG. 6.—Curve of growth of British female infants born in England. The smooth curve is calculated from Equation 13 and the observed weights are indicated by dots.

tions of thermostasis, constancy of osmotic conditions, etc., under which reactions in living tissues take place and also in the statistical methods employed in obtaining the individual observations, which automatically exclude, by mutual cancellations, individual and accidental deviations from the average.

The foregoing data may be compared with those obtained by Schmidt-Monnard[276] for the growth of 100 breast-fed infants in Frankfurt. The autocatalysis equation for the infantile growth-cycle in males (data for the

first nine months only being employed in the computation of the constants) proves in this case to be:—

$$\log_{10} \frac{x}{314.6 - x} = 0.142\,(t - 2.65) \quad . \quad . \quad . \quad . \quad . \quad . \quad (14)$$

In the following table the observed and calculated weights are compared:—

TABLE XVII

South German Males

Age in months	Average weight in ounces	
	Observed	Calculated
1	122	116
2	145	141
3	171	167
4	200	192
5	207	215
6	240	236
7	248	254
8	251	268
9	267	279
10	293	288
11	297	295
12	303	300

The equation for females is found to be:—

$$\log_{10} \frac{x}{290.0 - x} = 0.156\,(t - 2.25) \quad . \quad . \quad . \quad . \quad . \quad . \quad (15)$$

In the following table the observed and calculated weights of the female infants are compared:—

TABLE XVIII

South German Females

Age in months	Average weight in ounces	
	Observed	Calculated
1	114	113
2	141	138
3	169	164
4	191	189
5	207	211
6	227	230
7	242	245
8	245	257
9	261	266
10	266	273
11	268	278
12	274	282

Here the agreement between theory and observation is not quite so exact as in the case of the British infants, possibly because the criteria of normality applied to the infants from which the data were obtained were not so rigid. The agreement is, however, of a very satisfactory order, and it is evident, from the three groups of infants which we have reviewed, that the curve of autocatalysis does faithfully represent, in all its essential features, the first year of extra-uterine and the late months of intra-uterine growth.

5. **Influence of Race and Environment.**—A comparison of the results enumerated in Tables XI and XII with those enumerated in Tables XV and XVI, shows that the initial superiority in weight of British infants born in Australia over British infants born in England is maintained throughout the first twelve months of extra-uterine growth. The origin of this superiority lies primarily in the fact that A, the constant expressing the maximal growth attainable by the cycle, is larger for Australian than for English infants.

Reverting to Equations 5 and 7 in the preceding chapter we see that A is expressive of or proportional to the total mass of available nutrition, the "nutritional level," in other words, of the environment. For while it is true that the prime factor in inducing stoppage of growth in any cycle is the accumulation of the products of the process rather than the exhaustion of the materials for its continuance, yet the ultimate growth attainable in any cycle is nevertheless **proportional** to the totality of available foodstuffs. In the mammals this corresponds to the concentration of nutritive materials constantly maintained by the activity of the digestive organs, in the blood and tissue-fluids, a concentration which, under given environmental conditions, may be assumed to be approximately and in the long run constant. The probable

mechanism which maintains this constancy of the available nutritional materials in the tissue-fluids is indicated by the investigations of Van Slyke[500,501,502,503] and others, who have shown that the proportion of free amino-acid groups unutilized by the tissues and stored as a reserve is characteristic of each tissue and maintained at a relatively constant level, notwithstanding the diversity of materials absorbed from the digestive tract. That a similar equilibrium between tissues and tissue-fluids is also maintained for the carbohydrates is shown by the relative constancy, under normal conditions, of the blood-sugar content and by the fact that when the blood is robbed of sugar, as in phloridzin glycosuria, the tissues immediately yield up sugar to the blood in the effort to maintain the normal equilibrium.

The value of A in the growth-equation, therefore, must be primarily determined by the average concentration of nutritional materials available for the growth or repair of cells. We should expect to find, therefore, that A is dependent, to a large extent, upon nutritional and other conditions constituting the environment of the growing animal.

The reverse is the case with the constant which is the specific velocity constant of the growth-process itself, *i.e.*, expressive of the velocity of transformation of unit mass of nutritional materials into tissue substance. This parameter of the growth-curve is internal rather than external in character and is expressive of the intimate nature of the growth-process itself, independently of the nutritional level of the tissues. We should expect to find, therefore, that the constant $k\left(=\frac{K}{A}\right)$ is dependent upon sex or race but not upon environmental factors.

In the following table the values of A and k for British infants born in England and Australia and for South German infants are compared:—

TABLE XIX

COMPARISON OF THE EFFECTS OF RACE AND ENVIRONMENT UPON THE PARAMETERS OF THE GROWTH-CURVE

	Males		Females	
	A	$k \times 10^6$	A	$k \times 10^6$
British (born in England)	318.0	399	312	340
British (born in Australia)	341.5	398	350	317
South German	315.0	451	290	537

It will be seen that the parameters are affected in the sense indicated by our theory by the factors of sex, race, and environment. While the value of A is not greatly affected by sex or by dissimilarity of race, since values obtained in the similar environments of Frankfurt and London are very much alike, it is greatly affected by dissimilarities in environment, as a comparison of the values of A in Australia and in Europe shows. On the other hand, k is comparatively unaffected by environment, being practically identical for British males, whether born in Australia or in England, and very nearly the same for British females born in these two environments; whereas it is profoundly affected in magnitude by sex and race, as indicated by the marked difference in the values of k for males and females, and for the South German as compared with British infants.

When it is remembered that these parameters are not calculated arbitrarily, but are computed by the method of least squares from all of the observations and therefore partake in some measure in the errors incident to the observations, it will be admitted, I think, that the above data afford a very striking confirmation of the correctness of the view that growth is determined by an underlying autocatalyzed chemical process. It is furthermore clear that the form of the curve of growth in infants is determined by two separate factors; the one, analogous to the absolute mass of reacting substances in a chemical reaction,

being dependent upon environment and probably upon the abundance or deficiency of the habitual dietary; while the other, analogous to the specific velocity of a chemical reaction, is relatively, if not absolutely, independent of environmental or nutritional conditions, and, being expressive of the nature of the growth-process itself as distinguished from the availability of the materials for growth, is distinctively modified by race and sex.

6. **The Juvenile and Adolescent Cycles of Growth.**—The second, or Juvenile, and third, or Adolescent cycles of human growth do not permit of such precise formulation as the infantile cycle, because they are partially fused with one another at their extremities. Under such circumstances the analysis of the complex curve into its constituent cycles, each defined by three mutually independent parameters, A, k, and t_1, is a matter of considerable difficulty and tedium. The only method which I have hitherto found applicable to all cases consists of a series of successive approximations and adjustments each of which, in itself, involves a lengthy series of computations. The parameters so obtained can only be regarded as approximations to the exact values.

Nevertheless the quantitative comparison of theory with observation, even when subject to the limitations which have been outlined, is not without value, for the measure of agreement which is thus attainable displays the autocatalytic character of the processes which determine growth-velocity in definite and unmistakable terms and not merely as a casual impression, gathered from the outline of the curve; while the values of the parameters themselves, although subject to subsequent correction by more exact methods of computation, are evidently sufficiently close to the true values to justify certain conclusions regarding their relative magnitudes which are of great importance in the interpretation of the influence of sex and race upon growth, and may ultimately

furnish a series of quantitative criteria for the evaluation of the effects of external factors such as diet or climate.

The British Anthropometric Committee[19] have computed the average weights[d] of large numbers of individuals at various ages between 3.5 (that is from 3 to 4) and 30.5 years. The total growth of man occurs in three cycles, of which the first is almost completed by the end of

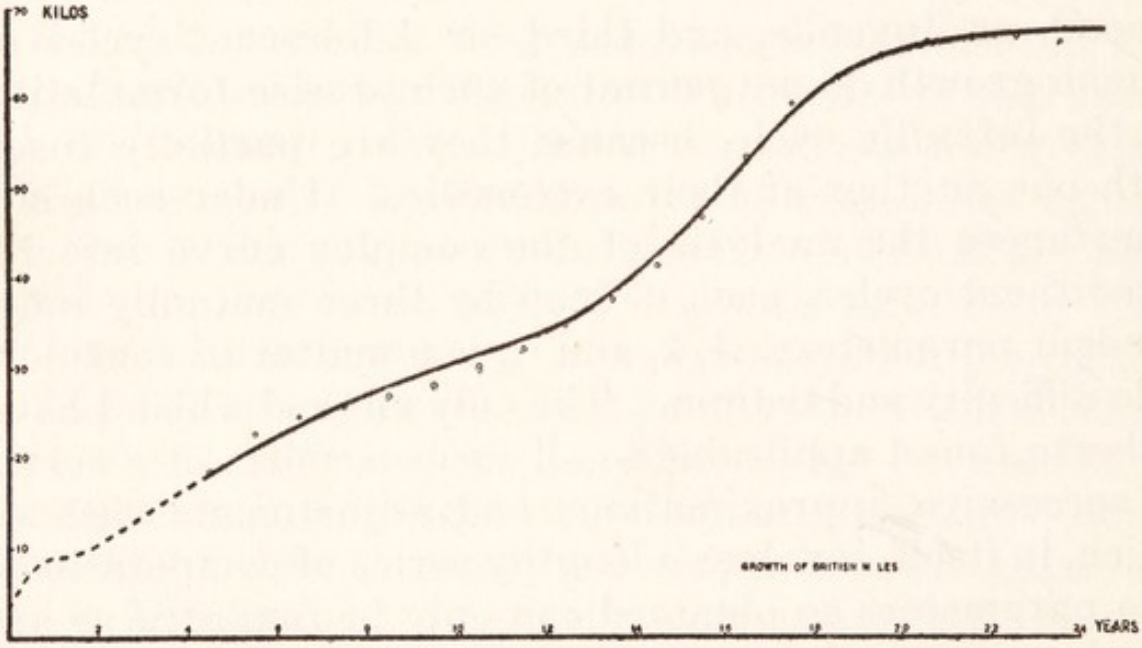


FIG. 7.—Calculated curve of growth of British males. The observed weights are indicated by the dots within the small circles.

the first year and may confidently be regarded as complete by the end of the third year. We have seen (Section 4) that for British males born in England the total growth attained in this cycle is 318 ounces, or almost exactly 9 kilos.[e]

I find that the Anthropometric Committee's results

---

[d] The observed weights include that of the clothing. According to Bowditch [45] these constitute, on the average, a constant proportion, namely, about 8 per cent. of the actual weight of the individual irrespective of age. The effect of this inclusion will, therefore, be to increase the apparent value of A in each cycle to 8 per cent. above its true value.

[e] The infantile cycle of growth was disregarded in a former comparison [38b] with the result that the agreement between theory and observation in the earlier years was very poor. Obviously, the effect of this omission would decrease as the weight increased.

for British males may be defined by the following equations:—

$$x^{\mathrm{I}} = 9.0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (16)$$

$$\log \frac{x^{\mathrm{II}}}{24 - x^{\mathrm{II}}} = 0.188\,(t - 5.5) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (17)$$

$$\log \frac{x^{\mathrm{III}}}{35 - x^{\mathrm{III}}} = 0.272\,(t - 16) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (18)$$

$$x = x^{\mathrm{I}} + x^{\mathrm{II}} + x^{\mathrm{III}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (19)$$

where x is the weight in kilograms at t years of age, and x^{I}, x^{II} and x^{III} are the weights due to the first, second and third cycles of growth, respectively. The weights computed from these equations are compared with the actual body-weights in the following table and in the figure (Fig. 7).

TABLE XX

Growth of British Males

Age in years	Body-weight in kilos	
	Observed	Calculated
4.5	18.6	18.5
5.5	22.7	21.1
6.5	24.6	23.7
7.5	25.9	26.1
8.5	27.0	28.2
9.5	28.3	30.0
10.5	30.4	31.7
11.5	32.3	33.4
12.5	35.0	35.0
13.5	37.9	38.5
14.5	41.7	42.5
15.5	47.1	47.5
16.5	53.8	52.9
17.5	59.6	58.4
18.5	62.5	62.5
19.5	64.4	64.0
20.5	66.5	66.0
21.5	67.1	66.8
22.5	67.2	67.4
23.5	66.5	67.7

Quetelet[19] has similarly computed the average weights of Belgian males and females at yearly intervals from birth. Unfortunately we have not, in this instance, the precise knowledge of the magnitude of the infantile cycle

which we possess in the case of the British infants, and it becomes necessary to assume a value which permits a satisfactory fitting of the computed curves for the second and third cycles with the values established by observation. As it chances this is in each instance just double the birth-weight as given by Quetelet (3.1 kilos for males and 3.0 kilos for females). It is probable therefore, that these values for the magnitude of the infantile cycle are rather low, for British infants, at all events, do not attain maximum growth-velocity ($x = \frac{1}{2}A$) in the infantile cycle until from one to two months after birth.

I find that Quetelet's results for Belgian males may be represented by the equations:—

$$x^{\mathrm{I}} = 6.2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (20)$$

$$\log \frac{x^{\mathrm{II}}}{18.7 - x^{\mathrm{II}}} = 0.169\,(t - 5.5) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (21)$$

$$\log \frac{x^{\mathrm{III}}}{41.4 - x^{\mathrm{III}}} = 0.175\,(t - 16.5) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (22)$$

$$x = x^{\mathrm{I}} + x^{\mathrm{II}} + x^{\mathrm{III}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (23)$$

the various symbols having the same meanings as in the preceding comparison. The calculated and observed results are compared in the following table:—

TABLE XXI

GROWTH OF BELGIAN MALES

Age in years	Body-weight in kilos	
	Observed	Calculated
1.5	9.0	9.6
2.5	11.0	10.8
3.5	12.5	12.3
4.5	14.0	14.1
5.5	15.9	16.1
6.5	17.8	18.1
7.5	19.7	20.1
8.5	21.6	22.1
9.5	23.5	24.0
10.5	25.2	26.4

GROWTH OF BELGIAN MALES

Age in years	Body-weight in kilos	
	Observed	Calculated
11.5	27.0	28.2
12.5	29.0	30.7
13.5	33.1	33.7
14.5	37.1	37.3
15.5	41.2	41.3
16.5	45.4	45.3
17.5	49.7	49.4
18.5	53.9	53.1
19.5	57.6	56.5
20.5	59.5	59.1
21.5	61.2	61.2
22.5	62.9	62.7
23.5	64.5	63.7
25.5	66.2	65.2
30.5	66.1	66.0

We could hardly expect the agreement to be closer if the parameters had been determined by the method of least squares, and it is especially noticeable that the agreement obtains over the entire period of growth, during which the weight increases over 600 per cent. The rather large divergence at 1.5 years may be attributable to lack of completion of the infantile cycle at this age, or to an error in the estimation of its total extent (6.2 kilos) which would, of course, affect the agreement with this measurement more than any other.

For Belgian females the corresponding equations are:—

$$x^{\mathrm{I}} = 6.0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (24)$$

$$\log \frac{x^{\mathrm{II}}}{13.9 - x^{\mathrm{II}}} = 0.15\,(t - 4.5) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (25)$$

$$\log \frac{x^{\mathrm{III}}}{36.1 - x^{\mathrm{III}}} = 0.171\,(t - 14.5) \quad . \quad . \quad . \quad . \quad . \quad . \quad (26)$$

$$x = x^{\mathrm{I}} + x^{\mathrm{II}} + x^{\mathrm{III}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (27)$$

The calculated and observed values of the body-weight are compared in the accompanying table:—

TABLE XXII

GROWTH OF BELGIAN FEMALES

Age in years	Body-weight in kilos	
	Observed	Calculated
1.5	8.6	9.8
2.5	11.0	11.0
3.5	12.4	12.2
4.5	13.9	13.6
5.5	15.3	15.2
6.5	16.7	16.7
7.5	17.8	18.4
8.5	19.0	20.2
9.5	21.0	22.2
10.5	23.1	24.6
11.5	25.5	27.2
12.5	29.0	30.4
13.5	32.5	33.8
14.5	36.3	37.5
15.5	40.0	41.7
16.5	43.5	44.5
17.5	46.8	47.4
18.5	49.8	49.7
19.5	52.1	51.6
20.5	53.2	52.9
21.5	54.3	53.8
22.5	55.2	54.9
25.5	55.8	55.5
30.5	55.3	56.0

Comparing the parameters for Belgian males and females, recollecting that the velocity-constant $k = \frac{K}{A}$ we find (Table XXIII) that the magnitude of each cycle is more than double that of the preceding, being in

TABLE XXIII

	A (kilos)		$k \times 10^4$		t_1 (years)	
	Males	Females	Males	Females	Males	Females
Infantile cycle	6.2	6.0	—	—	—	—
Juvenile cycle	18.7	13.9	90	108	5.5	4.5
Adolescent cycle	41.7	36.1	42	47	16.5	14.5

each instance less for the female than for the male. The specific velocity of growth, however, is much less in the Adolescent than in the Juvenile cycle, and it is in each

instance slightly greater in the female than in the male. The most striking sexual difference, however, resides in the relative values of t_1, the time of half-completion of the cycles. In each cycle the female anticipates the male, in the Juvenile cycle by one year, and in the Adolescent cycle by two years. This obviously corresponds with the earlier onset of puberty in girls, and accounts for the fact which has been commented upon by many observers, that for a brief period at puberty girls exceed boys in physical dimensions.[f]

[f] This indicates a way in which hormones may profoundly affect growth and development without altering either the magnitudes or, to any considerable extent, the specific velocities of the individual growth-cycles. For if, by any means tantamount to a slight early increase of the autocatalyst sufficing only to increase the apparent value of the specific velocity constant by a small proportion of its true value, the initial stages of the cycle can be accelerated, then the rapid accumulation of autocatalyst which would ensue would result in a degree of anticipation of the period of maximum growth-velocity out of all proportion to the magnitude of the initial acceleration brought about by the hormone.

CHAPTER III

THE PHYSICAL MANIFESTATIONS OF GROWTH IN ANIMALS AND PLANTS

1. **The Growth of the Dairy Cow.**—Investigation of the extra-uterine growth of the dairy cow, from the point of view of the Autocatalytic Theory of Growth, has been undertaken by Brody and Ragsdale,[47] from whose article the following summary of their results has been compiled.

From the differential equation:—

$$\frac{dx}{dt} = k\,x\,(A - x) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (28)$$

it follows that the velocity of an autocatalyzed reaction progressively increases from zero to a maximum value, when $x = \frac{1}{2}A$, and thereafter decreases to zero again with the completion of the cycle ($x = A$). Now on referring to the integrated form of the equation:—

$$\log \frac{x}{A - x} = k\,A\,(t - t_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (29)$$

it will be evident that the maximal velocity is attained when $t = t_1$.

On plotting a diagram, therefore, in which the velocities of the autocatalyzed reaction form the ordinates and the corresponding times the abscissæ we obtain the following curve (Fig. 8). A close approximation to the velocity of growth at any age is furnished by the increment of weight in a unit of time. When this velocity-curve of growth is plotted for the Jersey cow we obtain the diagram shown in Figure 9; the curve for the Holstein cow being very similar in outline (Fig. 10).

These curves yield very clear evidence of the existence of two rather clearly demarcated cycles of growth with maxima at about 5 and 20 months of age respectively. The

slight asymmetry of the velocity-curve of cycle A is probably attributable to interference by cycle B which begins before the completion of cycle A, thus resulting in the superimposition of the two cycles, the sum of their ordinates representing the total growth. The velocities in the second cycle are somewhat irregular, a fact which the authors attribute to the small number (12 or 14) of

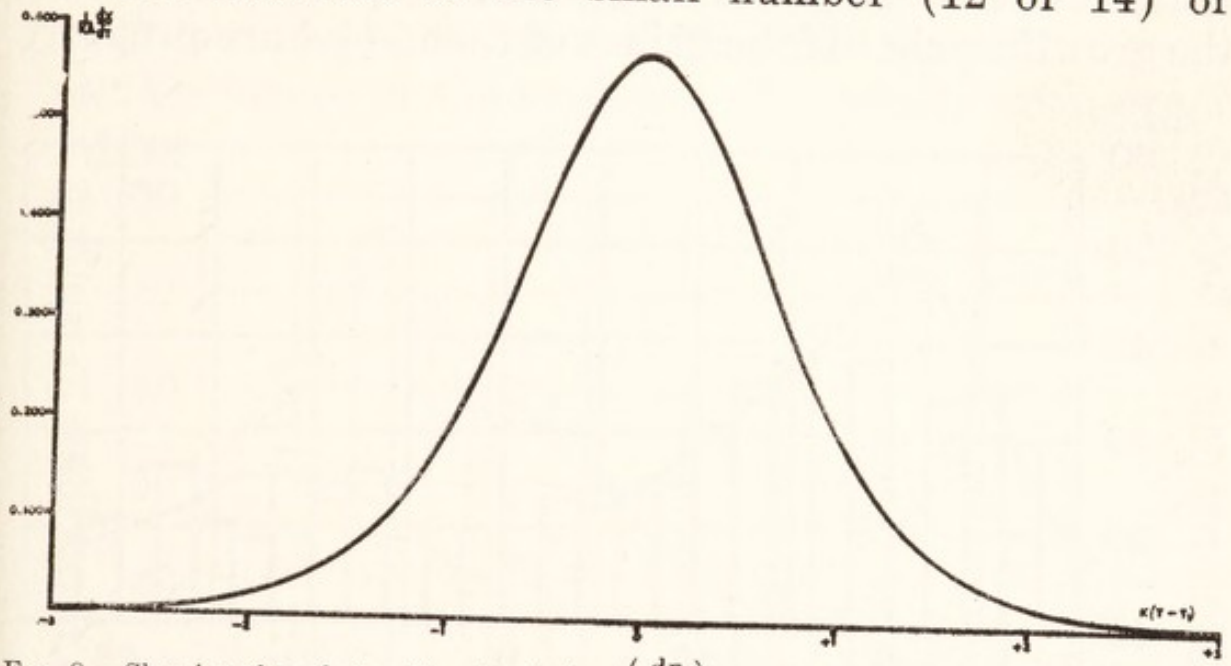


FIG. 8.—Showing the relationship of velocity $\left(\frac{dx}{dt}\right)$ to time in an autocatalyzed monomolecular reaction.

animals weighed, and to the fact that the animals were bred at 20 to 21 months of age, calving at the ages of 29 to 30 months.

In Equation 29, x is the weight gained from the beginning of any cycle up to any age t of that cycle; while A is the total growth accomplished at the completion of the cycle. If the cycle under consideration is preceded by one or more other cycles then the growth of the preceding cycles must be subtracted from the weight of the animal at the age t in order to obtain x. Let w be the weight gained up to the beginning of the cycle under consideration, and x_1 the total weight gained up to the age t. Then Equation 29 becomes:—

$$\log \frac{x_1 - w}{A - (x_1 - w)} = K(t - t_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (30)$$

where $K = kA$.

Brody and Ragsdale then proceed as follows:—The highest point of the velocity-curve in any cycle is assumed to occur at $t = t_1$.[a]

Having thus evaluated t_1, a reasonable beginning or end of the cycle is chosen by inspection; the growth made between the ages t_1 and the beginning or end of the cycle is the value of $\frac{1}{2}A$, since t_1 is the centre of the growth-cycle. If the values of t_1 and $\frac{1}{2}A$ are properly

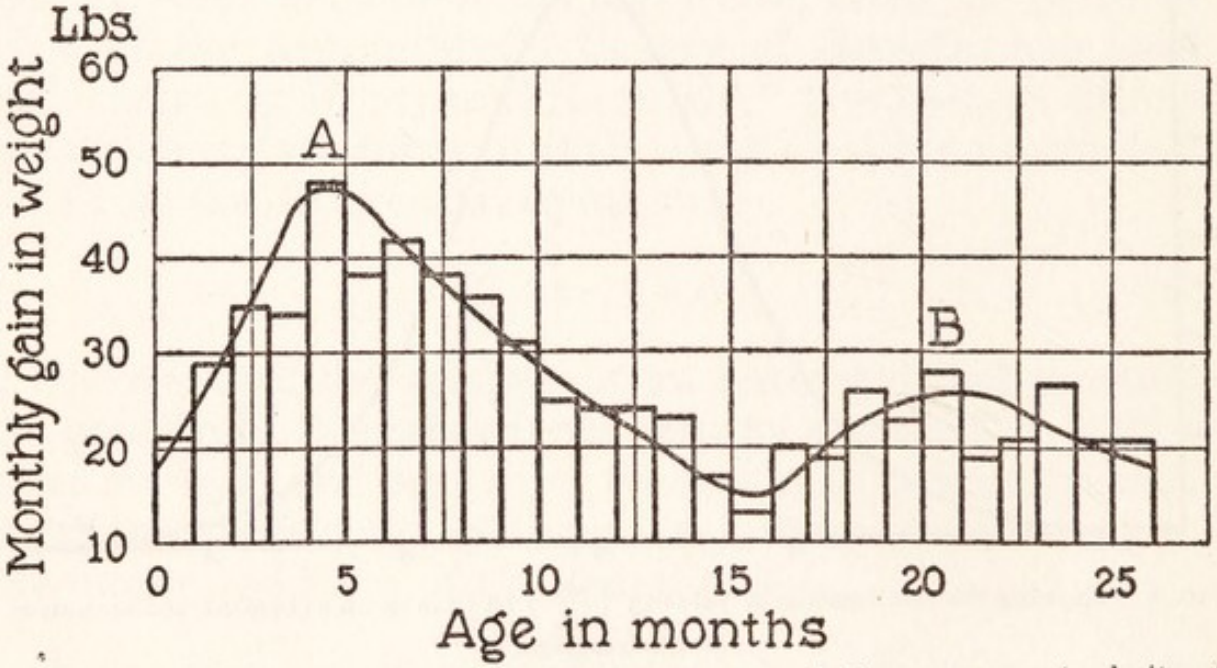


FIG. 9.—Velocity curve of growth of the Jersey cow. Ordinates represent velocity of growth, the height of the rectangles representing monthly gains in weight; abscissae represent age in months. (After Brody and Ragsdale.)

chosen, the values of K for different values of x should be nearly constant. If the value of K deviates systematically then a slightly smaller or slightly larger value of $\frac{1}{2}A$ is chosen until the systematic deviations disappear; the value of A can be estimated still more easily by changing Equation 29 to the form:—

$$y = K(t - t_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (31)$$

where $y = \log \frac{x}{A - x}$. Since this represents a straight

[a] Unfortunately, this is only true when, as in the present instance, the cycles are so far separated from one another that the total growth is not, to any important extent, receiving simultaneous contributions at any time from both cycles. This latter case actually occurs, as we shall see, in the white rat, and in a less degree, as we have already seen, in the juvenile and adolescent cycles of human growth.

line, therefore $\log \frac{x}{A-x}$ if plotted against t, should also give a straight line for the proper value of A. A few values of A are chosen and few values of $\log \frac{x}{A-x}$ ascertained from the table of ordinates of the curve of autocatalysis (see Appendix). The value of A giving the straightest line is the one chosen for computation.[b]

It is stated by Brody and Ragsdale that while there is some evidence of the existence of a wholly intra-uterine

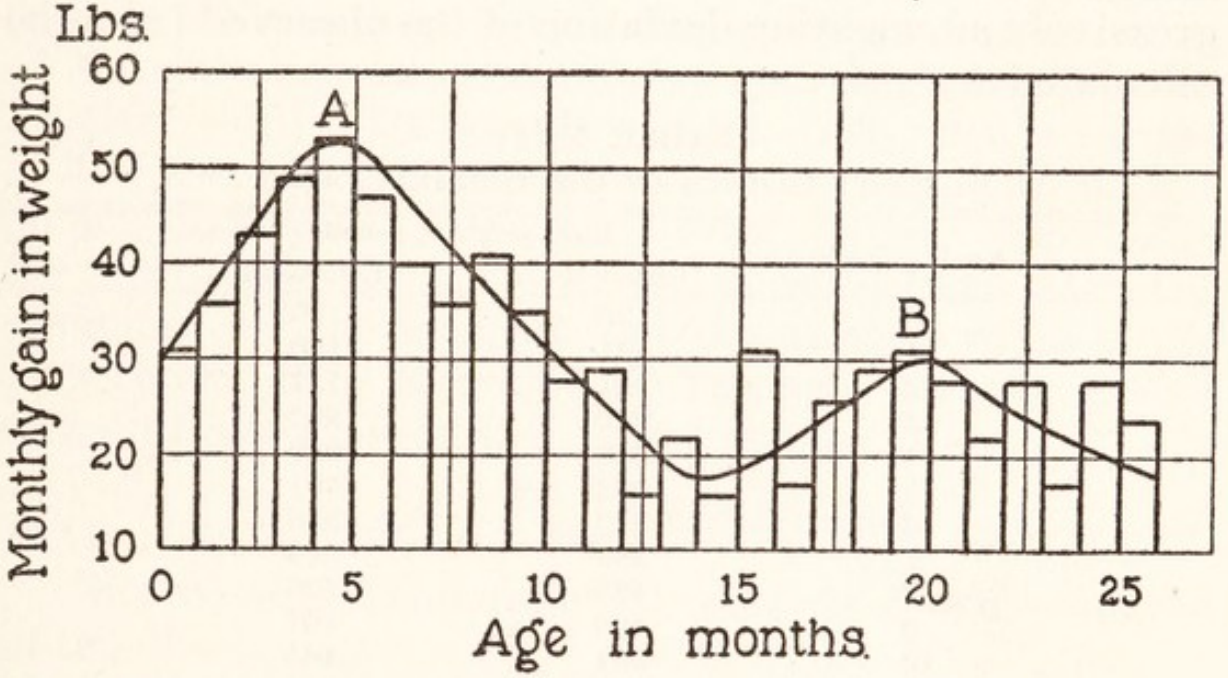


FIG. 10.—Velocity curve of growth of the Holstein cow. Ordinates and abscissae have the same meaning as in Figure 9. (After Brody and Ragsdale.)

cycle of growth in the cow, thus completing the three cycles generally observed in the growth of vertebrates, its magnitude, relatively to the extra-uterine cycles, is very small. Accordingly for the first extra-uterine cycle they assume w in Equation 30 to be zero. Assuming the value of t_1 for the Holstein cow to be 4.5 months, when the animal weighs 275 pounds (Fig. 10) we get the value for A, $275 \times 2 = 550$ pounds, Equation 29 for the first extra-uterine growth-cycle of the Holstein cow takes the form:—

$$\log \frac{x}{550-x} = 0.158\,(t-4.5) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (32)$$

[b] This graphic method was suggested to Brody and Ragsdale by Dr. E. R. Hedrick.

where x is the weight in pounds and t the time in months. By the same method the equation for the Jersey cow is found to be:—

$$\log \frac{x}{444 - x} = 0.169\,(t - 5) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (33)$$

The agreement between the observed and calculated weights, displayed in Tables XXIV and XXV, is as satisfactory as could be desired. After 9 months of age the intrusion of the second extra-uterine cycle leads to a progressively augmenting deviation of the observed from the calculated weights:—

TABLE XXIV

EARLY GROWTH OF THE HOLSTEIN COW

Age in months	Body-weight in pounds	
	Observed	Calculated
0	90	90
1	121	120
2	157	157
3	200	202
4	249	250
5	302	301
6	349	348
7	389	393
8	425	430
9	466	461
10	501	485
11	529	503

TABLE XXV

EARLY GROWTH OF THE JERSEY COW

Age in months	Body-weight in pounds	
	Observed	Calculated
0	55	55
1	76	76
2	105	105
3	140	140
4	174	180
5	222	222
6	260	265
7	302	305
8	340	340
9	376	367
10	407	389

According to Brody and Ragsdale the velocity curves in the last cycle, B (Figs. 9 and 10), are very irregular and

consequently they believe that no significance can be attached to the fit of Equation 30 for the data of that period. However, they apply the equation to the later growth of the Jersey cow in order to illustrate the method of procedure. They assume from the growth-velocity curve that the value of t_1 for the second extra-uterine cycle is in this case 20.5. At this age the animals weigh 635 pounds. They further assume from previous measurements of Eckles[113] that the weight of the Jersey cow at maturity is 902 pounds. From these figures they compute that the value of A for the second extra-uterine cycle is probably about $2 \times (902 - 635) = 534$ pounds. If this is so, then A for the first cycle, which was previously ascertained to be 444 pounds, appears to be $902 - 534 = 368$ pounds. The reason for this divergence of values will be considered later, but assuming for the moment that the smaller value is the correct one then Equation 30 becomes, for the second extra-uterine cycle in the Jersey cow:—

$$\log \frac{x_1 - 368}{534 - (x_1 - 368)} = 0.0765\,(t - 20.5) \quad . \; . \; . \; . \quad (34)$$

The calculated and observed values of x_1 are compared in table:—

TABLE XXVI

LATE GROWTH OF THE JERSEY COW

Age in months	Body-weight in pounds	
	Observed	Calculated
15	520	513
16	533	533
17	553	553
18	572	575
19	598	598
20	621	621
21	649	645
22	668	669
23	689	690
24	716	713
25	737	734
26	758	754
27	770	771
28	784	788
29	804	803

The agreement between the observed and calculated values is surprisingly exact when one recollects in the first place the disparity between the assumed and actual magnitudes of the first extra-uterine cycle, to which attention has been drawn above, and in the second place the fact that these cows were bred at from 20 to 21 months and calved at from 29 to 30 months. One is led to surmise that there may be some connection between these two sources of error of such a nature that they counterbalance and nullify each other. Now it will be noted that the time at which gestation began exactly coincided with the period of maximum growth-velocity in the second extra-uterine cycle. Hence the intra-uterine growth of the calves, totalling 55 pounds at birth, occupied the period comprised in the second or autostatic half of the second extra-uterine cycle. The effect of this would be to render the descending limb of the growth-velocity curve (Fig. 8) less steep than it would otherwise have been. Now the autocatalytic (monomolecular) formula demands symmetry of the velocity curve, and any formula fitting the descending limb of the curve would therefore of necessity indicate an ascending limb less steep than it really was. In other words the formula would ascribe to the first half of the cycle just as much excess of growth as gestation communicated to it in the second half. This excess of growth, attributed by the formula to the second extra-uterine cycle, would actually be derived from the latter part of the first extra-uterine cycle, of which the corresponding beginning portion is intra-uterine. We are now in a position to understand why the total magnitude of the first extra-uterine cycle, deduced from the apparent magnitude of the second cycle, is so considerably less than its true magnitude, for the apparent magnitude of the second cycle exceeds the true magnitude by the amount of intra-uterine growth which occurs during gestation.

Furthermore, we must infer that the last portions of the first cycle reproduce exactly its earliest portion which is intra-uterine, for the exact symmetry of the apparent velocity curve for the second cycle, which is implied in the precision with which the autocatalytic formula represents the measurements, could not otherwise be observed. The intra-uterine growth of the cow, therefore, so far as

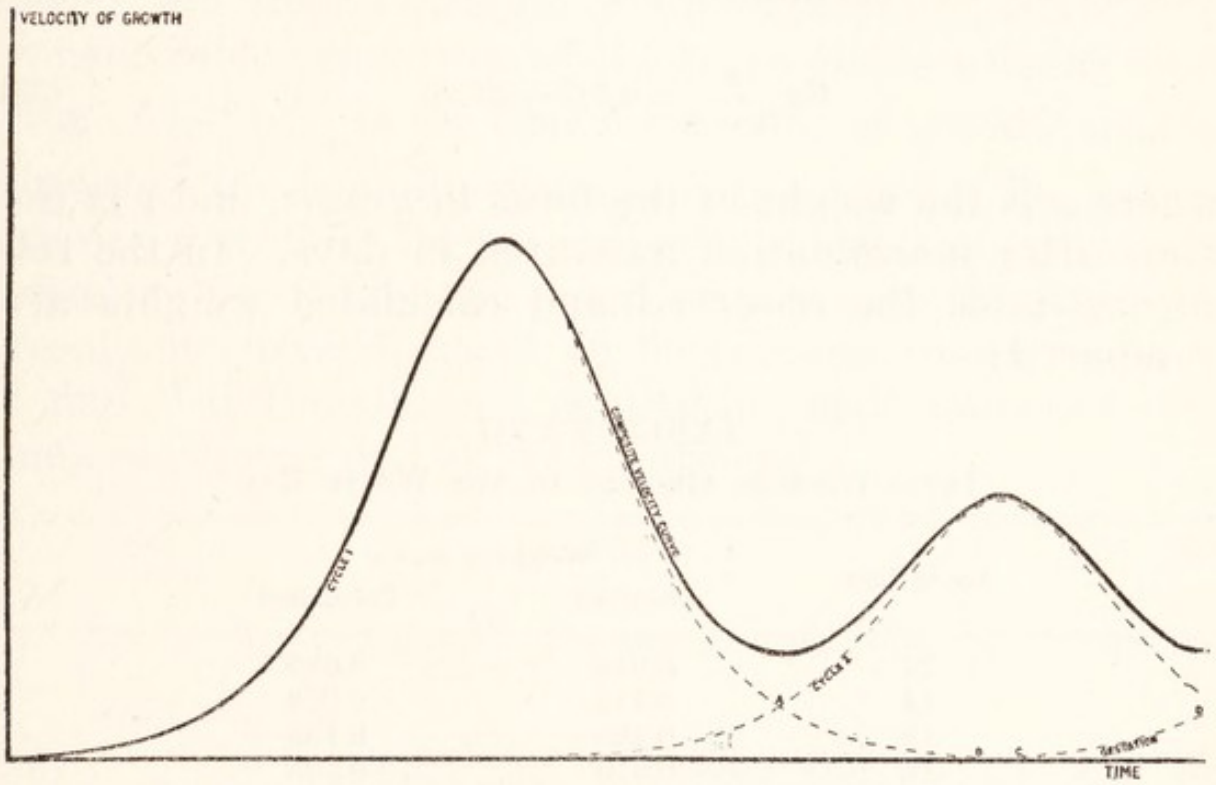


FIG. 11.—Composite velocity curve of three successive growth cycles. Unless the curve c - d, representing the inital stages of the third cycle, reproduces exactly the terminal portion a - b of the first cycle, the composite curve cannot be symmetrical.

any growth of considerable magnitude is concerned, appears to constitute a continuation of the first cycle backwards from birth. The manner in which the composite growth velocity curve is thus built up is illustrated in the accompanying figure (Fig. 11) and it will be evident that unless the intra-uterine curve $c - d$ exactly reproduces the latter portion $a - b$ of the first cycle, the apparent velocity curve for the second cycle could not be symmetrical. The closeness of fit of the autocatalytic formula found by Brody and Ragsdale, therefore, demonstrates not only that this formula applies to the second extra-uterine cycle,

but also that the growth velocity curve for the first cycle is symmetrical throughout its duration.[c]

2. **The Growth of the White Rat.**— The intra-uterine growth of the white rat has been measured by Stotsenburg[480] from the 13th day after insemination, when the fetus is large enough to be directly weighed, until birth on the 22nd day. I find that the results may be represented by the formula:—

$$\log \frac{x}{6-x} = 0.3\,(t - 20.25) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (35)$$

where x is the weight of the fetus in grams, and t is the time after insemination measured in days. In the following table the observed and calculated weights are compared:—

TABLE XXVII

INTRA-UTERINE GROWTH OF THE WHITE RAT

Age in days	Weight in grams	
	Observed	Calculated
13	0.040	0.040
14	0.112	0.078
15	0.168	0.156
16	0.310	0.303
17	0.548	0.576
18	1.00	1.05
19	1.58	1.78
20	2.63	2.74
21	3.98	3.74
22	4.63	4.62

The growth-curve of the white rat subsequently to birth, as determined by Donaldson,[99] does not display any irregularities of curvature such as to suggest that it consists of more than one cycle. Superficially it appears, in fact, to constitute a single sweep of an autocatalytic curve

[c] The actual formula of the composite curve would not be strictly that of a simple curve of autocatalysis, but the quantitative departures from the autocatalytic formula would not necessarily be large enough to be appreciable.

(Fig. 2). Closer inspection, however, shows that the terminal portion of this curve, instead of remaining horizontal to the base-line, slowly and progressively rises and, in fact, no autocatalytic formula can be found which, singly, will fit the whole of the observed curve. If we plot values of $\log \frac{x}{A-x}$ against times, in the manner suggested by Brody and Ragsdale, no value of A can be found which will yield a straight line for the graph, although a considerable proportion of the curve yields a linear relation of $\log \frac{x}{A-x}$ to the time for a value of A considerably less than the final dimensions of the animal. The extra-uterine growth of the rat evidently consists of two cycles which are so intimately fused with one another that the resultant curve displays no fluctuations in its contour. I find that Donaldson's results for male unmated rats may be represented by the equations:—

$$x^{\mathrm{I}} = 6.0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (36)$$

$$\log \frac{x^{\mathrm{II}}}{185 - x^{\mathrm{II}}} = 0.0252\,(t - 69) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (37)$$

$$\log \frac{x^{\mathrm{III}}}{90.5 - x^{\mathrm{III}}} = 0.01125\,(t - 170) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (38)$$

$$x = x^{\mathrm{I}} + x^{\mathrm{II}} + x^{\mathrm{III}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (39)$$

where x is the weight in grams at t days after birth, and x^{I}, x^{II} and x^{III} represent the growth due to the first (intra-uterine), second and third growth-cycles, respectively.

The observed and calculated values are compared in Table XXVIII and the accompanying figure (Fig. 12) displays the growth due to each of the two extra-uterine cycles separately (discontinuous lines) and the composite curve of total growth which represents the sum of their separate effects. The experimental observations are indicated by the small circles on the diagram.

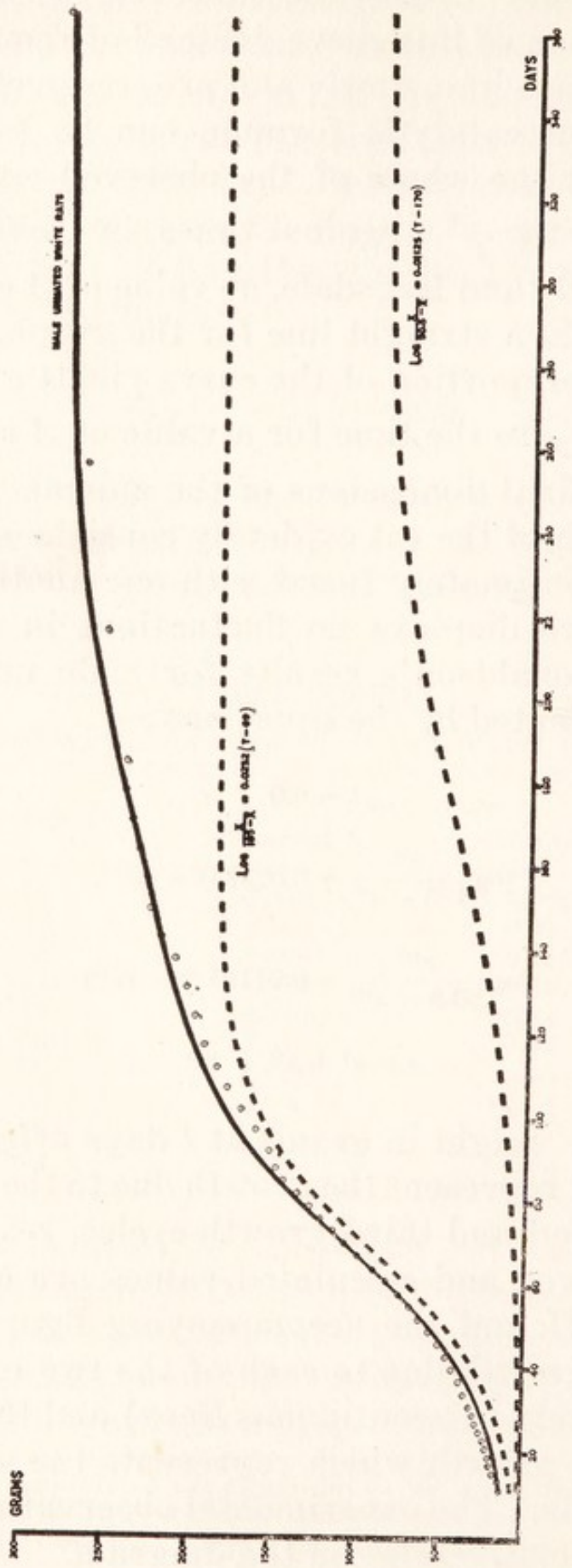


FIG. 12.—Extra-uterine curve of growth of male, unmated white rats (full line), showing the two cycles out of which it is built up (broken lines). The observed weights (after Donaldson) are indicated by the dots within the circles.

TABLE XXVIII

THE GROWTH OF MALE, UNMATED WHITE RATS

Age in days	Body-weight in grams	
	Observed	Calculated
11	13.3	13.6
12	14.8	14.0
13	15.3	14.4
14	15.2	14.8
15	16.5	15.4
17	17.8	16.4
19	19.5	17.4
21	21.2	18.6
23	22.9	20.0
25	25.3	21.4
27	27.4	23.1
29	29.5	25.0
31	31.8	26.7
34	34.9	30.1
37	37.8	33.7
40	42.2	37.9
43	46.3	42.7
46	50.5	48.0
49	56.7	53.9
52	62.5	60.4
55	68.5	67.3
58	73.9	74.7
61	81.7	82.5
64	89.1	90.6
67	99.3	99.0
70	106.6	107.5
73	113.8	116.0
76	121.3	124.3
79	128.2	132.4
82	135.0	140.2
85	143.8	147.6
88	148.4	154.6
92	152.3	163.1
97	160.0	172.5
102	168.8	180.6
107	177.6	187.5
112	183.8	193.3
117	191.4	198.6
124	197.3	204.8
131	202.5	210.2
138	209.7	215.2
143	218.3	218.5
150	225.4	223.1
157	227.0	227.6
164	231.4	232.0
171	235.8	236.2
178	239.4	240.5
185	239.8	244.7
216	252.9	260.4
256	265.4	272.7
365	279.0	281.0

The correspondence of the observed and calculated values is extremely close, the deviations in no case exceeding the probable error of the measurement as indicated by the range of variation of the animals employed. There can be no doubt that these autocatalytic formulæ do actually represent the time-relations of the growth-process in this animal, and the case is especially instructive because it shows so clearly what a remarkable degree

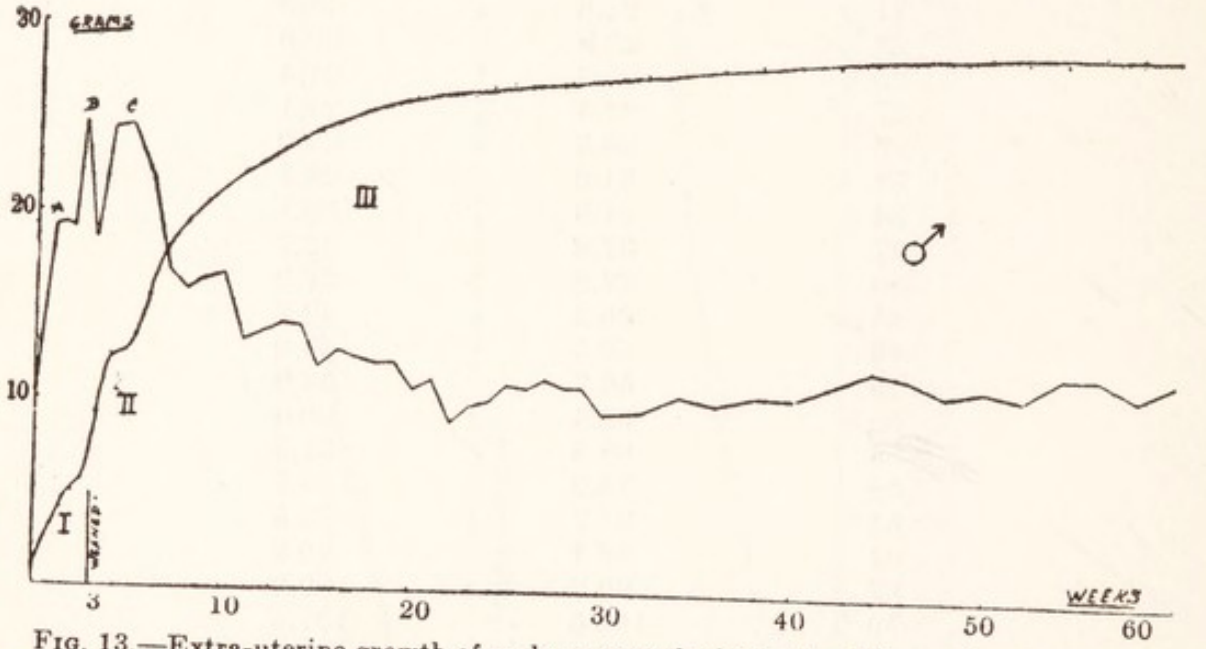


FIG. 13.—Extra-uterine growth of male, unmated white mice. The continuous curve represents the change of weight with age. The actual observations are indicated by dots. The broken curve represents the percentage of variability (ratio of standard deviation to mean weight).

of fusion of successive cycles may occur and how little a mere optical impression of the form of such a curve may be trusted to certify its autocatalytic character or the simplicity of the underlying processes which determinate it. It is for this reason that the reiteration of merely graphic representations of growth-curves adds but little to our certainty of the autocatalytic character of the processes which determine growth-rate. Only a quantitative comparison of the curve of autocatalysis with the observations can furnish any measure of certainty.[d] In this connection it cannot be too strongly emphasized that

[d] An attempt was formerly made to represent this curve by a single autocatalytic formula, but the agreement with the observations was, as might be expected, exceedingly poor in the later portions of the curve.[389]

the application of the autocatalytic formula to the time-relations of growth is not merely a matter of fitting an arbitrary equation to a series of observations, but that, on the contrary, it involves the analysis of the whole growth-history of the organism into its constituent parts, an analysis which we are as yet but rarely in a position to perform.

It will be noted, as in the cases previously considered, that the total growth of the rat is tricyclic. It will ob-

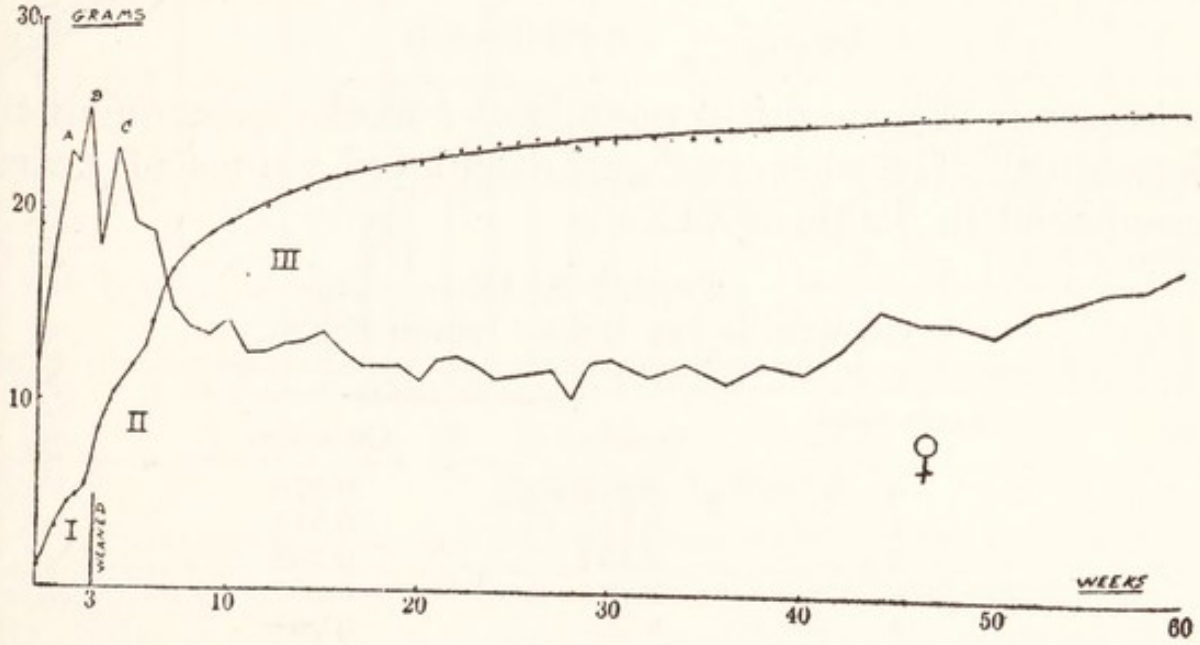


FIG. 14.—Extra-uterine growth of female, unmated white mice. The continuous curve represents the change of weight with age. The actual observations are indicated by dots. The broken curve represents the percentage of variability (ratio of standard deviation to mean weight).

viously be of importance in the future to determine the effects of dietary deficiencies and the like upon each of the growth-cycles separately and to quantitatively estimate the effects in terms of the alterations induced in the parameters of these cycles.

The growth of the white mouse similarly consists of three cycles[362,402] but the analysis of the composite curve into its constituent cycles has not yet been performed (Figs. 13 and 14).

3. **The Growth of the Domestic Fowl.**—It has been shown by Brody[46] from the measurements of Card and Kirkpatrick[56] that the growth of the Rhode Island fowl takes

place in successive cycles, of which two are post-embryonic and one, or possibly two occur during the period of development within the egg (Fig. 15). The first post-embryonic cycle attains maximum growth-velocity at 8.5 weeks after hatching, and the second about fourteen weeks. The equation to the first cycle has been computed by Brody in the manner described in connection with the growth of the dairy cow, and he finds it to be, for the Rhode Island breed:—

$$\log \frac{x}{2.76 - x} = 0.182\,(t - 8.5)$$

where x is the weight in pounds at t weeks subsequent to hatching. The observed and calculated values of x are compared in Table XXIX:—

TABLE XXIX

GROWTH OF THE RHODE ISLAND FOWL

Age in weeks	Weight in pounds	
	Observed	Calculated
0	0.082	0.078
1	0.115	0.114
2	0.162	0.168
3	0.264	0.251
4	0.364	0.363
5	0.538	0.516
6	0.737	0.717
7	0.962	0.961
8	1.228	1.233
9	1.525	1.521
10	1.805	1.800
11	2.014	2.040
12	2.290	2.240
13	2.388	2.390
14	2.566	2.505

It is stated by Brody that similar results were obtained with other breeds of fowl, the several breeds differing only with respect to the parameters of the curve. It may here be suggested that the magnitudes of these parameters may ultimately be found to afford, under given dietary conditions, a means of defining the several races quite as trustworthy as their external characteristics, while the behavior of the parameters in hybridization may

be expected to throw an important light upon the mechanism of size-inheritance.

4. **The Growth of the Frog.**—Davenport[93] has made numerous measurements of the growth of frog embryos. On examining his results we find that they are mostly concerned with the first and second growth-cycles, and that they are made at intervals too infrequent to enable us to determine with accuracy the time at which the

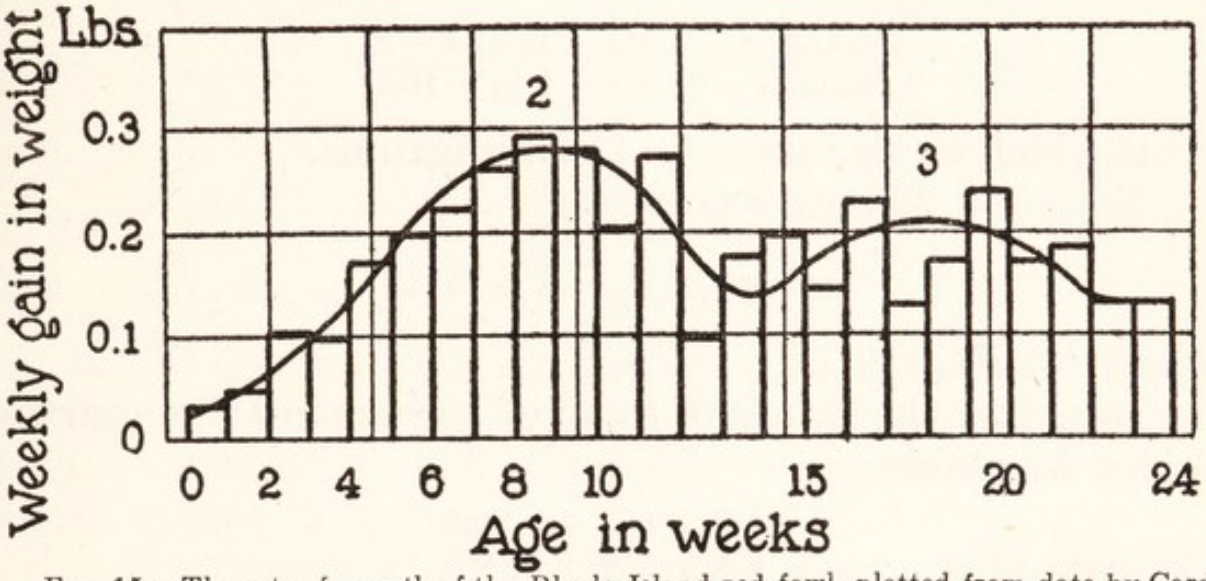


FIG. 15.—The rate of growth of the Rhode Island red fowl, plotted from data by Card and Kirkpatrick. Ordinates represent the weight in pounds gained per week; abscissae represent the age in weeks of the birds from the time of hatching. (After Brody.)

growth which is due to any particular growth-cycle is half completed. We can, however, apply Equation 29 in the following manner:—

From Davenport's table we find that:—

At	the	age	of	2	days,	body-weight	= 2.0	milligrams
"	"	"	"	7	"	"	= 5.05	"
"	"	"	"	9	"	"	= 10.4	"
"	"	"	"	14	"	"	= 23.52	"

Hence, applying Equation 29 we have:—

$$\log \frac{2}{A-2} = K(2 - t_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (40)$$

$$\log \frac{10.4}{A-10.4} = K(9 - t_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (41)$$

$$\log \frac{5.05}{A-5.05} = K(7 - t_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (42)$$

$$\log \frac{23.52}{A-23.52} = K(14 - t_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (43)$$

Subtracting the first from the second of these equations we have:—

$$\log \frac{10.4\,(A-2)}{2\,(A-10.4)} = 7K \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (44)$$

Subtracting the third from the fourth we have:—

$$\log \frac{23.52\,(A-5.05)}{5.05\,(A-23.52)} = 7K \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (45)$$

Whence we have:—

$$\frac{23.52\,(A-5.05)}{5.05\,(A-23.52)} = \frac{10.4\,(A-2)}{2\,(A-10.4)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (46)$$

from which we find that $A = 112$ milligrams.

Equation 29 now becomes:—

$$\log \frac{x}{112-x} = Kt - b \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (47)$$

where $b = Kt_1$.

Inserting the values of t and of x given in Davenport's tables we obtain:—

$$\begin{aligned} K - b &= -1.7796 \\ 2K - b &= -1.7404 \\ 5K - b &= -1.5004 \\ 7K - b &= -1.3263 \\ 9K - b &= -0.9899 \\ 14K - b &= -0.5754 \end{aligned}$$

Omitting the remaining observations on account of the possible interference of a second growth-cycle, and applying the method of least squares to the above, we obtain the normal-equations:—

$$36K - 6b = -7.9120 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (48)$$

$$178K - 19b = -19.5056 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (49)$$

Whence $K = 0.0962$ and $b = 1.9279$; but $b = Kt_1$, hence $t_1 = 20$.

Equation 47 now becomes:—

$$\log \frac{x}{112-x} = 0.0962\,(t-20) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (50)$$

where x is the weight in milligrams and t the time in days.

Giving to t the values 1, 2, 5, 7, etc., we can calculate the corresponding theoretical values of x. In Table XXX the theoretical and observed body-weights are compared.[e]

TABLE XXX

GROWTH OF TADPOLES

Age in days	Body-weight in milligrams	
	Observed	Calculated
1	1.83	1.64
2	2.00	2.03
5	3.43	3.90
7	5.05	6.00
9	10.40	9.01
14	23.52	23.46
41	101.0	110.9
84	1989.9	112.0

It will be seen that, save in the case of the last observation, the agreement is good. The discrepancy in the last observation is evidently due to the superimposition of another growth-cycle.

5. **The Growth of Annual Plants.**—Reed and Holland[385] have measured the growth of sunflowers (*Helianthus*) at weekly intervals until no further elongation occurred. Fifty-eight plants were employed for the purpose. As soon as the seedlings had reached an average height of more than 10 centimetres the fifty-eight plants were selected at random and removed from those adjacent so as to leave a space of about 20 centimetres between any plant and its nearest neighbor. Each plant was marked with ink at a distance of 10 centimetres below the growing tip. This mark served as a point from which subsequent measurements were made.

The sunflower is usually unbranched, and it was selected for investigation on this account, since it appeared probable that in an unbranched plant the total

[e] The age is given in days after hatching. The embryos were weighed after the removal of superficial water and the weights given are the average weights. The number of embryos weighed is not given.

height of the stem would be more closely proportional to the growth of the entire organism than in a form developing many branches. Seventeen of the fifty-eight plants, however, developed branches. As the average heights of the branched and unbranched individuals were close enough together at maturity to fall within the range of the probable error, Reed and Holland do not believe that the validity of the measurements is affected to any appreciable extent by the admixture of branched stems.

The average length of stem was taken as a measure of the total growth of the plant. If the stem is cylindrical then excluding roots and leaves, this assumption is evidently valid, but the growth of roots and leaves doubtless stands in a fairly definite proportion to the growth of the stem, and in that case the growth in length of the stem would stand in a fixed proportion to the growth of the organism as a whole. While this proportionality was not definitely established by measurement the strict applicability of the autocatalysis formula to the growth makes it appear very probable that the stem, in this plant, actually represents a fixed proportion of the growth of the whole organism.

The maximum observed height of the stems was 254.5 cm. and the time at which this height was attained was 34.2 days. Equation 29 therefore becomes:—

$$\log \frac{x}{254.5 - x} = K\,(t - 34.2) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (51)$$

Inserting the observed values of x and t into the equation a series of values of K were computed of which the average was 0.0421. Employing this value of K theoretical values of x may be computed from the formula by inserting the corresponding values of t. The calculated and observed values of x (= height in centimetres) are compared in Table XXXI:—

TABLE XXXI

GROWTH OF SUNFLOWERS

Age in days	Height in centimetres	
	Observed	Calculated
7	17.93	17.05
14	36.36	31.43
21	67.76	55.35
28	98.10	90.09
35	131.00	132.21
42	169.00	173.06
49	205.50	205.64
56	228.30	227.01
63	247.10	239.74
70	250.50	246.87
77	253.80	250.56
84	254.50	252.46

The correspondence between the observed and theoretical values is very satisfactory. The observed and calculated curves of growth are also compared graphically in Figure 16.

Further comparison of the calculated and observed values was made by testing the "closeness of fit" of the theoretical to the observed curve by the statistical method of Elderton.[122] It was found that in over ninety-two cases out of a hundred a random sampling would give values diverging more widely from the theoretical than those actually found. In other words the "fit" of the calculated curve to the observed values was almost certainly not accidental.

Reed and Holland infer from these results that the growth of the sunflower is largely determined by internal rather than external factors.[f] They point out that there was no correlation between the weekly height-increments and that proportion of the total area of a simultaneous thermograph record which lay above 40° F. There was also no trace of correlation between the weekly growth

[f] The value of A must, however, be regarded as depending very largely upon external factors, namely, those which determine the "nutrient level," among which must be reckoned the fertility of the soil. This factor was, however, constant for the duration of the experiment.

increment and the evaporating power of the air as determined from the readings of the wet and dry bulb thermometers. On the other hand growth ceased with the oncoming of the reproductive processes, or perhaps it would be more correct to say that reproductive processes ensued when growth had attained a maximal extent and a minimal velocity.

Many data concerning the growth of oats, and not only of the whole plants but their constituents as well, have

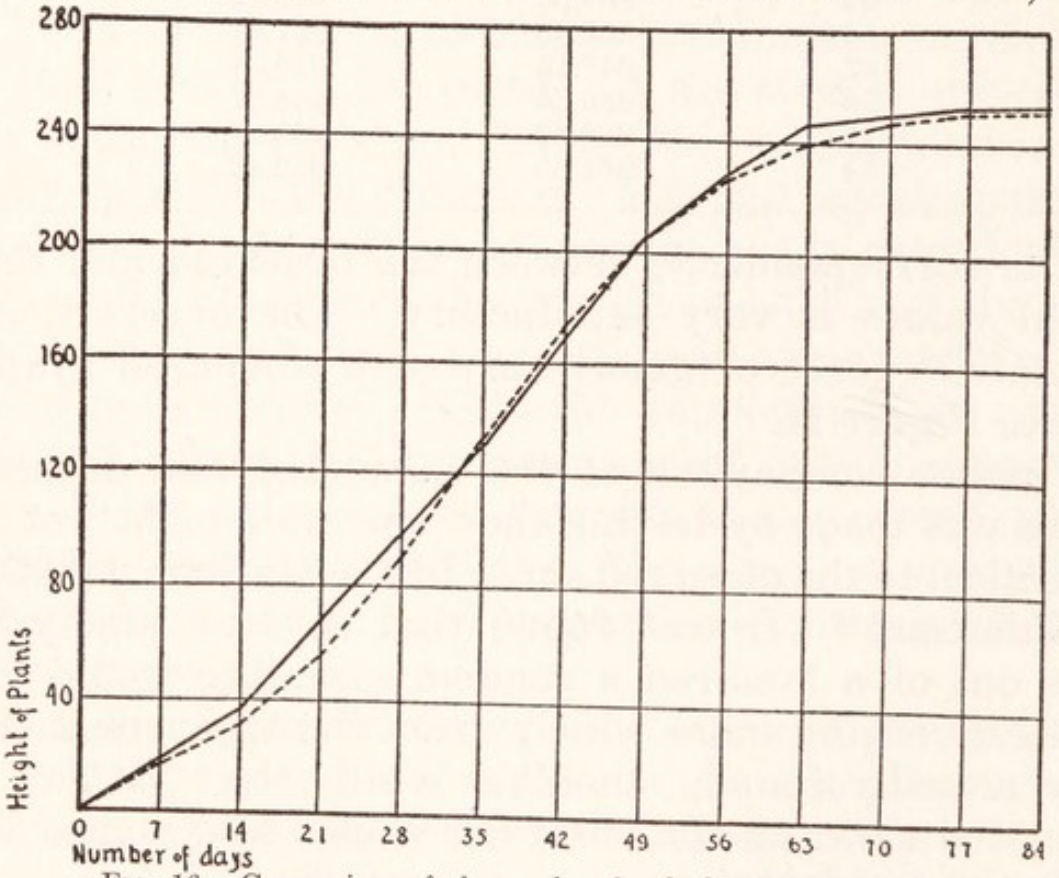


FIG. 16.—Comparison of observed and calculated values for the mean height of *Helianthus*. Full line indicates the observed heights; the discontinuous line the calculated heights. (After Reed and Holland.)

been collected by Monnier.[310] The seeds of the oats were planted in the beginning of May, and the first determinations were made upon the 16th of May; from this date determinations were made every few days until the 29th of July, when the growth of the plants was nearly complete. Nothing is said in Monnier's paper concerning the conditions under which the plants were grown; presumably they were grown under approximately constant conditions of soil, temperature, moisture, etc., but the results above cited of Reed and Holland indicate that

small fluctuations in these factors would not introduce any appreciable error into the results.

In applying Equation 29 to these results t is taken as zero on the 16th of May, and thereafter is estimated in days. This procedure is perfectly correct, since it is not the time from the beginning of growth (*i.e.*, germination) but the period separating the moment of observation from that of half completion of growth which appears on the right-hand side of the equation, and this is obviously unaffected by the date which we consider as zero time.

Fifty plants were weighed and analyzed in each set of determinations. The weight of the fresh plants having been determined, they were dried in an oven at 110° until no further loss in weight occurred, and the weight of the dried plants was then determined. The dried plants were then powdered and the nitrogen in the powder was determined by the Kjeldahl method; another portion of the powder was ignited to a dull red heat and the weight of the ash determined; in the ash the P_2O_5, the K_2O and the CaO were determined separately.

I have determined the value of A, the final growth, from two pairs of observations in the manner illustrated in the application of the formula of autocatalysis to the growth of the frog (Section 4). This procedure, of course, introduces a possibility of error in the determination of the parameters which most accurately define the data, since the four observations are necessarily chosen more or less at random, and one or more of them may be erroneous. In order to obviate this source of error as far as possible, each group of data was plotted upon squared paper and a curve drawn smoothly through the points; two pairs of points equally distant from one another were then chosen, the pairs being separated as widely as possible from one another, in such a manner that the points chosen lay as closely as possible to the smoothed curve; these were the points utilized to determine the most probable value of A for that group of data.

The value of A having been thus estimated, the experimentally determined values of t and x (time and weight) were then inserted in Equation 29, and the most probable values of K and t were determined from all of the observations by the method of least squares. Subsequently to ripening these plants begin to lose water and consequently decrease in weight. The weights after this diminution begins to occur were therefore rejected in the determination of the constants. The most probable values of A, K, and t_1 for each group of data having been thus determined, the theoretical values of x (= weight in grams) are calculated for the various values of t (= time in days). These calculated values are compared with those experimentally observed by Monnier in Tables XXXII, XXXIII, and XXXIV.

The agreement between the calculated and observed values is very satisfactory except in the later measurements of the weight of the whole plants, when the loss of water introduces a factor unforeseen in the equation and consequently results in more or less marked deviations from it.

TABLE XXXII

OATS. GROWTH OF FRESH PLANTS

$A = 349.35$; $K = 0.0777$; $t_1 = 33.3$

Time in days	Weight in grams	
	Observed	Calculated
0	0.935	0.905
4	1.78	1.81
8	3.58	3.76
16	14.51	15.20
21	34.70	34.96
25	52.20	64.55
29	141.20	110.89
35	185.70	200.84
39	243.30	255.65
42	314.34	288.93
47	304.00	320.44
51	254.62	335.34
57	229.90	346.40
64	224.30	346.40
70	180.35	347.76
74	190.45	348.36

TABLE XXXIII

OATS. GROWTH OF DRIED PLANTS

$A = 61.8$; $K = 0.0702$; $t_1 = 37.75$

Time in days	Weight in grams	
	Observed	Calculated
0	0.167	0.138
4	0.260	0.261
8	0.473	0.501
16	1.77	1.78
21	4.29	3.86
25	5.42	6.98
29	14.49	12.09
35	23.77	24.19
39	29.99	34.08
42	42.36	41.07
47	50.44	50.55
51	54.43	55.25
57	59.74	59.28
64	71.30	60.91
70	65.11	61.47
74	68.00	61.62

TABLE XXXIV

OATS. GROWTH OF ORGANIC MATERIAL

$A = 56.51$; $K = 0.0664$; $t_1 = 38.7$

Time in days	Weight in grams	
	Observed	Calculated
0	0.164	0.152
4	0.270	0.281
8	0.425	0.513
16	1.48	1.71
21	3.70	3.54
29	12.48	10.41
35	20.70	20.45
42	37.23	35.20
47	44.79	44.08
51	48.41	49.07
57	52.75	53.39
64	64.10	55.62
70	59.11	56.13
74	62.63	56.31

The growth of the mineral constituents did not by any means conform to the time relations indicated by the formula of autocatalysis.[390] From this it appears that their concentration in the plant is determined by quite

other factors than those which determine the rate of growth of protoplasm. There are indications afforded by his data, also, that an exchange of ions occurs during growth, between the soil and the plant, with the result that certain ions (possibly magnesium ions) leave the plant and return to the soil, while others (potassium and calcium) are taken up from the soil to replace them (*loc. cit.*).

The estimates of the nitrogen contents of the plants were highly irregular and while the increase of nitrogen followed approximately the growth of organic material, a detailed comparison of the results with those indicated by the formula of autocatalysis yields no additional information.

6. **The Growth of Fruits.**— While the growth of the plant as a whole (in animals) comes to a stop with the onset of reproductive activity, the growth of the fruit itself probably represents a separate autocatalytic cycle. Unfortunately very few accurate observations have been recorded, but Anderson[15] has published a series of observations upon the growth in weight of the fruit of *Cucurbita pepo*. The vine was grown in the open air and its terminal portion was trained into the laboratory, which was ventilated in such a manner that the conditions of moisture and humidity were practically the same as those of the outside air. The fruit rested upon cotton wool in the scale-pan of a self-registering balance. The date of pollination is given, and the daily increase in weight for a period of 47 days. The fruit was completely ripened in 30 days, but still continued to show fluctuations in weight. The temperature varied, but usually fluctuated between 14° and 22°. Once or twice, however, it rose as high as 30° and fell as low as 6°. Sunshine, etc., were subject to the inconstancy of the weather. Since, in addition, the experiments were carried out only upon a single individual, it is evident that the conditions were not favorable for a comparison between theory and experiment. Nevertheless, the curve of growth, constructed from Anderson's

measurements, resembles so closely the curve of autocatalysis (Fig. 17) that a detailed comparison of the observed and calculated weights is of interest.

The maximum daily increment occurred at between ten and eleven days after pollination. After 25 days the fruit

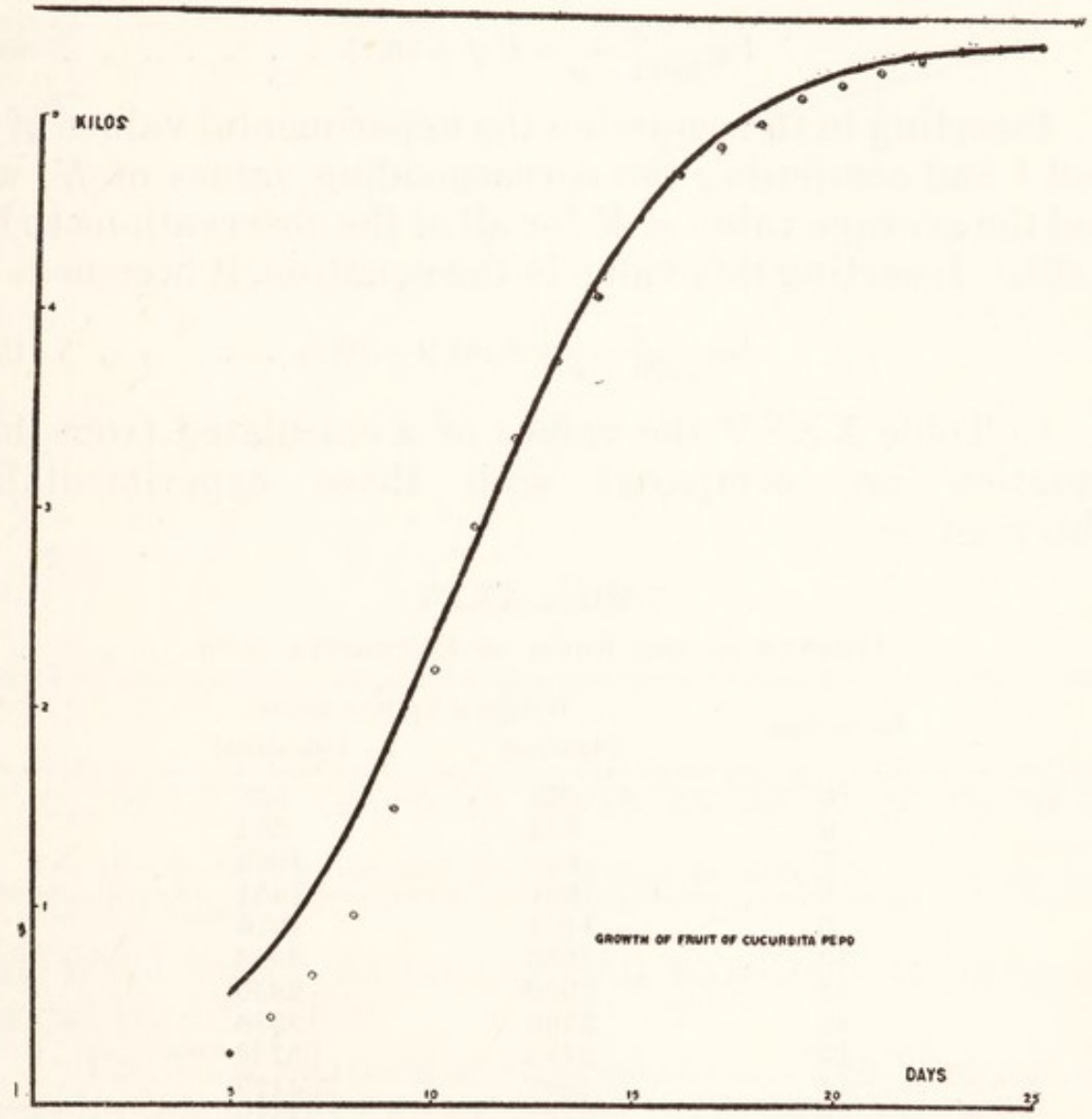


FIG. 17.—Comparison of observed and calculated values for the growth of the fruit of *Cucurbita pepo*. The observed weights are indicated by the dots within the circles.

had attained the maximum weight of 5,366 grams, and the weight then gradually sank to 5,216 grams at the end of 47 days. This loss of weight (probably due to migration of water) was very irregular, a marked increase and corresponding decrease in weight not infrequently taking place during one day, so that the net result was a very trifling

alteration in weight. Taking, therefore, the true maximum weight as 5,400 grams, half this weight was attained between the 10th and 11th days, in fact, calculated by interpolation, in 10.7 days, thus coinciding with the time of maximum daily increment. Equation 29 therefore becomes, for this case:—

$$\log \frac{x}{5400 - x} = K\,(t - 10.7) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (52)$$

Inserting in this equation the experimental values of x and t and computing the corresponding values of K, we find the average value of K for all of the observations to be 0.161. Inserting this value in the equation, it becomes:—

$$\log \frac{x}{5400 - x} = 0.161\,(t - 10.7) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (53)$$

In Table XXXV the values of x calculated from this equation are compared with those experimentally observed:—

TABLE XXXV

GROWTH OF THE FRUIT OF CUCURBITA PEPO

Age in days	Weight of Fruit in grams	
	Observed	Calculated
5	267	583
6	443	804
7	658	1093
8	961	1451
9	1498	1876
10	2200	2352
11	2920	2850
12	3366	3338
13	3758	3786
14	4092	4172
15	4488	4488
16	4720	4736
17	4864	4923
18	4980	5062
19	5114	5162
20	5176	5234
21	5242	5284
22	5298	5336
23	5352	5344
24	5360	5361
25	5366	5373
47	5216	5400

The observed and calculated values of x are further compared in Figure 17. The agreement in the second half of the cycle (subsequent to 10 days) is excellent, and on the whole is as good as could be expected between theory and the results of observations made upon a single

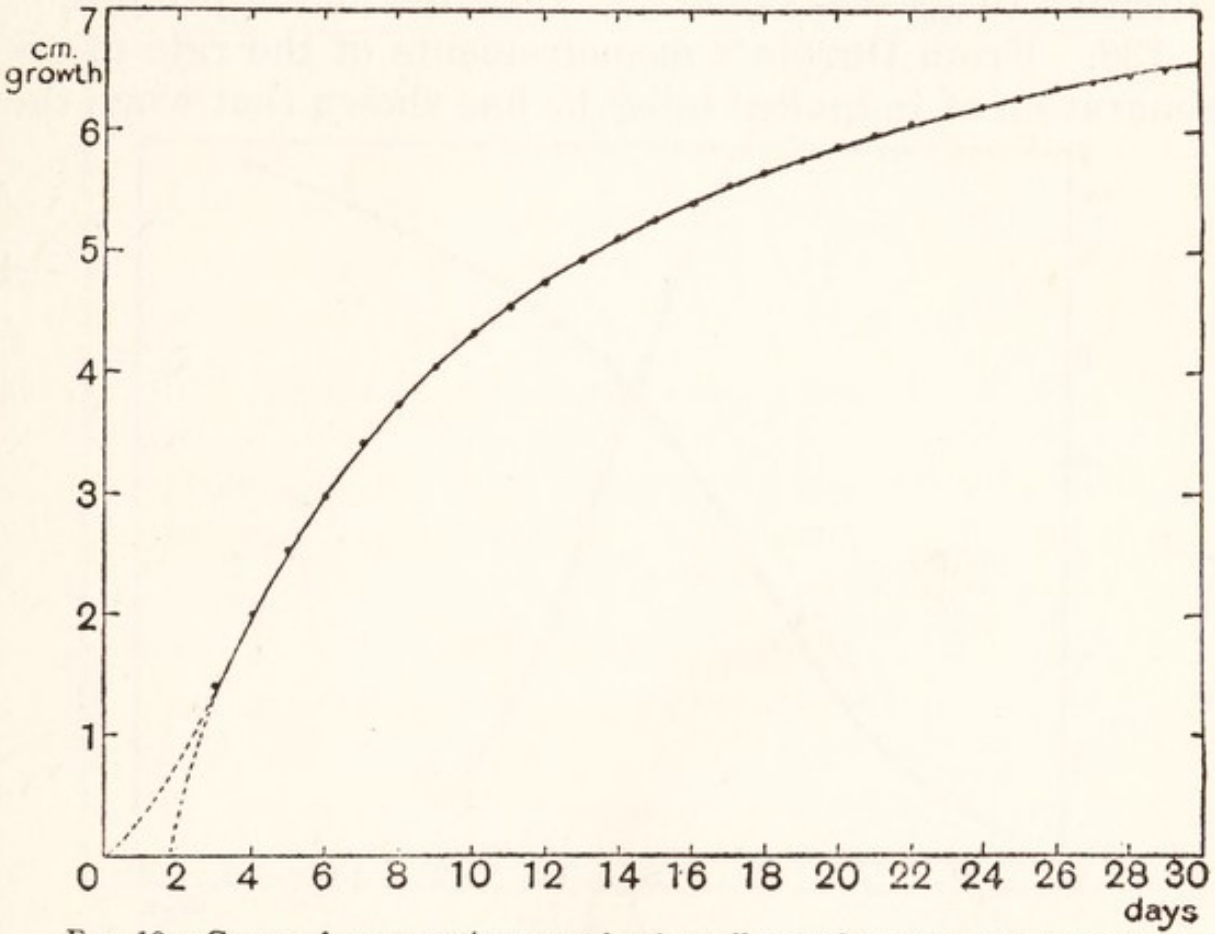


FIG. 18.—Curve of regenerative growth of small tadpole's tails. The dotted lines represent alternative hypothetical extensions of the observed curve. (From D. W. Thompson, after M. L. Durbin.)

individual under fluctuating and uncontrolled environmental conditions.

7. **The Time-relations of Regeneration.**—The renewal of non-vital portions of an organism is succeeded by more or less effectual efforts towards replacement varying from the complete restoration of the lost parts, in many invertebrates, to mere covering of the wound as in the higher vertebrates.[g] The inciting cause of regeneration, as we will subsequently see (Chapter VIII), is the removal of the products of the reactions which underlie and de-

[g] Certain organs, for example the liver, may, however, be almost completely regenerated after extensive destruction even in mammals.

termine growth-rate, and the removal of these products, as in other chemical equilibria, reinaugurates the forward reaction.

The time-relations of this phenomenon have been very thoroughly analyzed by D. W. Thompson, who has drawn attention to several important features of the process,[490] p. 138. From Durbin's measurements of the rate of regeneration of tadpoles' tails[h] he has shown that when the

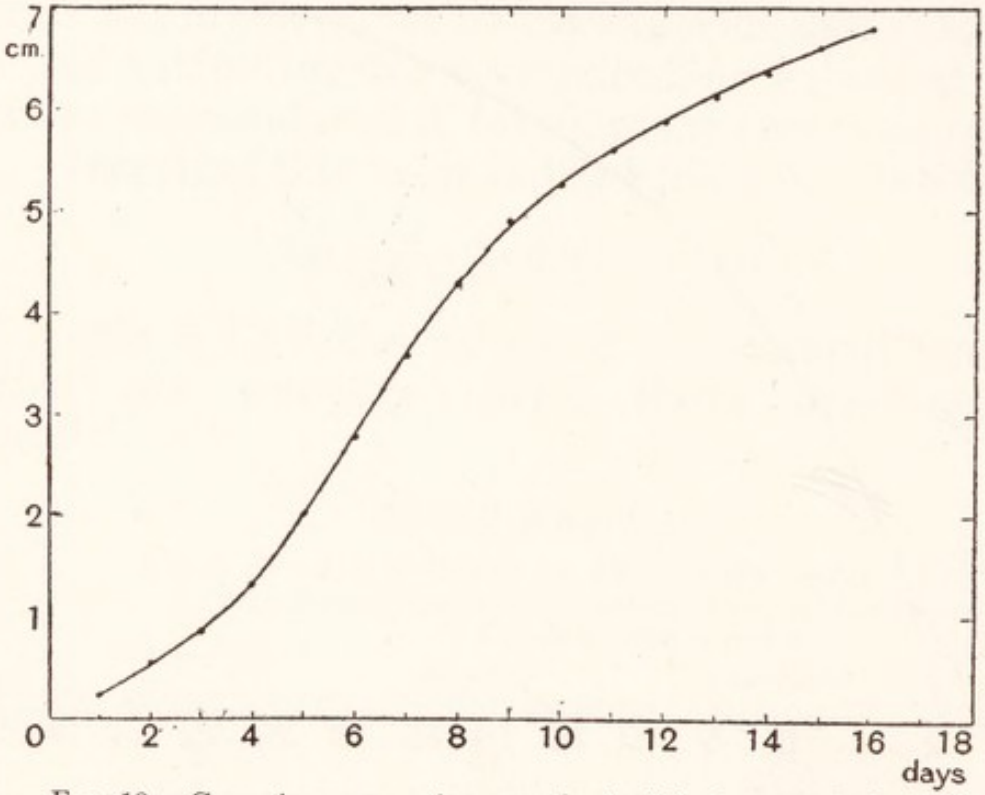


FIG. 19.—Curve in regenerative growth of tail in larger tadpoles. (From D. W. Thompson, after M. L. Durbin.)

tadpoles are relatively small (34.2 mm. in length, tails 21.2 mm. in length) and not much over fifty per cent. of the tail is removed (11.5 mm.), the curve representing the consequent regeneration from day to day yields little or no evidence of any inflexion due to autoacceleration (Fig. 18). When, however, larger tadpoles were employed (49.1 mm., length of tail removed not specified) a distinct point of inflexion was noted in the curve of regeneration (Fig. 19) which therefore resembles in outline the normal

[h] Since the earlier volumes of the *Journal of Experimental Zoölogy* are at present inaccessible to me, I have relied upon the full account of the experiments of Durbin [110] and Ellis [123] contained in D. W. Thompson's *Growth and Form.*

curve of growth of an entire organism.[i] From this it is inferred by Thompson that the curves of growth and regeneration are in reality similar, although superficially different, and he suggests that even in the case of smaller tadpoles a point of inflexion might be found if the

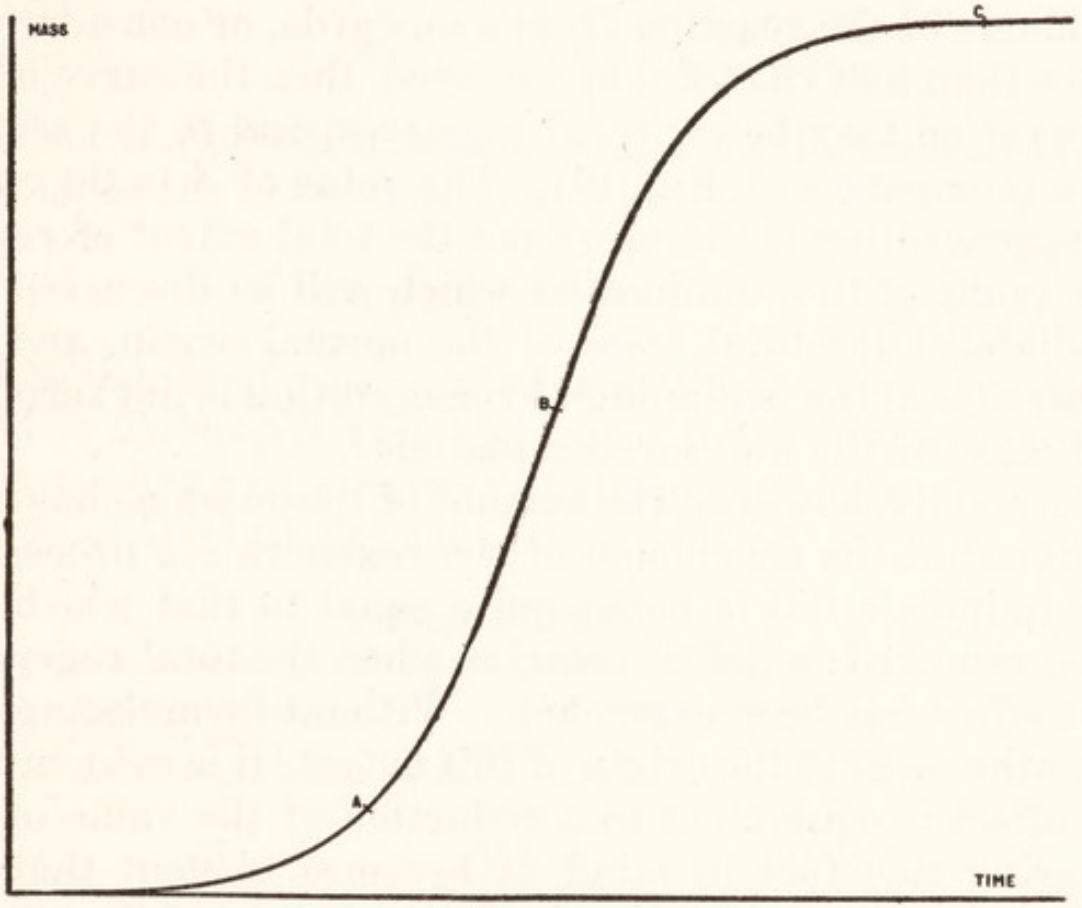


FIG. 20.—Curve of transformation in an autocatalyzed monomolecular reaction. The segment B-C has no point of inflexion, but the longer segment, A-C, includes a point of inflexion.

measurements were made at a sufficiently early stage of regeneration.

It is quite possible, however, that apart from a relatively brief "lag-period" (Chapter IV) no inflexion or autoacceleration of the curve might occur. This will be evident if we revert to the view which has just been expressed, that regeneration is due to the reinauguration of growth by the removal of its products. On examining the

[i] The rate of regeneration was measured in terms of increment in length. If we regard the cross-section of the tail as uniform throughout its length, an assumption which is doubtless approximately, although of course not exactly valid, then the growth in length may be considered to have been proportional to the growth in weight or volume.

curve of autocatalysis (Fig. 20) it will be evident that if the products of the reaction from b onwards be removed, the curve of regeneration may be expected to correspond to the section of the whole curve of autocatalysis lying between b and c (compare with Fig. 18), while if the products of the reaction from a onwards, or considerably more than half the total be removed, then the curve of regeneration may be expected to correspond to the section $a - c$ (compare with Fig. 19). The value of A in the curve of regeneration is therefore not the total extent of repair but (subject to a diminution which will be discussed immediately) the total mass of the normal organ, and the value of x at the beginning of regeneration is not zero, but the mass of the uninjured remainder.

Actually, however, the amount of tissue which has been restored at the conclusion of the regenerative process in the tadpole's tail is never quite equal to that which has been removed; a defect remains when the total regenerative effort has been expended. Without formulating any hypotheses as to the origin of this defect,[j] it is evident that its effect is equivalent to a reduction of the value of A.[k] Bearing this fact in mind, it becomes evident that the value of A in regeneration is the total mass of the part or organ at the conclusion of regeneration, while x may be regarded as the total mass, inclusive of the portion originally uninjured, which is attained at any intermediate period of regeneration.

In Durbin's experiments upon smaller tadpoles (Fig. 18) the maximum regeneration recorded is 6.5 mm. at 30

[j] It may arise in diverse ways in different tissues. In the present instance it may be primarily due to the abstraction of nutritive materials from the tail which subsequently culminates in metamorphosis. In more adult animals accumulations of autocatalyst in the pericellular fluids derived in part from other tissues, may possibly assist in abbreviating the growth which is attainable before equilibrium of the forward and reverse reactions of autocatalysis is reached (Chapter VIII). In this latter case x as well as A would be affected.

[k] Equivalent, that is, to the effect which would be exerted by a reduction of the "nutrient level."

days, by which time the daily increment had fallen to 0.05. We may therefore assume, with a probable approximation to exactitude, that the value of A is 6.6 mm. plus the uninjured remainder of the tail, which was 9.7 mm. Equation 29 therefore becomes:—

$$\log \frac{x}{16.3 - x} = K(t - t_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (54)$$

Determining the values of K and Kt_1 by least squares from all of the observations we find:—

$$\log \frac{x}{16.3 - x} = 0.0581\,(t + 3.13) \quad . \quad . \quad . \quad . \quad . \quad (55)$$

The values of x calculated from this formula are compared in Table XXXVI with those actually observed by Durbin, adding in each case the length of the unmutilated portion of the tail (9.7 mm.).

TABLE XXXVI

Period of regeneration in days	Total length of tail	
	Observed	Calculated
3	11.1	11.3
7	13.1	13.0
10	14.0	13.9
14	14.9	14.8
18	15.2	15.3
24	15.9	15.9
30	16.2	16.1

The agreement, it will be seen, is almost perfect. The reason why t_1 has a negative value is clear. The mutilation of the tail virtually reinaugurated a growth-cycle at the point corresponding to the mass of uninjured tail plus the accumulation of pericellular autocatalyst derived from other tissues. If, as in the experiments, this point on the autocatalytic curve lies beyond the centre then it corresponds to a period in the reaction later than that at which $x = \frac{1}{2}A$. The centre of the curve or point of inflexion will therefore antedate the moment of mutilation. It is

for this reason that no point of inflexion appears on the curve of regeneration. In case a larger proportion of the tail were removed, then the point of inflexion or maximal growth-velocity might occur at some period succeeding the moment of mutilation and t_1 would then be positive.[1]

[1] I have been unable to institute a quantitative comparison between the curve of autocatalysis and Durbin's curve of regeneration for older tadpoles, owing to the fact that I have been unable to refer to her article for details essential to such a comparison (length of tail removed and length of remainder).

CHAPTER IV

THE REPRODUCTION OF UNICELLULAR ORGANISMS[a]

1. **The Allelocatalytic Effect.**—The problems presented by the multiplication of organisms which consist of a single cell would appear, at first sight, to be very much simpler than those involved in the growth of the multicellular aggregates which compose the higher animals or plants. When a single cell which is capable of asexual reproduction is inoculated into a nutrient medium, after a longer or shorter period of delay, generally designated the "lag-period," reproduction commences, and continues until a maximum density of population is attained, when, it is generally supposed, the nutrient capacity of the medium has become exhausted, or suffices only to maintain existence without permitting the excess of assimilation which is required for multiplication.

As soon as we examine the phenomena in more detail, however, it becomes apparent that no such simple interpretation will suffice. In the first place, the multiplication of unicellular organisms in a limited volume of nutrient medium is autocatalyzed, just as the multiplication of cells in a higher animal or plant is autocatalyzed. Attention has been drawn to this fact by McKendrick, who studied the multiplication of bacteria inoculated into culture media,[295] and he sought to interpret it by supposing that each cell is alike in its inherent capacity for synthesizing new protoplasm. The number of centres of synthesis, and consequently the rate of synthesis, would thus obviously multiply in proportion to the number of

[a] Throughout this book the term "*cytoplasm*" has been employed to designate that living part of the cell which is external to the nucleus, and the word "*protoplasm*" has been employed to designate the totality of living matter in the cell, exclusive of non-living reserve materials.

cells, while at the same time the available substrates, *i.e.*, the food-materials, would diminish in the same proportion. Evidently this mode of interpretation is quite consistent with, and in fact is an alternative mode of expressing the view that cellular multiplication is autocatalyzed, that is, that it is a process which manufactures its own catalyzers, which, however, are conceived as being wholly endocellular. A similar view of the chemical mechanism underlying the growth of plants is attributed by Monnier to Chodat in the following words:—"On peut bien, ainsi que M. Chodat l'a proposé, considérer l'accroissement comme une réaction chemique complexe, dans laquelle la catalysateur est la cellule vivante, et les corps en présence sont l'eau, les sels, et l'acide carbonique."[310] To Professor Chodat, therefore, we must accredit the origin of the view that the rate of growth is governed by an autocatalyzed process, but the catalyzer, it will be noted, is considered to be endocellular and is, in fact, either the cell itself, as a whole, or some structure or essential constituent which is resident within the cell. This conception of the origin of the autocatalytic phenomena in growth does not suffice to interpret the whole of the facts, however, as the following considerations reveal:—

The multiplication of infusoria (*Enchelys*) arising from a single individual in a limited volume of nutrient medium is, like the multiplication of bacteria, an autocatalyzed process.[410] This is illustrated by the following curves (Fig. 21) which collectively represent the first or progressively accelerated phase of an autocatalytic reaction. As in the case of bacterial cultures[371] an isolated individual displays no reproductive tendency for a variable, and frequently considerable period. Once cell-division has occurred, however, succeeding cell-divisions recur, not at equal, but at progressively abbreviated intervals, so that a culture arising from one individual may contain in nineteen hours after isolation only four individuals, four hours later, eight individuals, three

hours after this, sixteen individuals, and forty-eight hours after the original individual was isolated over a thousand individuals may be present in the culture. Thus in this instance the first twenty-four hours produced but four individuals, and the succeeding twenty-four hours not merely four times as many but a thousand, or 250 times as many as were produced in the first twenty-four hours. Even if we subtract the "lag-period," or period preceding

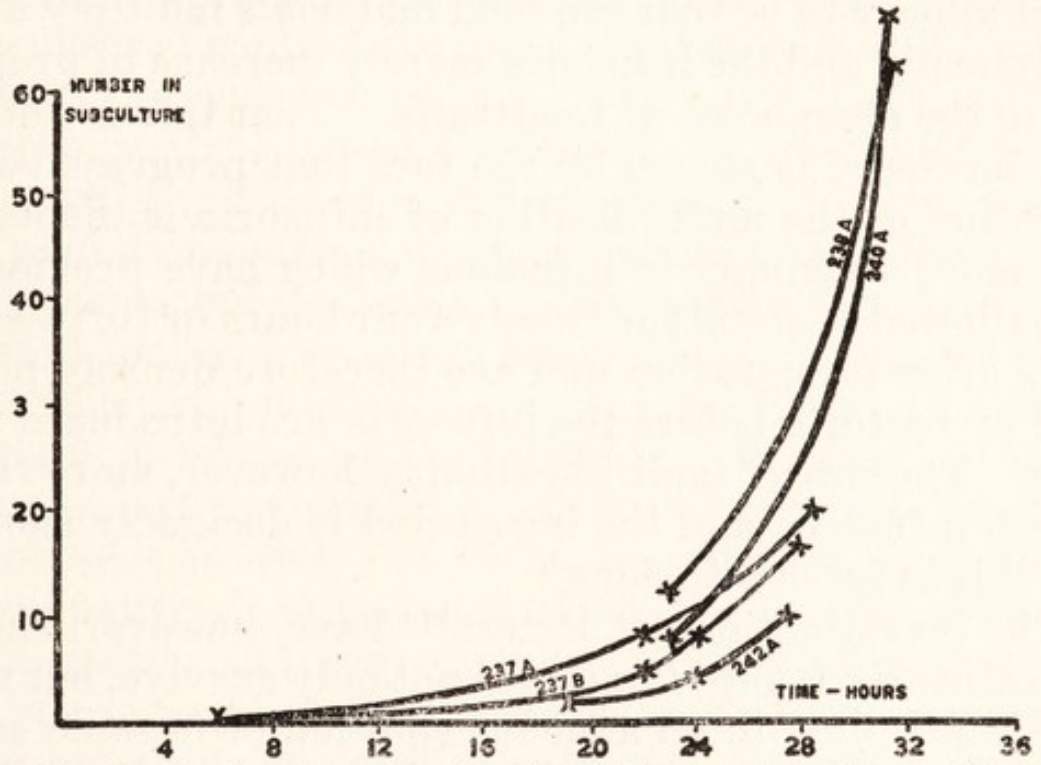


FIG. 21.—Showing the autoacceleration of multiplication rate in cultures arising from single isolated infusoria (*Enchelys*).

the first cell-division, a progressive increase in the frequency of divisions may readily be observed in the early stages of multiplication in such cultures.

The progressive abbreviation of the period which elapses between succeeding early divisions in infusorial cultures, shows that the products of multiplication do not at all times possess like capacity for reproduction, as the theory of Chodat and McKendrick would imply, but that the capacity of the individual cells to reproduce (within a given period) increases up to a certain maximum with the population of the culture.

Infusorial cultures in hay infusions are accompanied

by a population of bacteria which increases very rapidly with the age of the culture, at all events in the early stages. The question therefore at once suggests itself whether the autocatalysis in the multiplication of infusoria may not merely be a reflection of the autocatalysis which is known to occur in the growth of bacteria. If we were to suppose, what was formerly believed, that the food of infusoria consists of bacteria, then the explanation of the above facts might appear to be that the food materials multiply autocatalytically and the infusoria merely increase in proportion to the abundance of foodstuffs. That this is not the case, however, is shown by the fact that progressive acceleration of the multiplication of infusoria is displayed even more strikingly in infusions which have previously been allowed to stand for twenty-four hours or forty-eight hours after preparation and are therefore densely populated by bacteria before the infusoria are introduced into them. The rate of multiplication is, however, more rapid in such infusions and the lag-period is decidedly shorter than it is in fresh infusions.[b]

The investigations of Peters[372] have, however, shown that infusoria (*Colpidium*) will not only survive, but multiply indefinitely in an aqueous solution of organic salts, containing a source of carbon with not less than three atoms of carbon in the molecule, an ammonium salt as the source of nitrogen, and a phosphate as the source of phosphorus. The enhancement of reproductive rate and abbreviation of the lag-period which are observed in infusions which have previously been bacterized, therefore, is probably attributable to the production of a greater proportion of foodstuffs which are immediately available to the infusoria, without previous digestive elaboration,

[b] Prepared by mixing 5 grams of chaffed hay with 100 c.c. of tap-water, heating to boiling and then standing on a boiling water-bath for half an hour. After filtration 0.4 c.c. of $\frac{N}{10}$ Na_2CO_3 solution is added to every 10 c.c. of the filtrate in order to communicate a favorable reaction ($P_H = 7.7$).

through the hydrolysis of polysaccharides and proteins and deaminization of amino-acids which are brought about by the metabolic activities of the bacteria. This interpretation of the favorable influence of preliminary bacterization upon the multiplicative rate of infusoria is supported by the fact that it is displayed quite as well after the bacterized infusions have been filtered through a porcelain filter, thus removing both the bacteria and their spores. It is true that the infusoria (*Enchelys*) inhabiting these filtered media have the elongated cylindrical outline of infusoria which have been "starved" in old and comparatively "exhausted" cultures, or which have been cultivated in distilled water to which a suitable reaction and tonicity have been communicated by the addition of a "buffer mixture" of salts. This, however, is readily accounted for by supposing, what indeed is very probable, that the nutrition of infusoria may be either saprophytic, if soluble predigested foodstuffs are abundant in the medium, or partly saprophytic and partly holozoic if bacteria are present as well. In the latter case the infusoria become engorged with digestive vacuoles containing bacterial bodies and the products of their digestion, and the infusoria therefore appear more bulky and pyriform or even globular in outline, but they are not necessarily better nourished than the infusoria in a bacteria-free medium which contains an abundance of predigested foodstuffs.

The true origin of the progressive enhancement of reproductive rate in infusorial cultures becomes very evident if we compare the reproductive rate in cultures arising from single isolated individuals with the reproductive rate which is exhibited in cultures arising from two like individuals simultaneously isolated into the same volume of nutrient medium. The number of individuals produced in a given time (24 to 48 hours according to the speed of multiplication) is not twice, but from four to sixteen times the number produced in the cultures arising

from single individuals.[411] In other words, the reproductive rate of each individual is enhanced by the neighborhood of the other. Now, while conjugation is a normal event in infusorial cultures under certain special conditions, continuous observation has shown that it does not occur in the cultures which exhibit this mutually accelerative effect, and, in fact, Jennings has shown that when conjugation does occur in cultures of infusoria, the reproductive rate of the conjugants is not enhanced, but on the contrary, very decidedly diminished.[208] The effect is not due to the simultaneous seeding of the culture fluid with a double "charge" of bacteria associated with the infusoria, because it is even more than usually marked in infusions which have been previously bacterized, and in which the bacterial population is initially so dense that the bacteria introduced together with the infusoria must constitute a totally negligible fraction of the whole population. Hence the only possible inference that can be drawn from this phenomenon is that the infusoria discharge into the culture-medium some substance which accelerates their own multiplication. We thus have evidence of the existence, not merely of an internal supply of autocatalyst such as that contemplated in the hypothesis of Chodat and McKendrick, but also an external supply of catalyzer, which is communicated to the pericellular fluid by each of the cells which inhabit it.

A phenomenon which reveals very clearly the importance of the part played by the accelerative material which is shed from multiplying infusoria into the surrounding medium, is the noteworthy increase of reproductive rate which is brought about by mere reduction of the volume of the culture medium. The following experiment, one of the very many of the same kind, will serve to illustrate the results obtained in all of them:—

Single infusoria were isolated into fresh hay infusion from two six-day-old parent-cultures, A and B, which were thickly inhabited. After washing by reisolation, they

were separately placed in varying volumes of the hay infusion, the volume of infusion in each case being determined by weighing the slide before and after the drop was placed upon the depression in its centre. The slides were kept upon pads of wet filter paper in closed Petri dishes to prevent evaporation. After forty-four hours the counts in the various cultures were as follow:—

TABLE XXXVII

Culture number	Weight of culture fluid, milligrams	Number of individuals 24 hours	Number x weight
372A	5.0	250	1250
374A	10.0	31	310
375A	13.6	104	1414
373A	23.4	64	1498
376A	34.6	33	1142
372B	1.4	64	90
373B	7.6	4	30
375B	13.0	8	104
374B	23.8	6	143
376B	32.2	4	129

Thus, if we except the irregular results obtained from cultures 374A and 373B, it is evident that the population attained in these cultures in forty-four hours was very nearly inversely proportional to their volume, and if in the A series a volume of about 1.5 c.c., or in the B series about 0.15 c.c. had been employed, we may infer that no multiplication at all would have occurred. Now as a matter of fact single individuals isolated into volumes exceeding one cubic centimetre very rarely survive, and failures to reproduce on the part of individuals isolated into cultures exceeding 0.1 c.c. in volume are not at all infrequent. This phenomenon has also been observed by Peters in the cultivation of *Colpidium*[372] and it is a fact familiar to those who are engaged in the cultivation of yeasts that the isolation of a single cell into too large a volume of culture medium results in the death of the isolated cell or at least a very great delay of its multiplication.[222,515]

We must infer that some necessary substance is dissipated by these cells into the surrounding medium, and that the reproductive rate of infusoria is nearly proportional to its concentration. When this substance is too dilute, *i.e.*, is dissipated into too large a volume of medium, then reproduction, and even maintenance, become impossible.

When an infusorian (*Enchelys*) has been isolated into freshly prepared hay infusion, or into distilled water to which a suitable reaction and tonicity have been communicated by alkaline salts, the presence of an autocatalyst of cell-multiplication in the medium can be demonstrated, directly after the first cell-division, by isolating one of the daughter-cells into a fresh medium. The reproductive rate of this reisolated cell is always much less than that of the cell which has been left undisturbed in its surrounding medium, and this is true even when the isolated cell has been transferred from a medium poor in foodstuffs, such as faintly alkalinized distilled water,[c] to one which is comparatively rich in foodstuffs, such as freshly prepared hay infusion.

Glass-distilled water which has been rendered faintly alkaline by the addition of one c.c. of $\frac{N}{10}$ Na_2CO_3 solution per 100 c.c.,[d] and which has been inhabited by multiplying infusoria for forty-eight hours, may be shown to contain an agent which accelerates the reproduction of infusoria, in the following manner:—The water is freed from infusoria by heating to 50° C. to immobilize them, followed by filtration through a double filter paper. The filtrate is

[c] Distilled water to which has been added one volume per hundred of a $\frac{m}{15}$ mixture of monopotassium and disodium phosphates having a P_H of 7.7.

[d] This medium is too alkaline for optimal multiplication of the infusoria. Distilled water containing a sufficiently small proportion of carbonate solution to communicate the most favorable reaction fails to support multiplication either because the tonicity is too low or because the "buffer action" of such a small proportion of carbonate is inadequate. However, energetic multiplication will occur in considerably hyperalkaline media, and it was desired to employ sodium carbonate in this experiment in order to facilitate the subsequent neutralization of the medium.

then neutralized to cochineal indicator by the addition of $\frac{N}{100}$ hydrochloric acid, and evaporated on a boiling water-bath to one-half its original volume. A small precipitate of coagulated protein is removed by filtration, and the fluid, stored in flasks plugged with cotton, is boiled several times at intervals of two or three days to sterilize it. This fluid, when added to "buffered" distilled water, or to freshly prepared hay infusion, greatly accelerates the reproductive rate of infusoria isolated into these media. The following experiments show the type of results obtained:—

Single infusoria from two parent cultures A and B, were isolated into 0.08 c.c. of each of the following media:—I. Distilled water buffered by a phosphate mixture of P_H 7.7; II. Five volumes of buffered distilled water mixed with one volume of freshly made up hay infusion; III. Hay infusion that had been inhabited for a month by infusoria, heated to 50° to immobilize the infusoria, filtered and then boiled and diluted with an equal volume of buffered distilled water. After 24 hours the numbers of individuals found in each culture were as follow:—

Distilled water	A.	1 individual
	B.	1 individual
Distilled water + hay infusion	A.	2 individuals
	B.	2 individuals
Distilled water + old culture medium	A.	5 individuals
	B.	43 individuals

Forty-eight hours later the maximum population attained in the fresh hay infusion was considerably greater than that attained in either of the other media, due to the greater abundance of substrates but, as the above figures show, the initial rate of multiplication was much greater in the medium which had been previously inhabited.

For reasons which will be clear in the sequel, the phenomenon of "lag," taken together with the autocatalytic character of their multiplication, indicates that bacteria,

as well as infusoria, discharge into the surrounding medium a material capable of accelerating their own reproductive rate. It has also been shown by Bottomley and others[40,41,42,309] that substances are produced by bacteria which are remarkably stimulative of plant growth. It has also been ascertained that a great variety of living tissues and preparations derived from living tissues, including yeast itself, contain substances which are capable of stimulating, to a very noteworthy degree, the multiplicative rate of yeast.[115,203,515]

It is neither a very congenial nor a probable assumption to suppose that each of these accelerative substances is specific for the cells which produce it, and indifferent towards cells of other types or species. In fact the experiments on yeast growth directly negative such a supposition. It is therefore probable that we are here dealing with some universal process which underlies and determines the multiplication of all types of cells, and to which the autocatalytic character of growth in widely divergent types of living matter is attributable.

It will be recollected that the multiplicative rate of infusoria is enhanced in hay infusions which have previously been inhabited by bacteria. The question now arises whether this effect is due, as we have supposed, to the greater abundance of immediately available foodstuffs in such media or, on the contrary, to direct acceleration of the multiplicative rate of the infusoria by accelerative substances discharged into the medium by the bacteria. That the availability of foodstuffs is actually increased by the metabolic activities of bacteria we can hardly doubt, and, as we shall subsequently see, the shortening of the "lag-period" which occurs in such media is confirmatory of this supposition. The existence of accelerative substances in bacterized hay infusions, other than mere abundance of foodstuffs, cannot at present be established because the substances responsible for the two types of effect have not been separated from one another. In the

case of yeast, however, an approach towards separation of these two factors has been achieved.

If compressed yeast be immersed in ether to cytolyze the cells, and then dried on a water-bath and ground up to a fine powder in a mortar, the addition of a small proportion of this powder to fresh hay infusion, followed by boiling to ensure initial sterility of the mixture, greatly accelerates the reproductive rate of infusoria isolated into it. Autolyzed yeast extract has a like effect. But if the yeast be thoroughly and repeatedly extracted with cold acetone, the residue is devoid of accelerative effect. We can hardly suppose that all soluble materials of a nutritive character have been removed from the yeast by this simple procedure, but the substance which is responsible for the accelerative effect obviously has been removed.

The substance which is discharged by infusoria into alkalinized distilled water, and which induces acceleration of reproductive rate, resists, as we have seen, prolonged boiling in neutral solutions. The accelerative substances for the multiplication of yeast which occur in a variety of tissues are similarly thermostable.[115] This quality, therefore, affords additional evidence of the identity or similarity of these substances in all the various cells from which they originate.

The autocatalytic character of reproduction in cultures of infusoria has thus been traced, in part to the inherent capacity of each cell to reproduce itself, due to the presence within it of a catalyzer capable of effecting protoplasmic synthesis, and, in part, to the shedding of a catalyzer into the surrounding medium, through the agency of which the cells are enabled to mutually facilitate each other's reproduction. This mutual acceleration of the multiplication-rate of contiguous cells has been designated the "Allelocatalytic Effect."

2. **The Growth of Individual Cells.**—We have seen that the reproduction of cells in a community is an autocata-

lyzed process, whether the community is differentiated into various types of cells, as in the higher plants or animals, or is merely an assemblage of similar cells, as in a culture of bacteria or infusoria, and we have furthermore found that the autoacceleration is due in part to the shedding of an accelerative agent by the cells into the medium which bathes them.

The question therefore presents itself whether the autoacceleration in growth may not be wholly due to this external agent or whether, on the contrary, the accelerative agent is partially retained within the cells. In the latter event we would expect the development of each individual cell to display the time-relations of an autocatalyzed reaction.

It was pointed out by J. Loeb in 1906, and again in 1907, that the production of nuclei in the early stages of embryonic development is essentially an autocatalyzed process,[238] for, as he observes, since successive cell-divisions occur at approximately equal intervals of time and the total mass of nuclear material is thereby increased by a similar proportion at each division, it follows that the time-intervals of successive cell-divisions form an arithmetical series, while the volumes of nuclear material synthesized in each interval form a geometrical series. The simplest illustration of this phenomenon, because it is not complicated by differentiation, is probably afforded by the multiplication of infusoria arising by repeated divisions from a single individual.[410] The production of nuclear material is thus not merely proportional to the time which is consumed in its synthesis but also, in a variable ratio, to the amount already produced, that is, to the mass of the product.

In certain instances, such as for example a colony of infusoria, the new nuclei produced by division are of approximately equal size at corresponding phases of the nuclear cycle, *e.g.*, just after or just before cell-division. In the early stages of the development of certain metazoa,

as, for example, the sea-urchin,[159] the nuclei which are successively produced are also equal to one another in size. In these instances, overlooking possible concurrent changes of nuclear composition, the mass of nuclear material is approximately doubled at each nuclear division, *i.e.*, at approximately equal intervals of time. In other instances, and these probably constitute the majority, the production of nuclear substance is not so rapid, so that in *Crepidula,* for example, Conklin finds that the actual increase of nuclear material, as estimated by the collective volume of the nuclei, is only about nine per cent. per division.[74] Nevertheless, the fact remains that the production of nuclear material stands in a certain proportion to the amount of nuclear material already formed, whether the ratio be expressed by 100 per cent. as in the early development of the sea-urchin, or only nine per cent. as in the early development of *Crepidula* and other forms investigated by Conklin. Moreover, in these latter instances a notable increase of nuclear density, due to diminution of the proportion of nuclear sap, renders the measurement of nuclear volume a far from complete estimate of the true increase of the mass of distinctively nuclear constituents. Thus the production of nuclear material, viewed as a whole, is accelerated by the nuclei already formed, or, in other words, is autocatalyzed.

Not only collectively, however, but also within the individual cells, the production of nuclear material is dinstinctively autocatalyzed. It has been shown by R. Hertwig[184] that immediately after the conclusion of cell-division a slight diminution of nuclear volume occurs[e] which is succeeded by a slow and almost uniform rate of growth which is termed by Hertwig the "functional growth" of the nucleus. During this phase of nuclear growth the cytoplasm grows both absolutely and relatively more rapidly than the nucleus. This is succeeded by a phase of extremely rapid nuclear growth, termed by Hertwig "divisional growth"

[e] This shrinkage, however, does not occur in every instance.[74]

during which the nucleus increases in volume at a relatively greater speed than the cytoplasm. In this way the ratio of nuclear to cytoplasmic material which obtained at the preceding division, the "kernplasma-relation" of Hertwig, is regained and at this moment cell-division normally recurs.

All observers are agreed that the most rapid nuclear growth occurs shortly before mitosis.[160] The rate of nuclear growth therefore undergoes acceleration as it

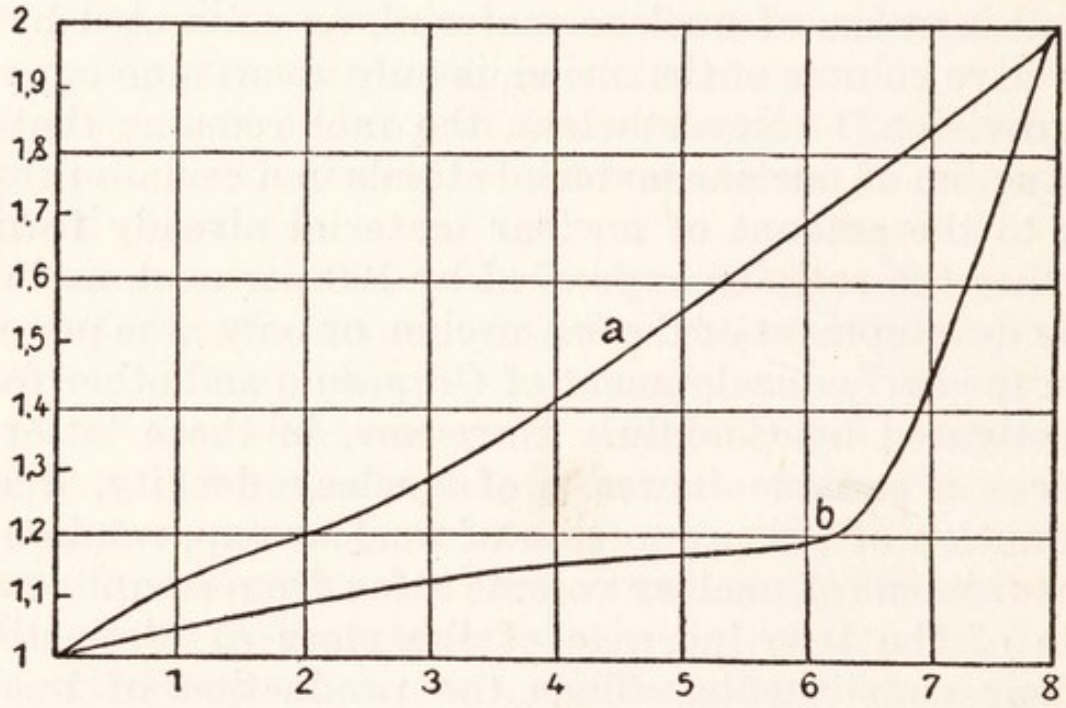


FIG. 22.—Comparison of the growth of cytoplasm (a) and nucleus (b). The abscissæ represent the time in hours since the last nuclear division, and the ordinates the growth in volume. (From Godlewski, after Popoff.)

proceeds. The velocity of cytoplasmic growth, on the contrary, is comparatively uniform. In the accompanying diagram (Fig. 22) embodying the measurements of Popoff[379] the nuclear and cytoplasmic volumes at the moment of cell-division are reduced to the same scale so that their relative rates of growth may be compared. An inspection of these curves shows that nuclear growth undergoes a remarkable acceleration which is almost absent in the growth of the enveloping cytoplasm.

The kernplasma-relation is by no means constant in the different cells comprising an organism, or in unicellular organisms under varying environmental condi-

tions. Thus Conklin, who agrees with Hertwig that "the growth of the nucleus is more rapid in the last stage of the resting period preceding mitosis than at any other time in the cell cycle," finds that the kernplasma-relation is extremely variable even in the earliest stages of embryonic development[74] and Popoff has shown that by exposing infusoria to low temperatures abnormally high kernplasma-ratios may be obtained.[160] We shall revert to the significance of these variations of the kernplasma-relation in later chapters, but for the present the fact which claims our attention is the autoacceleration which the growth of the nucleus displays, in contrast to the comparatively steady rate of accumulation of cytoplasm. Evidently we must infer that the whole of the accelerative agent is not shed from the cells into the medium which bathes them, but that a portion is also resident within the nucleus.[f]

There is also an entirely independent reason for ascribing the source of the autocatalyst of cell-multiplication to the nucleus. This is the fact, the experimental evidence of which will be described later, that the accelerative agent is only shed from cells into the pericellular fluid at the moment of division, when the nuclear membrane has been dissolved. The fact that it is shed at this period leads us to infer that no obstacle exists to prevent its issuance from cytoplasm, yet in the interdivisional periods it does not escape at all. Evidently, therefore, it is not present in the cytoplasm during the interdivisional period (at least in the necessary excess to permit issuance into the pericellular medium), and it must be prevented from escaping

[f] An apparent uncertainty attaches to this conclusion because the composition of the nucleus, especially the proportion of nuclear sap, alters progressively during each nuclear cycle. Conklin finds that "the most abundant constituent of a fully formed nucleus is nuclear sap, and this is scarcely present at all in the earliest stages of the nuclear cycle."[74] p. 44. He believes that the nuclear sap is actually a cytoplasmic constituent which is taken up into the nucleus by osmosis. Even if the whole of the "divisional growth" in volume of the nucleus is actually attributable to the absorption of nuclear sap, however, it is still indicative of a progressive alteration in the nucleus which is accelerated while it proceeds.

from the cell by the membrane which encloses the nucleus during this period.

It is to the nucleus, therefore, that we must look, alike for the source of the accelerative agent in cellular multiplication, and the source of the autocatalytic time-relations which distinguish all types of growth. With certain special exceptions, such as the maturation of ova, or spermatogenesis, or the occasional formation of polynuclear cells, the growth of cytoplasm is determined by the multiplication of nuclei, for the obvious reason that nuclear division precedes and determines the moment of cellular division, and the subdivision of protoplasm ensures the accessibility of nutrients to all its parts and hence the uninterrupted growth of cytoplasm. Thus, if the production of nuclear materials in a multicellular organism or a culture of unicellular organisms is autocatalyzed, then the total production of protoplasm must similarly display autocatalytic time-relations.

3. **The Significance of the "Lag-period."**—It appears to be universally true that an isolated cell, placed in no matter how rich a nutrient medium, displays a period of inertia or "lag" before it responds to the stimulus of abundant foodstuffs by the reproduction of its kind. Thus the lag-period in bacterial cultures has frequently been the subject of careful investigation;[66,371] it is prominent in *in vitro* cultures of vertebrate tissue-cells,[52] and it occurs to a noteworthy degree in infusorial cultures. The circumstances attending these different observations are so unlike in all other respects that the phenomenon of "lag" must be regarded as an inevitable consequence of isolation, since this is the only common factor in all of the experiments enumerated. Were the lag-period only observed in cultures of vertebrate tissue-cells we might be inclined to attribute it to the adaptation which is necessary to accommodate the cell to the physical and chemical novelty of its environment, and this has frequently been the explanation offered. But such an ex-

planation altogether fails to apply to a culture of bacteria inoculated into a medium of favorable reaction and abounding with immediately available foodstuffs. There must be a common reason for the phenomenon of lag in all of the instances and circumstances in which it occurs, and an explanation which does not suffice in the instance of a bacterial culture must also be inadequate when applied to the lag phenomenon in in vitro cultures of tissue.

We have seen that when two infusoria are isolated into the same small drop of hay infusion mutual accelera tion of their reproductive rate occurs, through the shed ding of an accelerative agent into the pericellular fluid by the infusoria themselves. This effect, however, is not visible during the period of lag. Two individuals isolated together into the same drop of hay infusion do not begin to divide any earlier than a single individual isolated into a drop of similar size. The lag-period is not appreciably shortened. But once division has occurred acceleration begins to manifest itself, although if the individuals originally isolated chance to have been derived from an old and densely populated parent-culture and bacterial products are absent or scanty in the infusion, the mutually accelerative effect may not become manifest until after two or three divisions have occurred. The following is a typical result of this kind:—

Into a drop, measuring about 0.08 c.c., of freshly prepared hay infusion was isolated an infusorian (*Enchelys*) derived from densely inhabited parent-culture which was seven days old. At the same time two separately isolated individuals from the same parent-culture were introduced together into a similar drop of infusion. The following results were obtained:—

TABLE XXXVIII

Culture number	Number of individuals initially introduced	Number of individuals	
		After 24 hours	After 48 hours
310A	1	2	16
311A	2	3	120

Thus, after 24 hours one of the cells in culture 311A, which initially contained two individuals, had actually lagged behind the other and the cell which had been isolated in culture 310A and had not yet completed division, so that this culture now contained but three individuals although it should have contained four if the division rate of both of its original components had been equal to that of the cell in culture 310A. Certainly no mutual acceleration had been displayed up to this time. Twenty-four hours later the picture presented by the two cultures was very different. Three cell-divisions had occurred in the culture which initially contained a single individual, and five in the culture which initially contained two individuals. In the second twenty-four hours the accelerative effect of the second individual was most pronounced; in the first twenty-four hours it was not evident at all. Now the greater part of the first twenty-four hours was consumed by the lag-period, which is very long when the individuals are isolated from such old parent-cultures, and the absence of allelocatalytic effect during this period shows that the lag-period was not abbreviated by the presence of the second individual. Repeated experiments of this type sometimes indicate a slight abbreviation, and no less frequently a slight prolongation of the lag-period in cultures of two individuals. These variations, however, are no greater than may be observed in cultures of single individuals and always stand in most evident contrast to the acceleration due to the presence of the second individual after cell-division has at last occurred.

Similarly, the lag-periods of single infusoria isolated into varying volumes of culture fluid do not appreciably differ, but once cell-division has occurred then the intervals between the succeeding divisions are much abbreviated in the smaller volumes of culture fluid, and multiplication is correspondingly accelerated. The accelerative agent which is discharged by multiplying infu-

soria into "buffered" distilled water does not abbreviate the lag-period of infusoria isolated into nutrient media which contain it. Again, yeast extract shortens the lag-period only to a relatively insignificant degree, while, subsequently to the first division, the reproductive rate is decidedly enhanced. The acceleration of the multipli-

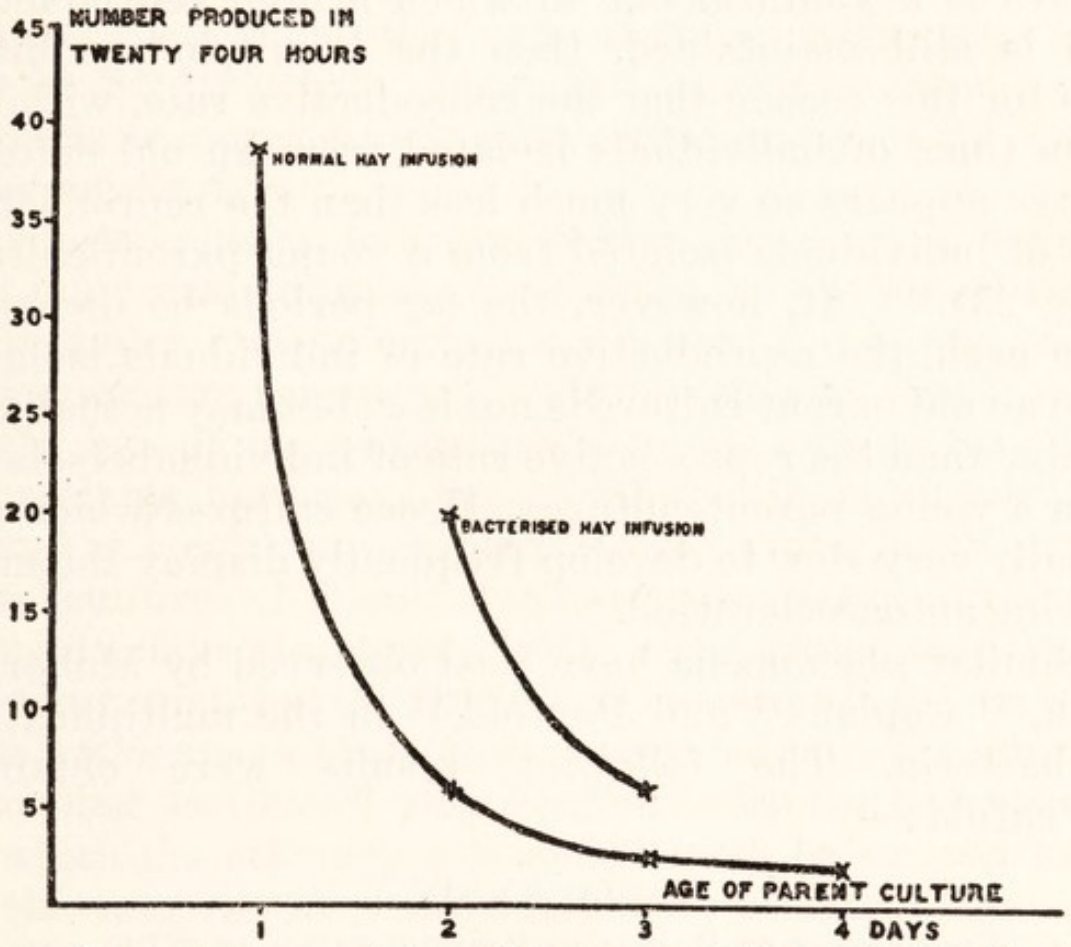


FIG. 23.—Showing the decrease of the multiplication rate of infusoria (*Enchelys*) in the first twenty-four hours after isolation from parent cultures of increasing age.

cation of infusoria which is induced by cholesterol or tethelin is similarly apparent only after expiry of the period of lag.

We must infer from all of these observations that the isolated cell is relatively insusceptible to agents accelerating nuclear synthesis during the period which precedes the first division. It is absolutely insusceptible to the accelerative agent which is shed into the medium by the infusoria themselves, or else this agent does not appear in the medium until a division has occurred. For the time

being the nucleus is closed to outside influences, and carries with it the characteristics of the nuclei of the cells which inhabited the parent-culture. If the parent-culture is old and densely populated, reproduction has ceased in it, and correspondingly we find that the lag-period is immensely prolonged. If, on the contrary, the parent-culture is a youthful one in which a high reproductive rate is still maintained, then the lag-period is brief. It is for this reason that the reproductive rate, within a given time, of individuals isolated from an old culture, always appears so very much less than the reproductive rate of individuals isolated from a young parent-culture (Fig. 23).[410] If, however, the lag-periods be deducted from each, the reproductive rate of individuals isolated from an old parent-culture is not less, but may actually be greater than the reproductive rate of individuals isolated from a young parent-culture. Hence cultures which are initially very slow to develop frequently display the most striking autoacceleration.

Similar phenomena have been observed by Muller,[323] Rahn,[382] Coplans[77] and Penfold[371] in the multiplication of bacteria. The following results were obtained by Penfold:—

TABLE XXXIX

CULTIVATION OF B. COLI IN PEPTONE-WATER AT 37° C.

Age of parent-culture	Number of bacteria in one drop of subculture after						
	0 mins.	60 mins.	80 mins.	100 mins.	120 mins.	180 mins.	300 mins.
17 hours	29	38	45	88	116	728	41307
4 days	28	28	26	27	29	136	7984
12 days	18	18	19	22	22	115	3623

Both autocatalysis and lag are very clearly displayed, but the lag-period is less than 60 minutes in the subculture from the 17 hour parent-culture, while it is 120 minutes or more in the subcultures from the 4 and 12 day parent-cultures. According to Penfold bacteria isolated from

the parent-culture during the period of maximal reproductive rate actually display no measurable lag at all.

If we may compare the age of an organized cell-community with the age of a culture of bacteria or infusoria, then the same principle prevails, for in vitro cultures of vertebrate tissue cells display a much longer lag-period when the isolated cells are derived from adult tissues than when they are derived from embryonic tissues.[52,59,60,61,112,136] Thus the lag-period appears invariably to increase in duration with the age of the parent cell-community.

There appears, however, to be a tendency, in bacterial and infusorial cultures at any rate, for the lag-period to asymptotically approach a maximum as the age of the parent-culture is increased. Thereafter a further increase in the age of the parent-culture does not result in any appreciable increase of lag. This is well shown in Figure 23 and in comparing the above-cited data for the growth of subcultures of B. coli from parent-cultures which were 4 and 12 days old, respectively. The duration of lag is, however, manifestly affected by the abundance of available nutrients in the culture medium (Fig. 23) and also according to Chick[66] and Penfold[371] by the temperature at which the subculture is maintained, being lengthened as the temperature is lowered.

Summarizing our main conclusions at this point, we have seen that the autocatalytic character of growth is attributable to the autocatalysis which occurs during nuclear synthesis; that the accelerative agent which is responsible for the autoacceleration of nuclear synthesis is in part retained within the nucleus, and in part shed into the surrounding medium, and, finally, that during the lag-period, before any cell-division has occurred, the nucleus is insensitive to accelerative agents in the surrounding medium, other than the indirect and comparatively slight acceleration due to the mere abundance of foodstuffs.

These considerations, taken together, unite in indi-

cating pretty clearly that the moment of distribution of the accelerative agent between the nucleus and the surrounding medium is that at which cell-division or the preceding nuclear division occurs. And this is, indeed, a very reasonable assumption, for the nuclear membrane is known to be relatively impermeable to many soluble materials[73] and the dissolution of the nuclear membrane which accompanies nuclear division may well render possible a redistribution of materials between the nucleus and the cytoplasm, and the admixture of nuclear material with those constituents of the pericellular fluid which are capable of penetrating cytoplasm.

The fact that the synthesis of individual nuclei is autocatalyzed shows, indeed, that the accelerative agent cannot issue from the nucleus during the period preceding nuclear division, for if it did, and were dissipated as rapidly as it was formed, no autoacceleration due to its accumulation could occur. It is also a very suggestive fact that the rate of nuclear growth, which attains a maximum immediately before nuclear division, falls to a minimum immediately thereafter.[74] It does not appear possible to interpret this fact except by supposing that during the process of division the nucleus has lost some constituent which was responsible for the high rate of nuclear growth just before division occurred.

We are thus led to the formulation of the following hypothesis:—

During the periods between nuclear division, each nucleus retains the charge of autocatalyst with which it was originally provided, and adds to it in the course of the nuclear synthesis which is rendered possible by its presence. At the next division the autocatalyst is shared between the nuclear materials and the surrounding medium in a proportion determined, in part by its relative solubility in the two media, and in part by its affinity for chemical substances within the nucleus. At the end of this redistribution the autocatalyst stands in equilibrium

between the external solvent or pericellular medium, on the one hand, and on the other hand the nuclear substances with which it combines or in which it is dissolved. The nuclear membrane is then reformed, and the autocatalyst within the nucleus is again shut off from dispersal into the surrounding medium until the occurrence of the next succeeding division.

This hypothesis reveals a close interrelationship between many hitherto obscure and apparently unconnected phenomena. Thus it has been observed by Wildiers[515] that yeast will not develop, even in a medium containing every necessary nutrient, if the volume of the medium is excessive in comparison with the number of yeast cells introduced into it. We have seen that the same phenomenon is encountered in the cultivation of infusoria and that in fact a single infusorian, isolated into too large a volume of fresh culture medium not only fails to propagate itself but even fails to maintain its existence. Evidently some essential substance which is contributed to the medium by the organisms themselves is too much diluted, or too completely extracted from the cells in large volumes of culture media to sustain nuclear synthesis, or even nuclear equilibrium. It has been found by Wildiers and others[115,116,203] that the necessary constituent or "bios" may be supplied by a yeast extract or by extracts of a wide variety of living tissues. According to Devloo[97] the "bios" is either a phospholipin ("lecithin") or is associated with and soluble in lecithin.

We may infer that the "bios" of Wildiers is actually the nuclear autocatalyst and that, if the volume of culture medium be excessive, the partition of the autocatalyst between the medium and the cell leaves an insufficient residuum in the cell to maintain the synthesis of nuclear material at a velocity comparable with that of its degradation. The addition of "bios" to the external medium acts like the addition of a solute to one of two immiscible solvents; the ratio between the final concentrations in the

two solvents remaining unaltered, its initial addition to one of them diminishes the quantity which is subsequently extracted from the other.

The mutually accelerative or "allelocatalytic" effect of contiguous cells is similarly attributable to the fact that each cell contributes less autocatalyst to the medium at the moment of nuclear division by reason of the neighborhood of the other. Each cell contributes its quota towards the "saturation" of the medium, and hence each parts with less than it would otherwise lose at the moment of nuclear division. Hence, also, the allelocatalytic effect is not displayed until after the first division has been effected. The amount of autocatalyst retained within the cells must mount with each successive division in proportion to the number of cells within a given volume of the medium, so that the total synthesis of nuclear material displays the time-relations of an autocatalyzed reaction. Thus the autocatalytic phenomena displayed by the growth of cell communities, even those comprising the most complex multicellular plants and animals, are traceable to the progressive accumulation of the nuclear autocatalyst within the cells which is a consequence of its augmenting concentration in the pericellular fluid. If one of the constituent cells of the community is isolated into a fresh nutrient medium it remains physiologically a member of the parent community until the first nuclear division occurs, and the only advantage which it gains by the transfer, during this interval, resides in the greater abundance of certain raw materials or substrates which are presumably consumed in the synthesis of the nucleus. Sooner or later this leads to the attainment of the critical nuclear ratio, at which division of the nucleus occurs. From this moment onwards the daughter cells assume a new physiological character in consequence of the redistribution of the autocatalyst between the nuclei and the medium which they inhabit. If the nutrient medium has not previously received accessions of autocatalyst from

other sources, the daughter cells will retain a minimal proportion and hence assume an essentially youthful character, reinaugurating an autocatalytic cycle.

These considerations enable us to understand how the autocatalytic character of the synthesis of individual nuclei can result in autocatalysis of the collective synthesis of nuclear and cytoplasmic materials in the vast community of cells which constitutes the body of a higher plant or animal. A series of separate and entirely independent autocatalytic syntheses would not fuse into a total effect displaying similar time-relations. But the individual nuclear syntheses are only transiently independent and, until the latest stages of development at all events, the intervals of isolation of the individual nuclei are brief in comparison with the total period which is occupied in the production of the adult organism. At each cell-division equilibrium between the individual nuclei and the pericellular medium is re-established and the individual rates of nuclear synthesis are by this means periodically readjusted to a rate which is determined by the development of the community as a whole. The rate of development of the community, therefore, reflects the rate of accumulation of the autocatalyst in the pericellular media. It follows from this that any growth not accompanied by multiplication of cells, such as, for example, the deposition of reserve-materials, will not necessarily display the autocatalytic time-relations. The slow "creeping" accretion of weight which mice undergo after the completion of the adolescent growth-cycle and which continues throughout adult life until senescence supervenes, is possibly of this character. The relationship of weight to time during this period appears to be approximately linear.

CHAPTER V

THE SUBSTRATES OF GROWTH; INORGANIC SALTS AND AMINO-ACIDS

1. **The Distinction Between Substrates and Catalysts of Growth.**— The final equilibrium which is attained in any growth-cycle is relative to the prevailing nutrient level, for it occurs when the forward velocity of nuclear synthesis is equalled by the reverse velocity of nuclear degradation, and we have seen that the forward velocity of synthesis is proportional to the nutrient level. If any particular essential for the manufacture of nuclei is absent or scarce in the dietary this will constitute the limiting factor of development, because all other nutrients will perforce be useless in its absence. So a house may be built of many materials and yet its construction absolutely prevented by lack or scarcity of some particular item. Moreover, since the nucleus is in the long run as dependent upon the cytoplasm as the latter is upon the nucleus, any essential for the construction of cytoplasm will also constitute, if it is scarce or lacking, a limiting factor in development. It is necessary therefore to consider at this juncture the nature of these factors.

One characteristic the substrates of growth must certainly display in common and that is their absolute essentiality. Without raw materials the finished product manifestly cannot be made and any one raw material will serve as well as any other to limit growth if it be absent or inadequate in amount. In certain instances, it is true, substitutes for the insufficient constituent might conceivably be found, but in that event we should more correctly consider the group of substances mutually capable of replacing one another as constituting collectively one of the substrates of growth.

With the autocatalyst of growth and substances resembling it in their effect, it is quite otherwise. This can never be an absolute essential in the dietary, because it is produced endogenously, that is by the tissues of the organism itself. Thus acceleration or retardation of growth might conceivably arise in consequence of the presence of excessive amounts of such substances in the pericellular fluids, either effect being obtained according to whether the growth-cycle is in the autokinetic or in the autostatic phase. But in no case will absence of the autocatalyst from the dietary, at all events in the multicellular organisms, lead to absolute suppression of growth. We have previously (Chapter IV) seen reason to provisionally identify the autocatalyst of growth with the "bios" of Wildiers,[515] and in this respect, also, the "bios" displays the characteristics which we would expect in a growth-catalyst. Thus Ide has pointed out[203] that two types of growth of yeast are possible, the one, a very slow growth without "bios," the other rapid growth with "bios." It is true that growth may be inhibited in unicellular organisms, and even maintenance may be rendered impossible by excessive dilution of the autocatalyst or "bios" through isolation into a disproportionate volume of a culture medium which lacks it. This occurs no doubt because the proportion of autocatalyst left within the cell is too small to support nuclear synthesis at a velocity sufficient to keep pace with the growth of cytoplasm, and the nuclear ratio typical of the cell cannot be maintained. Evidently this could not occur in multicellular communities bathed in intercommunicating pericellular fluids.

Our knowledge of the substrates of growth, while exceedingly inadequate in many directions, is nevertheless far superior to our present knowledge of the properties and effects of the nuclear autocatalyst or "bios." This arises from the very universality of the distribution of the latter, for attention has been called to the existence

and necessity of many growth substrates, particularly amino-acids and vitamins, by the irregularity of their dispersal in living matter and the fact that certain of them are totally absent from many common foodstuffs. The existence of an agent affecting growth which is present in all tissues and yet not essential in the dietary must obviously be much more difficult to establish.

2. **The Inorganic Substrates.**—The inorganic substrates of growth must evidently comprise all of the inorganic elements which are found in adult tissues. The essentiality of some of these has long been well established. Thus the experiments of Abderhalden have shown[1] that if rabbits are maintained upon a diet deficient in iron, by continuing milk feeding after the normal period of lactation, they fail to grow at the normal rate and display anæmia, quantitively measurable by the decrease of relative hæmoglobin content. Addition of inorganic salts of iron to the milk greatly improved growth, but did not alleviate the anæmia because certain substances necessary for the utilization of iron to manufacture hæmoglobin were not supplied in sufficient abundance by milk. Hence iron, possibly in association with nucleo-proteins, plays some other rôle in the body besides that of enabling the manufacture of hæmoglobin. Addition of green forage containing iron speedily corrected the anæmia. There is thus a daily wastage of iron from the body which must be replaced by a current supply in the dietary.

The necessity of iodine for the manufacture of the active constituent of the thyroid (thyroxin) is manifested in the disorders of thyroid function which supervene when the nutrient supply of iodine is deficient. That lack of iodine in the dietary may actually lead to hypofunction of the thyroid, is demonstrated by the preventive effect of frequent administration of small doses of iodides.[273] Under circumstances which throw excessive strain upon the thyroid, such as the pollution of water by certain micro-organisms,[280] then even the normal proportion of

iodine in the dietary may be inadequate, and under such conditions the administration of iodides does not seem to be so effective in preventing hypofunction of the thyroid, as it is when the disorder arises from simple inadequacy of the iodine in the dietary. It is not clear how and for what reason a wastage of iodine occurs from the body, entailing a continual renewal of this element.

In respect to other inorganic elements our information is more meagre. The necessity of lime and phosphates for the growing animal is evident from the fact that the former substance enters extensively into the formation of bone, while phosphorus is required for the manufacture of all nuclei and of the lipins which abound in all tissues and more especially in nervous tissues. Accordingly, Osborne and Mendel[349] have found that young rats fail to grow if deprived of lime and phosphates and that considerable proportions of these substances are requisite to maintain a normal growth-rate.

In respect to the remaining mineral constituents of protoplasm, Osborne and Mendel have shown that either sodium or potassium must be abundant in the diet, but provided potassium is abundant sodium may be reduced to 0.04 per cent. of the intake, while if sodium is abundant then potassium may be reduced to 0.03 per cent. No more than 0.04 per cent. of chlorine and 0.01 per cent. of magnesium are required to maintain growth.

These minimal requirements are conditional upon the ability of the animal to conserve its mineral resources. Thus Rosemann has shown[426] that a diet which is deficient in chlorides results only in a most insignificant reduction of the total chlorine content of the body. Excretion of chlorides under these circumstances is completely arrested. No deleterious effects are observed. If, however, the conservation of chlorine is prevented by actual removal of the hydrochloric acid in the gastric juice through a fistula, then signs of malnutrition speedily appear.

An early effect of total deprival of mineral constit-

uents from the diet is the development of acidosis with accompanying ketonuria.[488] This is doubtless attributable to inadequate oxidation of the fats, consequent upon deficiency of the bases necessary to maintain the alkali-reserve.

The importance of a well-balanced mineral dietary has been especially emphasized by McCollum and co-workers.[291] While animals succeed in maintaining themselves, and even grow fairly rapidly upon a mineral diet of abnormal composition, yet improvement of growth is commonly manifested when the proportions of the mineral constituents approximate more closely to the actual requirements of the animal, as for example, the saline constituents of milk. A possible explanation of this fact has been advanced elsewhere.[409] While the selective activity of the tissues may succeed in retaining and accumulating a mineral element from a dietary in which it occurs in merely insignificant proportion,[a] yet this retention, and the implied rejection of the excess of other constituents, involves the overcoming of the resistance constituted by their respective osmotic pressures. Osmotic work is therefore performed by the tissues and their performance of this work must consume energy, and, in consequence, detract from the calorific value of the food. The restoration of a normal mineral dietary results in the release of energy for maintenance and growth and has the effect of an addition to the calorific value of the intake.

The mineral requirements of unicellular animals have been investigated by Peters.[372] He finds that phosphates and chlorides are necessary, and also potassium and magnesium. Cultures were obtainable in media which could not be demonstrated to contain calcium, sodium or sulphur. Nevertheless these substances, although undetectable, may have been present in sufficient amount to enter into the formation of constituents of the protoplasm, because the quantities requisite, if wholly utilized, would

[a] The mechanism of this retention has been discussed elsewhere.[392]

not improbably lie beyond the boundaries of chemical detection. In the absence of other sources of nitrogen, ammonium salts were also found to be essential.

The inorganic requirements of the plants are not incidental, as in the animal, but fundamental, since they constitute the ultimate source of all their nutriment. A discussion of these requirements would consequently lead us far out of our province, and the reader is referred to current works on plant physiology and agricultural chemistry.

3. **Amino-acids.**—The organic requirements of the higher animals comprise the carbohydrates, fats, proteins and accessory foodstuffs or vitamins. Of these the carbohydrates and fats are not limited to specific forms, but, provided only that they are digestible, *i.e.*, reducible to monosaccharides in the one case, or to fatty acids and glycerol in the other, any carbohydrate or fat may be utilized for the purposes of maintenance or growth by the organism. Not only that, but provided the accessory foodstuff or "Vitamin A" which is commonly associated with fats, is present in adequate amount, fats may be wholly excluded from the diet and, with the fats, the carbohydrates may also be excluded[b] without loss of power to maintain bodily equilibrium or to grow.[355,366]

Neither lack of fat nor lack of carbohydrate, therefore, can constitute a limiting factor in the growth of an animal provided the total calorific value of the diet is more than adequate for its maintenance. Proteins, however, stand upon a very different footing in relation to growth. They constitute a large proportion of the dry weight of living tissue, and, since they are the only abundant nitrogenous constituents of the diet their place cannot be taken by any other class of foodstuffs.

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[b] Carbohydrates cannot be omitted from the dietary unless fats are previously excluded, for in the absence of carbohydrates the fats are imperfectly oxidized and the products of their partial oxidation give rise to acidosis. "The fats are burnt only in the fire of the carbohydrates." [212]

The proteins, however, are not transported to the tissues as such. The investigations of recent years have shown them to be absorbed from the intestine only in the form of the amino-acids which result from their complete hydrolytic cleavage by the digestive enzymes. Now the various amino-acids which may be found united with one another to form a protein are not less than nineteen in number and they differ from one another not as the various hexoses differ, merely in stereochemical configuration, but frequently in fundamental structure. The majority of them consist of a simple or terminally branched hydrocarbon chain, with a single carboxyl group and an amino group in the α position, but the length of the hydrocarbon chain differs in the different acids and in two of them (phenylalanine and tyrosine) it includes an aromatic radicle. Others, namely aspartic and glutamic acids, contain two carboxyl groups. Lysine contains an ω amino group as well as the amino group in the α position. Arginine contains the guanidine radicle, and histidine, proline, oxyproline and tryptophane (the heterocyclic amino-acids) contain closed chains of carbon and nitrogen atoms. Each of these is separately presented to the cell in the pericellular fluid by which it is environed, and each constitutes, in consequence, an elementary foodstuff.

Instead of a single dietary constituent, protein, there are therefore no less than nineteen separate nitrogenous food constituents which in many cases differ fundamentally from each other in structure. The question therefore presents itself whether each of these substances constitutes a separate requirement of the organism or whether, on the contrary, certain of them may be synthesized from others or from substances of even simpler construction.

So far as glycocoll, or amino-acetic acid, is concerned our tissues appear to be able to manufacture it without difficulty. If benzoic acid is administered to man or animals it appears combined with glycocoll to form hippuric acid in the urine and the amount so excreted may far

exceed the intake of glycocoll in the food.[125,387] There is reason to suppose that it is manufactured from ammonia derived from the deaminization of other amino-acids and the carbonic acid which abounds in living tissues.

It is much more difficult to ascertain whether other amino-acids may be synthesized or not. The difficulty and expense of preparing them in a state of purity has hitherto precluded the extensive employment of amino-acids as the sole source of nitrogen in the dietary. The experiment has indeed been attempted by Abderhalden,[3,4] who found that maintenance of bodily weight and nitrogen retention could be secured in a synthetic mixture of amino-acids, but the far more prolonged and extensive series of investigations required to investigate the separate requirement of each of the individual acids has never been attempted.

On the other hand the majority of the naturally occurring proteins contain all of the dietary amino-acids, although in widely varying proportions. Only in a few exceptional classes of protein do we find that certain amino-acids are lacking. Gelatin is a well known example of an incomplete protein lacking cystine, tyrosine and tryptophane, and it has long been realized that gelatin is not in itself an adequate protein for the maintenance of nitrogenous equilibrium, although it is a "sparer" of protein, *i.e.*, can furnish a portion, but not the whole of the protein in the diet. This, however, is not conclusive evidence of the inability of the tissues to synthesize some amino-acids from others, for cystine being the only amino-acid which contains sulphur manifestly cannot be synthesized from any other. In recent years, however, the researches of Willcock and Hopkins[8,193,516] and of Osborne and Mendel[335,336,339,340,345] have established the fact that certain other proteins lacking one or other of the amino-acids are similarly inadequate to maintain nitrogenous equilibrium or permit growth. Thus zein, the alcohol-soluble protein in maize, which lacks

tryptophane, lysine, and glycocoll, is inadequate to maintain nitrogenous equilibrium unless it is supplemented by tryptophane,[516] and even with this addition it will not support growth. Since glycocoll can be synthesized by the tissues, the limiting factor, when zein has been thus supplemented, must of course be lysine. Gliadin, the alcohol-soluble protein in wheat and rye, contains tryptophane but is lacking in lysine and glycocoll, and this protein permits the maintenance of protoplasm already synthesized, but, like zein which has been supplemented with tryptophane, it fails to support growth.[336] We may therefore infer that tryptophane and lysine are both capable of becoming limiting factors of growth when they are lacking or present in inadequate amounts in the diet.

It is usually supposed that the daily requirement of protein in the diet arises from the need to repair the wear and tear of the cells incidental to the performance of their functions. It is known, however, that the greater part of the amino-acids arising from the ingestion of protein are rapidly deaminized, probably before they enter into the composition of tissue proteins, the non-nitrogenous remainder being employed like a carbohydrate or a fat as a source of energy. Since an increased ingestion of protein accelerates the rate of deaminization, the proportion available for the replacement of "wear and tear" becomes less the greater the quantity ingested. The question has been raised by Osborne and Mendel whether there is any genuine wear and tear of the proteins of normal uninjured tissue, or whether, on the contrary, the daily necessity for certain amino-acids does not actually arise from the destruction of tissue proteins, but from specific requirements for the manufacture of hormones, enzymes, or other substances of special physiological significance which are consumed in the performance of their function. They suggest that apart from injury due to toxins or trauma (to which we may add senescence) the only reason for the destruction

of any tissue protein at all might be to liberate small amounts of amino-acids lacking in the diet, and which are required to engage in "some hormone-like physiological duty upon which proper metabolism might depend."[343] It may be for this reason, they suggest, that maintenance of nitrogenous equilibrium and bodily weight is possible upon a diet completely lacking in lysine, although growth is impossible. The manufacture of new protein evidently requires lysine, and so also it may be presumed would the replacement of tissue protein which has been destroyed. Tryptophane, on the contrary, is required for some other purpose than protein-manufacture and hence is subject to continual wastage even in an animal which is not growing.

There is however this alternative, namely, that the lysine liberated in the destruction of tissue protein is utilized over and over again for the replacement of the same quantity of protein as that from which it has been derived. If, in fact, we conceive the degradation and synthesis of tissue protein as a balanced reaction of hydrolysis and its reversion, this is what we would expect to occur, and failure of the reversion, and consequent disturbance of the equilibrium, would only happen when one or more of the amino-acids was drained away for other purposes. The theory of Osborne and Mendel, with this slight modification, would thus be consistent with our general conception of physico-chemical equilibria and the disturbance of this equilibrium would provide the obvious reason for the breaking down of tissue protein which occurs when the amino-acids which are subject to wastage are withdrawn from the diet.

Another method of ascertaining the amino-acid requirement of animals consists in admitting various proteins to the diet only in minimal amounts. The amino-acid composition of different proteins varies very greatly and while but few are completely lacking in any of the nineteen dietary amino-acids, many contain certain amino-

acids in relatively small amounts. When excess of the protein is administered such comparative deficiencies may be inappreciable, because a sufficiency of the least abundant amino-acid is supplied, while the others are present in the dietary in excess. If, however, the protein is administered in minimal quantities, then the more

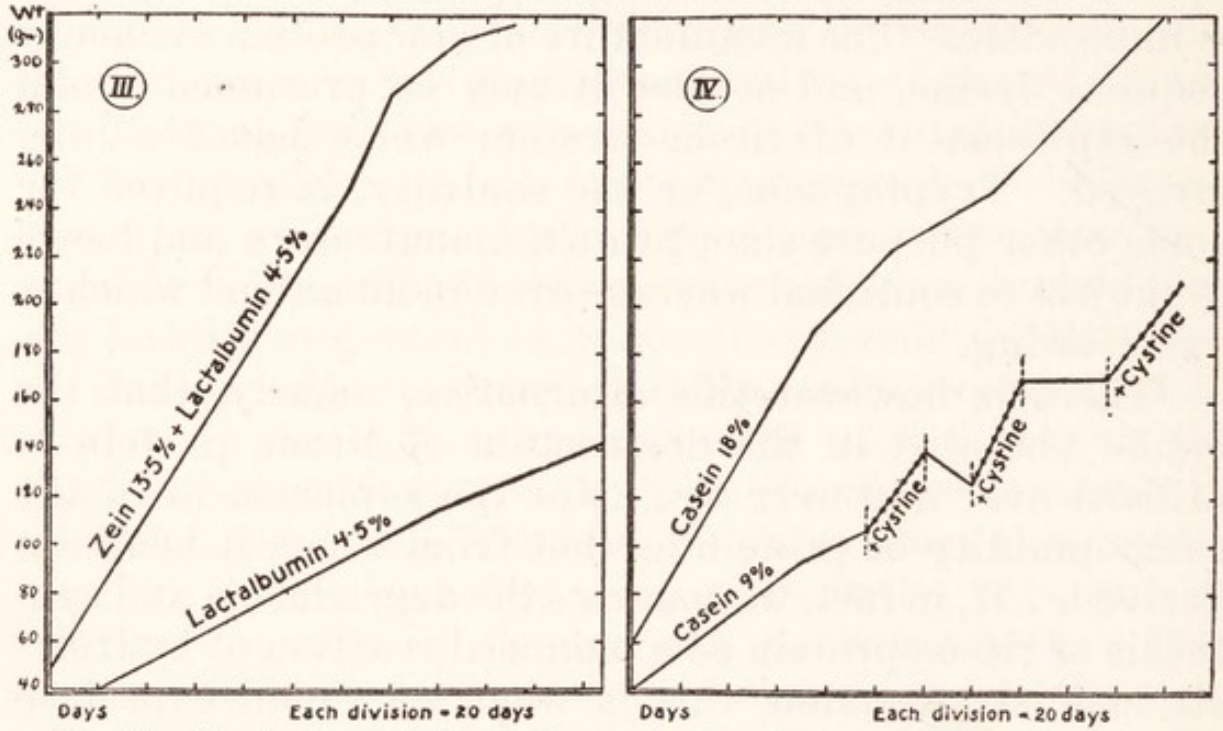


FIG. 24.—Showing the mutually supplemental action of zein and lactalbumin in the diet. No concentration of zein suffices to permit normal growth, and 4.5 per cent. of lactalbumin is also inadequate. The combination of 13.5 per cent. of zein and 4.5 per cent. of lactalbumin permits normal growth. The curves on the right show the supplemental effect of the addition of cystine to a diet containing an inadequate proportion of casein. (After Osborn and Mendel.)

abundant amino-acids may be present in sufficient amount, while the less abundant acids are now inadequate.

Thus, if only nine per cent. of the total calories in the diet are in the form of casein, and no other protein is admitted, normal growth cannot occur. The addition of cystine at once renders normal growth possible (Fig. 24). Such an animal eats more, because it is not merely replacing current wastage but also manufacturing new protein, but while eating only eight per cent. more protein it will grow four hundred per cent. more tissue than an animal receiving this small proportion of casein without addition of cystine.[340] The addition of alanine to the

casein is devoid of any effect, so that the effect of cystine is specific.[344] Similarly edestin, the globulin of hemp seed, fails to support growth when it constitutes only nine per cent. of the diet, but the addition of lysine renders it adequate. Of all proteins so far investigated, lactalbumin has been found to be the most efficient for the support of growth, and with but nine per cent. of this protein in the diet normal growth is achieved. It is also the most efficient protein for the correction of deficiencies in other proteins. Its occurrence in milk is therefore of special significance, and it is a fact of extreme practical importance that this protein is relatively much more abundant in human milk than in cow's milk.[331]

The amino-acid constitution of these proteins is illustrated in the following table:—

TABLE XL

Protein	Per cent. content of	
	Cystine	Lysine
Lactalbumin		8.10
Casein	0.1	7.61
Edestin	0.3	1.65
Gliadin	0.5	0.16

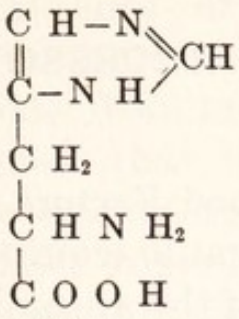
Any of these proteins is adequate to support growth when it constitutes 12.5 per cent. of the total dietary, and any excess over this proportion of the total calories conveys no advantage to the animal.[344] It might be supposed that an animal receiving but nine per cent. of its calories in the form of one of the less adequate proteins might achieve a satisfactory intake, even of the least abundant amino-acid, merely by consuming more food. While theoretically conceivable this does not actually occur, because the appetite of animals is related to the energy requirement of the body, and not to the need of particular tissues.[192,340,433] The appetite is satisfied, when no choice of diet is available, by a quantity which suffices to replace the calorific expenditure. Doubtless more would involve the animal in difficulties arising from imperfect

metabolism. On the other hand appetite does afford a guide to the proper composition of food when a choice is offered to the animal, and as between a deficient and an adequate diet of equal calorific value, the instinct of the animal leads it to choose the diet which most nearly permits normal growth.[350] Instinctive cravings for materials deficient in a one-sided dietary are experienced by the majority of human beings and are frequently manifested to a striking degree by children, but where such instincts cannot be satisfied the consumption of an additional amount of an erroneously constructed diet is evidently not a physiologically desirable method of correcting its deficiencies.

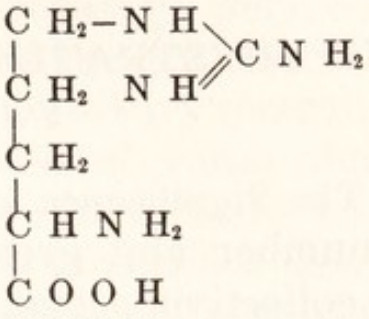
The attempt has several times been made to ascertain the amino-acid requirements of animals by hydrolyzing some complete protein until it is wholly converted into its constituent amino-acids and then, after removing one or more of them by chemical procedures, administering the residual mixture to animals as their sole source of nitrogen. An experiment of this kind was first performed inadvertently by Abderhalden[4] who hydrolyzed protein completely with acids and then fed animals with the resultant mixture of amino-acids. The animals failed to maintain a nitrogen balance and in consequence they lost weight. The tryptophane which was originally present in the protein had in fact undergone destruction during the procedure of hydrolysis and on restoring tryptophane to the mixture a nitrogen balance was obtained.

More extensive experiments of this nature were subsequently undertaken by Ackroyd and Hopkins[8,492] and later by Geiling.[157] Casein was completely hydrolyzed by 25 per cent. sulphuric acid and the mixture supplemented by the addition of cystine, which is deficient in casein, and tryptophane, which had been destroyed during hydrolysis. Arginine and histidine were now removed from the mixture. No growth could be obtained and in fact persistent loss of weight occurred. If either of these amino-

acids was reintroduced into the dietary, however, satisfactory growth occurred. The relationship of these amino-acids is shown by the following formulæ:—



Histidine



Arginine

Both are also related to guanine and therefore to the purine bases which enter into the formation of nucleic acids. Now the end product of the purine metabolism in rats is allantoin, which is probably derived from uric acid[200] and it is therefore an extremely suggestive fact that when arginine and histidine were both withdrawn from the diet a marked decline of allantoin excretion occurred. We may infer that these amino-acids are in part employed for the synthesis of purines.

Summing up, therefore, the present state of our knowledge concerning the amino-acid requirements of the higher animals, we may state that cystine, tryptophane and either histidine or arginine are necessary both for growth and for the maintenance of tissue already manufactured. Lysine is essential for growth but not for maintenance. Glycocoll is not essential for either purpose, since it can be manufactured, directly or indirectly, from other amino-acids. Regarding the thirteen amino-acids which remain, our ignorance of their necessity or replaceability in the diet is almost complete, although Abderhalden[3] suggests that proline may be derivable from glutamic acid and arginine from ornithine.

CHAPTER VI

THE SUBSTRATES OF GROWTH; ACCESSORY FOOD FACTORS

1. **The Significance of the Accessory Food Factors.**—The vast number and great variety of organic compounds which collectively constitute the tissues of the animal body compel us to assume either that animal tissues are possessed of very varied and extensive powers of organic synthesis, or alternatively that they must be dependent upon the vegetable kingdom for organic radicles of the most diverse description. For besides the amino-acids, nitrogenous compounds which may readily be derived therefrom, carbohydrates which may be manufactured, if necessary, from the deaminized residues of amino-acids, and neutral fats which may be manufactured from carbohydrates, our tissues contain, or certain organs produce, substances of a complexity not easily derivable from these. The phospholipins contain nitrogenous bases of a character differing from that of the amines which result from the decarboxylation of amino-acids. The sterols and other hydroxyaromatic derivatives, such as inositol, are not readily related to any of the amino-acids or even to the carbohydrates. The methyl pyrrole radicle in hæmatin, although closely related to proline, appears not to be derivable therefrom in the animal body, since, even with the addition of iron, Abderhalden failed to secure hæmoglobin synthesis in rabbits on an exclusively milk dietary.[2] The addition of chlorophyll-containing articles to the dietary enabled synthesis of hæmoglobin to occur, and since chlorophyll is also a methyl pyrrole derivative it was assumed by Abderhalden that the methyl pyrrole requisite for hæmoglobin synthesis is derived from chlorophyll, an assumption which appears the more probable

since Roaf has shown that urochrome, the yellow urinary pigment, is derived from chlorophyll in the diet.[388]

It is doubtless for reasons such as these that certain constituents of the dietary, ultimately derived from vegetable tissues, are essential for the growth and maintenance of animals, although the necessary quantities are so small that they must be devoid of value merely as sources of energy. These dietary constituents, which are requisite for reasons unconnected with their heat value and are at present chemically undefined, are termed by Gowland Hopkins[192] the "accessory food factors."[a] The number of ascertained accessories, at the present time, is three.

2. **The Fat-soluble Accessory Factor.**—The fat-soluble accessory is contained in butter, egg yolk and the body-fats of animals[286,288,357] with the exception of lard, in which it is usually deficient. If, however, the pigs have been fed with a lavish supply of green food, the lard derived from them may contain appreciable amounts of this accessory.[108] In vegetable tissues the fat-soluble accessory occurs more abundantly in leaves than in the reserve materials which are set aside for the subsequent nourishment of the embryo.[285] Thus it is abundant in alfalfa (lucerne), clover, spinach[464] and grass,[354] and it is generally deficient in seeds[296] and in the vegetable oils which may be derived therefrom.[168,292] The amount in

[a] It is unfortunate that the term "Vitamin" has come to be applied indiscriminately to all three classes of food accessory. Originally employed by Funk[143] to designate the water-soluble accessory, if this term had been retained for its original purpose then we might have felt confident that in the future the term "vitamin" would apply to a definite group of closely related substances. The divergent properties of the three classes of accessory factors indicate that they differ fundamentally in chemical character, so that, if it is applied to all of them, the term "vitamin" must eventually become chemically meaningless. Neither can the term "vitamin" be employed in a physiologically consistent sense to designate dietary essentiality, for in that event the term would also properly apply to the essential amino-acids. It would be preferable to employ the non-committal terminology of Hopkins, and designate these substances, collectively, the "accessory food factors," reserving the term "vitamin" for the substance or group of substances to which it was originally applied, namely, the water-soluble accessory, or antineuritic factor.

seeds is not increased by germination, but the green leaves which are formed subsequently to germination are rich in this substance. If, however, the seedlings are etiolated by growth in the dark, no fat-soluble accessory is produced, while green leaves form large amounts even in water cultures. Lower plants which contain chlorophyll also synthesize it; those which contain red pigment and manufacture less, and those which contain no pigment concerned in carbon assimilation, such as the fungi, synthesize none.[79] Similarly, it is almost absent from the leaves of etiolated cabbage and lettuce.[464]

Green leaves contain, besides chlorophyll, yellow pigments (xanthophyll and carotin) the presence of which, in green leaves, is masked by the coloration due to chlorophyll.[330] The occurrence of the fat-soluble accessory appears to be associated with the presence of the yellow pigments rather than with the presence of chlorophyll itself. Thus yellow maize contains a far greater amount than white maize.[462] Ripe peas of a green color contain more fat-soluble accessory than peas of a yellow color, but the former also contain more yellow pigment, the presence of which is masked by chlorophyll.[466] It is abundant in tomatoes.[465] In roots and tubers it is found in those which are colored, such as the carrot, or sweet potato,[463] but is lacking in those which are not colored.

The color of animal fats is due to the presence of carotinoid pigments (lipochromes) derived directly or indirectly from plants. In correspondence with the distribution of the fat-soluble accessory in vegetable tissues, we find that in butter fat and beef fats there is a general parallelism between their depth of color and their content of fat-soluble accessory. Nevertheless there is no exact proportionality between the two, and cod liver oil, which contains an exceptional abundance of the fat-soluble accessory, also contains very little yellow pigment.[467] The accessory is therefore not identical with carotinoid pigments, although it is extracted from vegetable tissues by

similar solvents.[473] Carefully purified carotin is devoid of effect in averting the consequences of deprival of the fat-soluble accessory.[363,427]

The fat-soluble accessory is contained in the non-crystallizable portion of the alcoholic solution of butter.[357] This fraction was at first supposed to be practically free from phosphorus or nitrogen, but Funk and Macallum[153] showed later that it contains appreciable quantities of nitrogen, although it has not been shown that nitrogen is invariably present in association with the fat-soluble accessory. Its abundance in butter is accounted for by the fact that it is about thirty times as soluble in cream as in skimmed milk, and hence the total quantity present in milk shared about equally between the cream which it yields and the skimmed milk which remains after its removal.[446] In green leaves it is not associated with the proteins, but is found in the non-saponifiable residue obtained after saponification of the fats.[79] It is soluble in alcohol, benzene and ether, and on partition between alcohol and petroleum ether it passes with the carotin fraction into the petroleum ether, while the xanthophyll fraction of the pigments, which remains in the alcohol, is free from the fat-soluble accessory.[462] The addition of the crude alcohol and petroleum extract of dried carrots to a fat which does not contain the accessory, confers upon it the power of protecting rats from the development of xerophthalmia, which results from deficiency of the fat-soluble accessory in the diet.[473]

The substance is thermostable, and is not destroyed by heating to 120° for twelve hours.[341,354,461] If, however, it is simultaneously heated and aerated it is rapidly destroyed, and even at room temperatures slow destruction occurs if solutions containing it are exposed in thin layers to the air.[107,194] It is destroyed by ultraviolet light in the presence of oxygen, but the destruction in this instance is due to the ozone formed by the action of the light upon oxygen. If the material is enclosed in quartz tubes in an

atmosphere of carbon dioxide, ultraviolet light is without effect upon it.[536]

The fat-soluble accessory is essential for the growth and reproduction of animals.[199,287,475] Adult rats are able to withstand its absence from their diet for over a year without loss of weight, but if previously, in the growth period, they have been confined to a diet deficient but not altogether lacking in it, then subsequently, when they are nearly adult, deprival causes, after some delay, a sudden decline of weight and ultimate death from apparent inanition or from intercurrent disease.[352] When young animals which have hitherto received normal nourishment are suddenly deprived of this factor they continue to grow for a considerable period, but growth cannot be sustained, and arrest of growth is followed by progressive loss of weight.[406] An amount of the factor which is just adequate to permit growth may not suffice to permit reproduction and it is not supplied to suckling young unless the mother is also receiving it.[293] These facts unite in pointing to an ability on the part of animals to store up reserves of this factor in their fats, upon which they are able to draw in order to supplement temporary deficiencies in the ration, so that with this assistance an allowance of the fat-soluble factor which would not by itself suffice for the animal's requirements, may remain adequate until the accumulated stores have been depleted, when its inadequacy is revealed either in the animal itself or in its offspring.

Many other evidences of malnutrition, besides arrest of growth, are afforded by animals which are deprived of the fat-soluble accessory. A disease of the eyes, variously known as "xerophthalmia," "keratomalacia," "keratitis," etc., occurs in from one-fourth to one-third of rats which have been deprived of this factor.[219,289,337,474] It appears to be attributable to a weakened resistance of the tissues, which renders them subject to invasion by bacteria which are ordinarily innocuous. It is immediately curable on adding the missing factor to the diet. There are no

histological changes, other than those due to infection, and no constant causative organism is found in the lesions.[474] The prevalence of eye diseases in many countries and localities in which fats are commonly deficient in the diet may therefore not improbably arise from this deficiency.[285,296]

It has long been noted by physicians that children who have received an inadequate proportion of fat in their diet are especially prone to develop rickets. Treatment of rickets by administration of cod liver oil has long been in vogue and with remarkable success. The following figures obtained by Hess and Unger[187] among negro children in New York are especially striking:—

TABLE XLI

Oil given average total	Duration of therapy	No. of infants	Infants not developing rickets	Infants developing rickets	Per cent. non-rachitic
54 oz.	6 months	32	30	2	93
23 "	6 "	5	4	1	80
21 "	4 "	12	7	5	58
Not given		16	1	15	6

It has been found possible by Mellanby[297] to induce rickets in puppies by a diet containing inadequate amounts of milk, with white bread, linseed oil and yeast or yeast and orange juice (to supply the water-soluble and antiscorbutic accessories). The addition to the diet of whole milk in sufficient amount (500 c.c. per diem), or of cod liver oil, butter, suet, olive oil, lard, cotton seed oil, meat, meat extract or malt extract, prevented the appearance of rickets. The majority of these substances contain an abundance of fat-soluble accessory, although lard, olive oil, cotton seed oil and the extractives have generally been found to be lacking or deficient in this factor. Shipley, McCollum, and co-workers also obtained a condition resembling rickets in rats deprived of the fat-soluble accessory,[447] although they are of opinion that lack of this substance is not the sole etiological factor in

the disease.[448] In the kitten it has been found that a diet deficient in the fat-soluble accessory, but otherwise adequate, produces emaciation and arrest of growth, with premature absorption of the thymus and characteristic enlargement and congestion of the costochondral junctions indicative of deficient ossification.[269,493] The animals develop a pendulous abdomen, with thinness and lack of tone of the stomach and intestine; a condition which somewhat resembles "cœliac disease" in children.[477] Pigs[537] and rats[186,530] similarly develop enlargement of the costochondral junctions on a diet which is deficient in the fat-soluble accessory, but if the diet is otherwise adequate the rats do not develop rickets.

The suggestion has been advanced that rachitis, like the xerophthalmia which is known to arise in consequence of a diet which is deficient in the fat-soluble factor, may originate in the bacterial invasion of weakened tissues, susceptibility to bacterial infection being a very characteristic feature of the rachitic condition.[366,367] Thus gastro-enteritis and pyelitis are frequently encountered among animals in receipt of a diet which lacks the fat-soluble factor.[50] In the opinion of Pritchard, however, rachitis is the ultimate outcome of a variety of dietary deficiencies,[381] while Hess attributes it to inadequate utilization of the inorganic phosphates in the dietary.

3. The Water-soluble Accessory Factor.—Our knowledge of the water-soluble accessory originates in the discovery by Eijkman[118,119] that avian polyneuritis, a disease resembling human beriberi, can be induced in pigeons by a diet of rice from which the husks have been removed by polishing, while the symptoms can promptly be cured by the administration of extracts prepared from the husks. The prevalence of beriberi in the rice-consuming countries of the East was traced to the introduction of modern milling methods, involving the total removal of the husk and germ from the grains. The antineuritic agent was subsequently found by Funk and others to be

exceedingly abundant in yeast, from which it can be extracted by alcohol after removal of the fats with ether.[142,143,144] To Funk, also, we owe the term "Vitamin," which should properly be applied to this accessory only. The identity of the antineuritic factor with the water-soluble accessory which is accessory for growth cannot be considered to be completely established, but must be regarded as highly probable.[306]

The water-soluble accessory occurs in leguminous seeds and in the "germ" of cereals. It is contained also in malt extract, potatoes, sweet potatoes, carrots and egg yolk.[296] It is absent from the sugar beet[463] and present only in small amount in meat (muscular tissue) and meat extract, although it is abundant in glandular tissues such as liver and kidney.[348] Milk is inferior to yeast in its content of the water-soluble accessory, 15 c.c. of milk containing no more than the equivalent of 0.2 grams of dried yeast.[353] In seeds it is situated in the outer (aleurone) layer of the endosperm, and in the embryo or the portion of the endosperm which is immediately adjacent to the embryo.[352]

Most of our knowledge of the chemical properties of this substance is derived from experiments upon the antineuritic factor. Assuming this to be identical with the water-soluble accessory we may state that it is soluble in water and in acidified alcohol or alcohol containing water. It is not soluble in ether or in absolute alcohol.[358,359] The fractions with which it is associated always contain nitrogen. It is incompletely precipitated by phosphotungstic acid[142] and is removed from aqueous solution by a variety of adsorbing agents, such as Fuller's earth, dialyzed ferric hydrate,[174] hydrated aluminum silicate, animal charcoal, etc. It is not destroyed by heating with strong acids, nor by standing in solution in tenth normal alkali for four days, but at 90° C. it is completely destroyed in one hour in tenth normal alkali.[334] It is not

affected by ultraviolet light,[535] but is partially destroyed by radium emanation.[481]

It has been ascertained by Funk[146] that several of the purines and pyrimidines have a mild curative action upon avian polyneuritis and his results have been confirmed by Chamberlin and Vedder.[63] The active substance in rice polishings and in yeast is probably an organic base since it resists hydrolysis by strong acids.[76,334] It is precipitated by phosphotungstic acid and by silver nitrate and baryta, reagents which also precipitate pyrimidines.[145] All active preparations yield a deep blue color with the special mixture of phosphotungstic and phosphomolybdic acids which Folin employs as a test for uric acid,[152] and the depth of color is proportional to the content of vitamin as determined by the curative effect upon avian polyneuritis.[114] Williams and Seidell prepared an active crystalline material which lost its activity on repeated crystallization. The inactive material was found to consist largely of adenine.[521] On heating with sodium ethylate, part of the antineuritic activity was regained, but pure adenine similarly treated does not display any antineuritic activity.[504] A highly active crystalline fraction prepared by Funk consisted largely of nicotinic acid which, however, when pure, proved to be devoid of activity.[147] This fact led Williams to investigate the effect of various hydroxypyridines upon avian polyneuritis. Several of these possessed slight curative power when freshly prepared, but upon standing they underwent a change of crystalline form and the curative action then disappeared.[520] Similar changes of crystalline form with loss of antineuritic activity have repeatedly been noted in attempts to purify vitamin. For this reason Williams suggests that the active substance is an isomeric (pseudo-betaine) form of a substance related to the hydroxypyridines. Harden and Zilva, however, were unable to confirm his results.[173] The nature of the active substance is therefore not yet known, but the evidence

which we possess points to its being a nitrogenous base which is similar in properties, if not in constitution, to the pyrimidine bases.

The water-soluble accessory is essential for the growth of young animals.[265,347] The effect of its deficiency in the diet is much more rapidly evident than the effects of deprival of the fat-soluble accessory. There is little ability to store it or the store is not available for the satisfaction of the requirements of the tissues, and in consequence animals require vitamin throughout the duration of their lives.[296,352] It cannot be supplied to suckling young unless the mother is in receipt of it and the consequences of its deprival are very disastrous to the young, especially if the deprival is pre-natal.[106] That some slight storage occurs, however, is indicated by the fact that the length of time an animal can survive deprival of it is proportional to the weight of the animal at the time at which the restriction is imposed, and also by the fact that the tissues of pigeons which have been deprived of this accessory until death occurs, nevertheless yield an alcoholic extract which has a curative effect upon avian beriberi.[148] The substance is contained in the milk of normal mothers but, as we have seen, not very abundantly, and in fact appears to be hardly sufficient in amount for the requirements of the young animal, so that addition of yeast to a milk diet usually promotes a more satisfactory growth.[176]

It has long been known that an excess of carbohydrate in the dietary aggravates the symptoms and promotes the onset of beriberi, and Funk has shown[149] that excess of either fats, carbohydrates, or protein accelerates death arising from deprival of the water-soluble accessory. This fact doubtless bears a close relationship to the experiments of McCarrison,[281,282,283] who has shown that monkeys which have been fed upon autoclaved rice (lacking in all of the dietary accessories) lose weight at the rate of about 18 grams per diem, and die in 23 days. If,

however, butter fat be added to the diet, thus replacing the fat-soluble accessory, the condition, so far from being improved, is actually aggravated by the development of gastro-enteritis. The animals lose weight twice as fast as those which are fed upon rice alone and die in 15 days. McCarrison believes that a diet which is deficient in accessories and protein lays the organism open to infection by the bacterial flora inhabiting the lower intestine, while a diet which is exceptionally rich in carbohydrates and fats probably encourages the production of toxic substances by fæcal flora.[169,220] On the other hand Findlay has shown that there is a definite disturbance of the carbohydrate metabolism in animals subsisting on a diet which is lacking in the water-soluble accessory and that this is correlated with a deficiency of glyoxalase in the liver.[135] Severe metabolic disturbance is also indicated by the fact that creatinuria occurs in animals which are deprived of the water-soluble accessory.[106]

Other abnormalities of development besides mere arrest of growth are displayed by animals fed upon a diet which is deficient in water-soluble accessory. Thus gametogenesis ceases and the animals are consequently sterile.[106,150,351] The lymphoid tissues undergo atrophy, premature involution of the thymus occurs and the lymphocytes in the blood become greatly diminished, the polymorphonuclears remaining unaffected.[80,81,530] It may be noted that both gametogenetic and lymphoid tissues are of a relatively undifferentiated type (*vide* Chapter VIII).

The question has been raised[23,141,151,486,517,518] whether the "bios" of Wildiers, which promotes the multiplication of yeast, may not be identical with the water-soluble accessory. Its solubilities resemble those of the water-soluble accessory, it is removed from solution by adsorbing substances, and at low concentrations its amount (or effect) is nearly proportional to the amount of water-soluble accessory as determined by experiments upon the growth of animals. However in high concentrations a

very remarkable and significant departure from the parallelism of these two substances is observed, for whereas the growth-promoting effect of yeast extract for animals increases up to a maximum with the amount administered and further amounts are devoid of effect, the "bios" effect upon yeast cultures increases to a maximum and then subsequently declines so that high concentrations actually inhibit multiplication.[115,116] This, as we have seen, is what might be expected of a growth-catalyst rather than of a growth-substrate. Furthermore, the water-soluble accessory is essential for the growth of animals, while the "bios" is not essential for the multiplication of yeast since a very slow multiplication occurs in its absence.[203,b] This, again, is precisely what we should expect to be true of the autocatalyst of growth. Moreover, extracts in which the water-soluble accessory has been destroyed by heating in alkaline solution, preserve the "bios" effect undiminished.[267,268,457] Hence the "bios" and the water-soluble accessory are not identical, the former being not improbably the nuclear autocatalyst, and the latter a substrate of growth. Considering the evidence pointing towards its relationship to the pyrimidines, its abundance in glandular tissues and comparative paucity in blood and muscular tissues, and the peculiar dependence of spermatogenesis upon its presence in the diet, we may perhaps hazard the conjecture that the water-soluble accessory is a substrate for nuclear synthesis.

4. **The Antiscorbutic Accessory.**—It has long been a matter of common knowledge that scurvy is a disease arising from a faulty diet, and the rapidity with which it

[b] Of course, the presence of associated traces, transmitted from culture to culture as a contamination, cannot be absolutely excluded, and indeed the origin of the non-essentiality of the autocatalyst of growth resides in the fact that it is contained in and manufactured by the cells themselves. But the animal soon consumes its internal supplies of water-soluble accessory and is then unable to maintain itself. If the non-essentiality of water-soluble accessory for yeast be attributed to its ability to synthesize it, then, for the same reason, it may not require it at all and the fact that the "bios" effect is undiminished in heated alkalinized extracts shows this to be the case.

is cured by a diet containing fresh vegetables and acid fruits was commented upon very frequently by early navigators, and particularly by the narrator of Anson's Voyages.[18] Two views, however, prevailed as to its origin, the one referring it to chronic poisoning or infection arising from inadequately preserved meat or fish, the other to deprivation of some necessary factor in the diet. The latter was demonstrated to be the true etiological factor in 1747 by Lind, a surgeon in the British Navy.[296] He established the curative value of orange and lemon juice and he also showed that cider possesses some measure of curative value, although inferior to that of orange or lemon juice.

Experimental scurvy was first induced in guinea-pigs by Holst and Frölich[191] by removing green food from the dietary and supplying the animals with grains and water only. Scurvy could be prevented by the daily addition of 30 grams of a variety of fresh raw leaves, roots or berries, but cooking greatly reduced their antiscorbutic value, and dried vegetables were found inadequate to protect the animals. The juices of acid fruits proved very effective, but it has since been shown that the presence of antiscorbutic factor in acid fruits is not invariable for it is very deficient in the juice of the "lime." The most valuable antiscorbutic foods are raw cabbage leaves, the raw juice of "swede" turnips, the juice of oranges or lemons[95] and the tomato whether cooked or raw.[158] The antiscorbutic accessory is absent from seeds, but is provided during germination,[67,284,524] a fact which indicates a ready means of preventing scurvy where fresh vegetables or fruits are not accessible. Sun-dried vegetables retain a certain measure of antiscorbutic value, which is further reduced by cooking, but factory-dried vegetables are entirely devoid of value.[449]

Among products of animal origin raw beef juice has no demonstrable antiscorbutic value.[111] Milk is very deficient in antiscorbutic accessory, the amount present being

barely sufficient to provide the needs of the infant or young animal.[68] Since cooking or drying greatly diminishes the content of this accessory it follows that infants fed exclusively upon cooked or dried milk or artificial foods must be supplied with raw orange or lemon juice or, failing these, "swede" turnip juice or tomato juice, otherwise scorbutic symptoms arise which, even if they are not immediately recognizable as such, produce severe disturbances of nutrition and arrest of growth.

In alkaline or neutral solution the antiscorbutic accessory is very rapidly destroyed by heating. It is much more slowly destroyed by heating in acid solution.[223] Tomatoes do not wholly lose their antiscorbutic value on heating, sterlizing, drying or keeping. About one-fourth of the antiscorbutic value is retained even after canning at fifteen pounds steam pressure for 30 minutes,[158] consequently commercial canned tomatoes contain the antiscorbutic factor.[27] According to Delf[96] cabbage juice loses 7/8 of its activity in twenty minutes at the temperature of boiling water. "Swede" turnip juice loses one-half, and orange juice less than one-half of its activity under similar conditions. The superior stability of orange juice is stated not to be wholly due to its acidity, although an alkaline reaction greatly accelerates the destruction of the antiscorbutic factor by heat. Hence the common domestic practice of adding carbonate of soda to green vegetables while cooking to preserve their color, results in practically complete destruction of their antiscorbutic value, a fact which may assume considerable practical importance in cases where the diet is limited in variety and contains but little fresh fruit or raw salads.

The antiscorbutic accessory, unlike the water-soluble accessory, is not "adsorbed" by Fuller's earth or dialyzed iron.[174] It is also not destroyed by ultraviolet light (or the ozone produced by its action upon oxygen).[535] The substance is diffusible and readily passes through a parchment dialyzer.

The immediate effect of deprival of the antiscorbutic factor is arrest of growth in young animals followed later by soreness and tenderness of the joints and muscles, swelling of the joints and hemorrhages in the limbs and frequently in the intestine. The gums become swollen and painful and the teeth are loosened. If an amount of antiscorbutic accessory be administered which is just sufficient to prevent the development of scorbutic symptoms, growth is nevertheless delayed, and there are indications that the utilization of the fat-soluble accessory is imperfect.[96] Conversely, it has been ascertained that if the diet is deficient in other respects, as in protein, inorganic salts or fat-soluble accessory, a larger ration of antiscorbutic accessory is required to permit the maximum growth and well-being attainable on the diet.[230] The incidence of scurvy varies in different animals and rats and mice appear to be immune. At all events, rats have been repeatedly fed upon diets lacking antiscorbutic factor for prolonged periods in the course of experiments directed to the protein requirements or the fat-soluble or water-soluble accessories. No manifest retardation of growth in these animals due to the absence of the antiscorbutic factor has been observed. It is not yet known whether this comparative immunity is due to a real difference of physiological requirements, or whether, on the contrary, it arises from a superior power of storing the factor, so that sufficient is available in the tissues to tide over a long period of deprivation. Typical scurvy has been observed in pigs deprived of raw vegetables, which proved curable upon admission of raw "swede" turnips to the diet.[377]

5. **General Considerations.**—The earlier workers upon the subject of accessory diet factors were very much impressed by the minuteness of the quantities which sufficed to maintain the health or growth of animals, and by a natural association of ideas the impression arose that these substances were not nutritive in the sense of supply-

ing materials for the manufacture of protoplasm, but rather "stimulative" or "catalytic." There is little evidence for the catalytic theory other than this slight analogy and much against it. Thus a catalyzer is not consumed in the process which it accelerates[c] and this indeed is the fundamental reason for the minute quantity of catalyst which is frequently sufficient to bring about large measures of transformation. The reaction accelerated proceeds more rapidly the greater the amount of catalyst provided and in direct proportion to it, but, on the contrary, after a certain maximum of growth-rate or well-being has been attained no further benefit is secured by increasing the supply of any of the dietary accessories. On the other hand the "stimulative" theory is very vague and can only be given definite meaning if the nature of the processes conceived under the term "stimulation" are defined. Little doubt can be entertained that the accessory foodstuffs actually enter into the manufacture of substances produced by tissues, whether these are structural elements of protoplasm, or substances elaborated by them (for example, enzymes) in the performance of their functions, or substances elaborated by a relatively small mass of specialized tissues for the advantage of the whole organism (for example, hormones). Having regard to the extremely small quantities of certain hormones which suffice to achieve their characteristic effects the latter supposition will appear to many the most probable. But it must not be forgotten that mere minuteness of amount is by no means proof that a substance does not enter into the composition of the protoplasm of every cell. Thus the daily loss of iron from the body of a man is but ten milligrams and the total amount in the body is only about 3 grams. The greater part of this is contained in the hæmoglobin, yet the universal occurrence

[c] An **autocatalyst** is consumed in the reverse reaction to that which produces it, but since animal tissues are unable to produce the accessory foodstuffs this possibility need not enter into our consideration.

of iron in the nuclei of cells has led many to assume that it forms an essential constituent of protoplasm. Even more striking testimony to the smallness of the amount of substances which may invariably accompany life is afforded by manganese, of which traces appear to be present in all higher animal and vegetable tissues and which is credited by some investigators with important functions in the life of the cell. Oxidizing enzymes are found in every cell and may therefore fairly be regarded as essential constituents of protoplasm, yet their amount is probably very small. The cystine-glutamic acid dipeptid isolated by Gowland Hopkins from a great variety of tissues is credited by him with important functions in the oxidations of the cell,[195] yet its amount is but 0.01 per cent. of the weight of the tissues. The daily wastage of a protoplasmic constituent of this character might be very slight and yet its replacement might very well be essential to the maintenance of the organism.

Finally, we are not possessed of any evidence which controverts the view that the hormones themselves may exert their actions by entering into the composition of the cells which they affect. On the other hand the ability of animals to "store up" excess of food accessories, particularly the fat-soluble accessory, affords a complete analogy to the storage of excess of other foodstuffs, namely, fats and carbohydrates. The idea that the accessory foodstuffs contribute any appreciable energy (fuel value) to the body need not of course be considered, but we have no reason to doubt, and every reason to believe, that they comprise raw materials for the manufacture of essential constituents of protoplasm or of products which are necessarily consumed in the course of its formation or repair.

When we consider the multitude of organic compounds which are contained in the body of an animal, the great variety of interreactions in which they participate and the limited synthetical power of animal tissues, it is not a matter for surprise that organic dietary constituents

should be required in addition to those necessary for the supply of energy or the manufacture of protein, but that they should apparently be limited in number to three. One is led to inquire whether additional accessories may not yet await discovery. It is true that animals have been maintained in health for long periods upon diets which are believed to contain no other than the three ascertained accessories, but we must remember that had we been limited to the investigation of the dietary requirements of the rat we would have inferred that the essential dietary accessories are but two in number, and in similar fashion others may as yet have escaped our notice. The ability to store up dietary requisites to be expended when necessary in satisfying subsequent requirements enables an adult rat, for example, to withstand deprival of the fat-soluble accessory for periods exceeding a year, or one-half the normal duration of its life. Accessories are transmitted to the young through the circulation and subsequently through the mammary secretion of the mother and the examples already familiar to us suggest the possibility that accessories may exist which would require one or two or even more generations to exhaust. The fact that the effects of unusual foods or even lack of food have in certain instances been traced for two or three generations after readmission of the normal diet[375] indicates that nutritive acquirements or deficiencies may exert effects extending far beyond the individual immediately affected. This aspect of the problem of the adequate dietary has been especially emphasized by McCollum, and from this point of view he and his co-workers are conducting experiments which extend over a series of generations.[290] Experiments of this character with purified foodstuffs, are essential before we shall be enabled, beyond doubt, to certify that we have ascertained the full dietary requirements of an animal.

CHAPTER VII

THE RETARDING INFLUENCES IN GROWTH

1. The Limitation of Bodily Dimensions.—We must now take up the consideration of the nature of those retardative influences which set a limit to the dimensions of every cell community. We have seen that each cycle of growth consists of an initial period of relatively slow growth, succeeded by a period of relatively rapid growth and terminated by a period of slow growth which tends asymptotically to the dimensions of the adult, or else merges into the initial phase of a fresh cycle. These characteristics are shared by those chemical processes which produce their own catalyzers, and we have seen that the progressive acceleration of growth which occurs during the first or autokinetic half of a growth-cycle, is due to the progressive accumulation, within and without the constituent cells of the community, of a substance which is manufactured by the cells themselves and promotes nuclear synthesis and the consequent multiplication of cells. It remains to ascertain what factors intervene to retard this process during the second, or autostatic phase of the growth-cycle, when the rate of cellular multiplication is undergoing the progressive retardation which reproduces in reverse order the phenomena of the autokinetic phase.

In the case of a community of unicellular organisms inhabiting a limited volume of nutrient medium, the most obvious supposition is that the population overtakes the food supply, until the available nutriment for each individual in the community no longer suffices to furnish the excess which is necessary to sustain the manufacture of new protoplasm. The origin of the retardation would therefore be, as in certain autocatalyzed reactions in glassware, the exhaustion of the raw materials or sub-

strates of the reaction. This, in fact, McKendrick assumes to be the limiting factor in the multiplication of bacteria.[295] That other factors may intervene even in this comparatively simple case is shown, however, by the investigations of Eijkman,[120,121] who has shown that substances are developed in bacterial cultures which inhibit their further multiplication. Naturally in certain cases such inhibitory substances may merely be toxic by-products of bacterial metabolism, such as the acids produced by milk-souring bacteria, and it has not yet been established that the growth-retarding substances discovered by Eijkman are not products of this character, which, however, are either volatile or thermolabile, for mere heating of the medium renders it again habitable by bacteria.

In the case of infusorial cultures, however, the origin of the retardation of multiplication in old cultures may readily be shown to reside neither in exhaustion of the available foodstuffs nor in the accumulation of toxic products within the medium.

If the infusoria are removed from an old hay infusion culture, by heating to a temperature somewhat above their thermal death point (50° C.) and then centrifuging, or filtering through two or three thicknesses of paper, it is found that this filtered, infusoria-free culture medium, which has been inhabited by a maximal population of infusoria for a prolonged period, is nevertheless capable of supporting the multiplication of infusoria isolated into it. Indeed, if these infusoria are derived from a relatively young culture, little or no diminution of reproductive rate in comparison with that in fresh hay infusion is observed and relative acceleration of reproductive rate may not infrequently be noted during the early stages of multiplication.

It is evident that these media contain no substances which are toxic for infusoria. On the other hand it is also evident that the food supply in the medium has not been

exhausted, because extensive multiplication of cells within it is still possible. It might be argued, of course, that the nutrients which barely suffice for the maintenance of a maximal population may well be superabundant for a small one, but it must be recollected that it is the concentration of nutrients which determines the availability of food to the cells which inhabit it, and this must have been very nearly the same for the cells which formerly inhabited the medium as for the cells newly transferred to it. If it be contended that the appropriation of food by the multitude of cells inhabiting the medium reduced the concentration of foodstuffs below the level necessary for protoplasmic synthesis, then this must also be true for the new cells introduced into the culture, unless indeed, and this, as we shall see, is the true solution of the dilemma, the new cells are so constituted as to be able to synthesize protoplasm at a lower "nutrient level" than the former inhabitants of the culture.

However this may be, the important fact which is established by these experiments is that cultures which have long been inhabited by a maximal population of infusoria do not contain any substances which are toxic for these organisms, nor are their nutrients exhausted. This being the case it is a very striking fact that the maximal population in such reinoculated cultures never attains the original magnitude and, moreover, if instead of inoculating the old culture with an individual derived from a young and vigorous culture, we employ one of the individuals which originally inhabited the old culture fluid, decided retardation of multiplicative rate in comparison with the rate in fresh hay infusion is observed.

To render this comparison accurate regard must be paid to the physical alterations which old culture fluids undergo during their inhabitation by infusoria and bacteria. Owing to the oxidation of carbohydrates which has occurred many soluble substances originally present in the medium have become converted into carbon dioxide

and water, the former having escaped into the air at the surface of the drop. Hence, old media have a much lower osmotic pressure than freshly made up hay infusions, and individuals from very old culture media, isolated into fresh hay infusion, lose water through the relative hypertonicity of the new environment and become laterally compressed and disc-shaped. Such a transfer is very badly endured by infusoria and individuals thus treated sometimes die, or, more usually, undergo an exceptionally prolonged "lag-period." On the other hand, transfer to a relatively hypotonic medium is well endured. It has been found that freshly made up hay infusion (see p. 84) diluted with four times its volume of distilled water containing two volumes per hundred of $\frac{m}{15}$ phosphate mixture ($P_H = 7.7$) provides a medium which is neither hypertonic nor markedly hypotonic for infusoria isolated from culture fluids which are from thirty to forty days old.

The rate of reproduction of individuals isolated from these old cultures is always far inferior, when they are isolated into the old culture medium itself, to the reproductive rate displayed by similar individuals isolated into hay infusion of similar tonicity, and usually even to the reproductive rate which is displayed in undiluted hay infusion, in spite of the handicap of hypertonicity. The maximal population is also soon attained in the old culture fluid, and is very scanty in comparison with the population which is attained in the fresh infusion, with or without dilution. It is also usually inferior to the maximal population attained in the old culture fluid when it is inoculated with an individual derived from a young culture. This fact, and the fact that maximal reproductive rate of individuals from young cultures is possible in the same medium, forbid the supposition that it contains any toxic substance, yet mere dilution of the old culture medium (with distilled water containing two volumes per hundred of $\frac{m}{15}$ phosphate mixture of $P_H = 7.7$), greatly enhances

the reproductive rate of individuals isolated into it which had previously inhabited the old culture. Also, despite the fact that dilution must result in diminution of the available nutrients, the maximal population attained in such cultures may actually somewhat exceed that attainable in undiluted cultures.

The following experiments will serve to show the type of effect obtained, one individual from a forty-day-old culture being in each instance isolated into the same volume (about 0.15 c.c.) of the culture media named:—

TABLE XLII

Culture number	Culture fluid	Individuals after			
		24 hours	48 hours	72 hours	96 hours
490A	Old culture medium	3	31	——	250
490B	Old culture medium	4	47	——	207
491A	Old culture medium + equal volume of buffered distilled water	5	171	——	234
491B	Old culture medium + equal volume of buffered distilled water	43	356	——	350
493A	Distilled water, $P_H = 7.7$	1	——	4	35
493B	Distilled water, $P_H = 7.7$	1	——	34	118
494A	Hay infusion + 5 volumes of buffered distilled water	2	——	2000	——
494B	Hay infusion + 5 volumes of buffered distilled water	2	——	4000	——

This, then, is the situation:—An old culture fluid contains no substances which are toxic for infusoria, nor does it retard the multiplication of infusoria isolated into it from young cultures. Nevertheless, infusoria from old cultures develop much more slowly in old culture fluid than in fresh isotonic hay infusion, and the maximal resultant population is very scanty. Mere dilution of the

old culture fluid with an equal volume of properly buffered distilled water enhances the reproductive rate of the individuals restored to it, and does not reduce the maximal attainable population.

In the light of these results we cannot attribute the failure of individuals inhabiting an old culture to reproduce indefinitely, either to loss of reproductive capacity of the cells themselves, or to inability of the culture fluid to sustain any larger population. The cessation of reproduction is evidently due to a reciprocal relationship between the old culture fluid and the individuals inhabiting it, whereby the fluid is no longer suitable for the reproduction of these particular individuals.

Precisely similar considerations may readily be shown to apply to the growth of the higher metazoa. In the first place, exhaustion of nutrient materials cannot well be the cause of cessation of growth in an animal which has free access to abundant and varied foods. Of course we might suppose, as indeed has been frequently assumed, that the dimensions of the alimentary canal limit and determine the dimensions of the animal, but if this be true then we must inquire what it is that determines the cessation of growth of the alimentary canal, which thus sets a term to the growth of the whole animal. We are ultimately driven in this way to the assumption of an hereditarily limited capacity for multiplication, either of the cells comprising the animals as a whole, or, in the last resort, of the cells composing its alimentary tract. Now an hereditarily limited capacity for multiplication does not exist. Repeated removal of parts of any tissue, whether alimentary in function, or other, results in repeated, and repeatedly successful repair so far as gross mechanical conditions permit repair. The transplantation of fragments of the most varied tissues to fresh nutrient media, or even to other parts of the body, results, as a multitude of experiments in recent years have shown, in the reassertion of multiplicative capacity by cells de-

rived from tissues in which, while *in situ,* no multiplication would have occurred. Moreover, as Uhlenhuth has pointed out[497] gigantism must, if we accept the mechanical hypothesis of limitation of animal dimensions, arise solely in consequence of possession of a greater absorptive area, while, on the contrary, pathological gigantism in human beings arises in consequence of lesions or abnormalities of the pituitary body,[85,272] and gigantism may actually be artificially induced in animals, as we shall see in a later chapter, by administering to them relatively small amounts of certain substances.

Besides this, the fundamental assumption underlying the idea that the dimensions of the adult are determined by the dimensions of its absorbing surface implies that the supply of foodstuffs to the individual cells of the body diminishes with their extension in space, so that outlying tissues would have to be regarded in the adult as struggling perpetually upon the verge of inanition. Nothing remotely resembling this is true. The actual nutrient medium bathing the cells is derived directly from the blood, and the whole of the alimentary and excretory machinery of the body, aided by endocrine activity, is devoted to the maintenance of the constant composition of the blood. The volume of the blood supply to the various organs differs, but it does not undergo any regular decrease in proportion to the distance of the organs from the alimentary surface. Thus the concentration of the substrates of growth, available to any animal which is not suffering actual deprivation, must be regarded as remaining substantially unaltered from birth, or before birth, to old age. Under these circumstances the velocity of the forward reaction of growth (or nuclear synthesis) must continually increase in proportion to the growth achieved, *i.e.,* to the mass of autocatalyst which has been produced. If in the adult the available foodstuffs no longer suffice to maintain growth, it is not because the supply of foodstuffs to the cells has diminished,

but because the requirements for the accomplishment of nuclear synthesis have increased.

It is evident that insufficiency of nutrients, as such, cannot determine the cessation of growth in the adult. The tissue fluids of the adult are, however, capable of supporting the vigorous multiplication of other types of cells, for example cancer cells, while, on the other hand, the latent capacity of the cells of the adult to multiply is testified in the phenomena of repair, or in the results following upon transplantation of tissues. We can only infer that, as in the case of an infusorial culture, the limit of multiplication is determined by a reciprocal unsuitability of the cells of the adult and the medium which they inhabit.

It is not to be denied that more specific or individual causes may govern the development of certain special tissues in the higher animals and thus indirectly modify the development of the whole. This is only what we might expect in view of the general tendency towards delegation and concentration of function which accompanies the higher planes of organic development. Some of these special influences will fall under consideration in a later chapter. But the phenomenon of growth-retardation in the adult is of far too universal occurrence to admit of one explanation in one biological type, and a different explanation in another. Underlying and modifying all the special instances of growth-acceleration or retardation which have been traced to endocrine activities in the higher animals, must be a much more generalized, and therefore more fundamental mechanism of growth regulation which governs the growth and ultimate dimension of all cell-communities. The phenomenon of growth-retardation in the adult occurs, and presents essentially the same features, in all of those animals, plants, and unicellular colonies in which growth occurs within a limited and continuous volume of fluid. Where the medium bathing the cells is unlimited, relatively to their mass, as, for

example, the open sea, and no closed circuits of nutrient media envelop the cells, as in the corals, then growth is indefinitely renewed,[527] and stasis is only imposed by gross external physical limitation, such as approach to the surface of the water, or insufficiency of food or oxygen. Limitation of growth in the higher metazoön is imposed by the contiguity of its units, and the fact that fluid intercommunication between them is achieved through the agency of a circumscribed volume of pericellular fluid. Mere interruption of this intercommunication, as by removal of a fragment of skin in a mammal, or section of a planarian into two equal parts, suffices to reinaugurate vigorous cell-multiplication which, in the latter instance, is sustained so far as to regenerate in their entirety the lost parts of the whole animal.

We have seen that the cessation of a chemical reaction may be brought about in either of two ways, namely, by exhaustion of the materials undergoing transformation, or by the accumulated velocity of the reverse reaction finally equalling the velocity of the forward reaction. The former factor is obviously inoperative in the growth of the higher animals, and it plays only a subordinate rôle in limiting the population of unicellular cultures. We are therefore driven to the acceptance of the latter factor, arising out of the mass-action of the accumulated products of growth, as the determining agency in the cessation of the growth of an adult animal.

In the case of a culture of unicellular organisms contained in a limited volume of culture medium, the food supply must obviously diminish with increasing population of the culture. The mathematical equation expressing this conception will evidently be that embodied in Formula 5. In the higher metazoa, however, in which special mechanisms exist for the maintenance of a constant nutrient level, the differential equation expressing the net velocity of growth will be:—

$$\frac{dx}{dt} = k_1 a\, x - k_2 x^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (56)$$

where k_1 is the velocity-constant of the forward reaction, and k_2 that of the reverse reaction. This, when integrated, yields the equation:—

$$\log \frac{x}{A - x} = K (t - t_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (57)$$

where $A = \frac{k_1}{k_2} a$ and $K = k_1 a$

The effect of increasing the nutrient level ($= a$) will obviously be to increase the net velocity of growth, because a appears only in the positive term of Equation 56. This is why an organism, isolated from an old culture into a fresh nutrient medium, is enabled to undergo division, for although it remains, until division occurs, a member of the parent culture in so far as the nuclear concentration of autocatalyst is concerned, the increase of a through the augmentation of foodstuffs enhances the forward velocity of nuclear synthesis, enabling it to exceed that of the reverse reaction sufficiently to permit the degree of synthesis requisite for division. For the same reason an animal which has been stunted by deprival of some normal dietary constituent even until an age approximating to the average normal life-duration, nevertheless reinaugurates vigorous growth directly the missing constituent is readmitted to the dietary.[342]

The effect of increasing the concentration of autocatalyst in the nutrient medium or pericellular fluids, however, will vary with the stage of growth at which the increase occurs. During the first half, or autokinetic phase of any growth-cycle, while the net velocity of the reaction is increasing with time and with the accumulation of the products of growth, increase in the concentration of the autocatalyst, as by the introduction of autocatalyst derived from other cells, will result in acceleration of cellular multiplication. During the second half, or autostatic phase of the cycle, however, while the net velocity of the reaction is decreasing with time and the accumulation of the products of growth, increase in the concentration of the autocatalyst will result in retardation of

growth. This arises from the fact that while the concentration of the autocatalyst enters into the positive term of Equation 56 only in its first power, it enters into the negative term in its second exponent. Hence, while x is less than $\frac{1}{2}A$, the net rate of growth increases with increase of x, but when x becomes greater than $\frac{1}{2}A$, the negative term in Equation 56 increases more rapidly than the positive, and the net rate of growth decreases.

We can now interpret the phenomena which occur when an infusorian is isolated into an old culture, formerly densely populated by infusoria, but freed from them by filtration or centrifugalization. This medium is loaded with autocatalyst, and consequently the discharge of autocatalyst into the medium at each division will be a minimum. Just as, in the partition of a dissolved substance between two immiscible solvents, the amount entering into or departing from either solvent is determined by its initial concentration in each of them, so in the partition of the autocatalyst between the nucleus and the surrounding medium, the amount discharged by the nuclei will increase in proportion to its concentration in the nuclei, and decrease in proportion to its concentration in the medium. The phenomena are not essentially different if dissociable compounds of the dissolved substance are formed in one of the solvents.

An infusorian isolated into such a medium, therefore, parts with less of the autocatalyst at each nuclear division than it would part with in a fresh infusion, or it may even acquire additional autocatalyst from the medium. The effect of this upon the rate of nuclear synthesis, and consequently upon the rate of multiplication, will depend upon the magnitude of the "charge" of autocatalyst already accumulated within the nucleus. If it be less than the amount corresponding to $x = \frac{1}{2}A$, the cell is in the autokinetic phase of growth, and the more rapid accumulation of autocatalyst will be advantageous. Hence, infusoria isolated from young parent cultures multiply

rapidly at first in old media which had formerly been densely populated, but the rate of multiplication rapidly falls off. On the other hand, if the accumulated "charge" of autocatalyst is already in excess of the amount corresponding to $x = \frac{1}{2}A$ and does not fall below this amount during the period of redistribution, then the cell is in the autostatic phase of growth, and the more rapid accumulation of autocatalyst will retard multiplication. For this reason, individuals isolated from old cultures into old culture media multiply slowly, and soon cease to multiply altogether, the net velocity of nuclear synthesis falling to zero before the critical nuclear ratio, or "kernplasma-relation" can be attained. Mere dilution of the old medium, reducing as it does the external concentration of autocatalyst, permits a greater loss of autocatalyst by the nuclei, and therefore a greater net velocity of growth.

We have seen that cessation of growth occurs in higher animals at a time when the nutrient level is still adequate to maintain tissue synthesis. The capacity for growth has been shown to be retained throughout life[342] and only its previous occurrence prevents the continuation of growth at any age. We must conclude, therefore, that the limiting factor which inhibits the multiplication of the metazoan cell-community is of the same nature as that which ultimately inhibits the multiplication of a protozoan cell-community, namely, the progressive accumulation of autocatalyst in the pericellular fluid. That this is the true explanation of the phenomenon is shown, on the one hand, by the fact that tissues removed from the community of which they formed a part and cultivated in media which are constantly renewed, are thereby enabled to multiply indefinitely,[112] and on the other hand by the fact recently discovered by Carrel that when vertebrate tissue cells are cultivated in blood plasma, the rate of cell multiplication varies in inverse ratio to the age of the animal from which the plasma was taken.[59,61] It is evident that a substance accumulates in the blood with advancing age

which has the effect of retarding cell multiplication. Similarly, the rate of healing or cicatrization of superficial wounds diminishes in proportion to the age of the subject.[60] The reinauguration of developmental activity, as in the ovum, is only rendered possible by the isolation of the developing tissues from the nutrient media of the parent.[a]

2. **The Physiological Differentiation of Tissues.**—We have hitherto discussed the phenomena displayed in growth as if all of the cells in the community were alike in all of the particulars which determine their multiplicative rate. This is not even true in a culture of infusoria, as we shall shortly see, and it is very remote from the truth in the higher plants and animals. In the first place it is to this complexity of cell types that we may probably attribute the multiplicity of growth-cycles which occur in the development of a vertebrate animal. The three main cycles which impress themselves upon our attention by their prominence in the growth of these animals may not be all that actually occur, but their presence demonstrates that at least three widely differing cell types comprise the tissues of the adult bird or mammal, and that when conditions become unfavorable for the development of the earlier types, they are still such as to permit multiplication of the later types.

Three factors enter into the determination of the multiplicative capacity of a cell, and the influence of two of these, the nutrient level and the concentration of the

[a] It is hardly necessary to point out that even in the placental animals the developing offspring do not share the nutrient circuit of the mother, although foodstuffs and excretory products pass through the chorionic epithelium from the one nutrient circuit to the other. The actual nutrient medium, in the one case as in the other, however, is not the blood, but the intercellular fluid derived from blood. There is much in the phenomena attending the transplantation of tissues to suggest the idea that the autocatalyst of nuclear synthesis does not wholly escape into the blood stream but remains partly localized in the tissue in which it arises. However this may be, it is evident that it does not escape from the body through the renal tubules, and we may thus understand its failure to traverse chorionic epithelium.

autocatalyst, has been discussed in this and the preceding chapters. The third is the magnitude of the critical mass of nuclear constituents at which division of the nucleus occurs. The multiplicative capacity of a cell, in other words, is determined by three variables, A, x and the "kernplasma-relation." Diminution of the "kernplasma-relation" will obviously permit the multiplication of cells in the presence of accumulations of autocatalyst which would totally inhibit nuclear synthesis before the attainment of larger values could occur.

While we may conveniently regard alteration of the kernplasma ratio as the archetype of the various internal factors which may modify the response of the cell to alteration of the nutrient level or the autocatalyst concentration, it must nevertheless be borne in mind that other internal factors may possibly differ from one cell to another, which would modify the multiplicative rate in a manner similar to the effect of diminution of the kernplasma ratio. Thus, if any physical or chemical condition arising in a given cell were to result in a selective acceleration of the forward in preference to the reverse reaction of nuclear synthesis, that is, if k_1 were increased without proportionate enhancement of k_2, the effect of this would be precisely similar to the effect of diminution of the kernplasma-relation in so far that division (*i.e.*, attainment of the kernplasma-relation) would be possible for these cells, while remaining impossible for cells not similarly affected. Since, however, all the internal factors which may hasten or delay the attainment of the kernplasma-relation cannot at present be enumerated, we may, for the sake of brevity of expression and concreteness of illustration, consider all of these factors as affecting the kernplasma ratio, although in particular instances it may possibly happen that the kernplasma ratio is not actually diminished but only attained more quickly, or conversely, that it is not actually increased but attained more slowly. We are justified in doing this because, as we shall see, direct obser-

vation demonstrates that alteration of the kernplasma-relation occurs in many instances of cell differentiation, and if any alteration of the ratio $\frac{k_1}{k_2}$ should ever actually occur, its effect upon the rate of multiplication of cells might be expected to resemble the effect of a corresponding diminution or increase of the kernplasma ratio.[b]

Now it must always be borne in mind that the opportunity of dividing confers upon the cell the possibility of rejuvenation through the dissipation of its accumulations of autocatalyst. If, then, a drastic diminution of the nuclear ratio, or any internal modification leading to an earlier attainment of the nuclear ratio, should occur in the production of any member of a cell-community, the individual so constituted will be able to propagate itself, possibly through a number of generations, until the increase of autocatalyst in the pericellular fluid becomes so great as to prevent the new cells from losing sufficient autocatalyst at division even to permit the synthesis of the lesser amount of nuclear material which is requisite to attain the new critical nuclear ratio. At the same time the possibility of multiplication by the older individuals of the community would become more remote than ever. The new type of cell could therefore compete most advantageously for place or nutriment with the old.

In this connection the observation of Jennings[206] that races of *paramœcia* may be obtained which differ in reproductive rate under like conditions, and transmit these differences to their descendants, assumes especial significance. The basis of these differences must lie within the cell, and the most obvious internal variation which could affect the reproductive rate in this manner would

[b] Any agency affecting the velocities of the forward and reverse reactions of nuclear synthesis unequally cannot, of course, be catalytic, for not merely the rate of attainment of equilibrium, but equilibrium itself would be affected. An alteration of physical conditions, such as a rise or fall in temperature, or an alteration of the reaction or consistency of the nuclear contents might be conceived, however, to have the ability of bringing about a change of this character.

be a modification of the nuclear ratio. Other internal changes may also, as we have seen, bring about analogous results, but these are most readily conceived, in our present lack of more precise knowledge, in terms of their effect, which would be precisely similar to that of a diminution of the nuclear ratio at division. From this point of view, also, we can interpret the observation of Peters[372] that in old infusorial cultures the population does not remain at a steady maximum, or even steadily decline, but fluctuates between successive maxima and minima of varying magnitude. In other words, certain members of the population die off and are succeeded by others better adapted for propagation in the medium which they inhabit. Not all of the infusoria or bacteria inhabiting a culture are therefore alike, or equally successful in propagation of their kind, and it has been observed that the individuals isolated from very old cultures frequently display an abnormally high reproductive rate, after expiry of the lag-period, when they are isolated into fresh hay infusion. Similarly, hereditary variations have been observed in various species of bacteria[14] and fluctuations of the population of densely inhabited cultures, analogous to those which occur in cultures of infusoria, have also been observed in cultures of bacteria.[66]

The importance of these observations resides in the fact which they reveal, that the morphologically similar elements of a unicellular community may differ from one another in their ability to multiply, and that to so great an extent that certain types are thereby enabled to supplant others in the community, or to continue multiplying where others are checked.

In these facts we appear to possess the key to the origin of differentiation in the development of the higher animals. By their mere multiplication in a circumscribed medium, cells of any one type render that medium unsuitable for their further multiplication. If, however, the variability of the cells is sufficient to enable them to

give rise to a type differing from the parent type in the possession of ability to divide at a lower nuclear ratio, or to attain this ratio under conditions prohibiting its attainment in others, then a fresh outburst of reproductive activity will ensue. In this way a **physiological differentiation** into varying types of cells of differing developmental potentialities may be conceived to occur, the fundamental inherited trait, which is responsible for their appearance, being an inherent tendency to vary on the part of the cells comprising the primitive embryonic tissues.

Morphological differentiation is in the main a cytoplasmic manifestation[305] but its origin must, in the first instance, be attributable to differences which arise between morphologically similar cells at the moment of their production, *i.e.*, at nuclear division. In final analysis we come back therefore to nuclear events and the results of modern investigation of the cytological aspects of inheritance have taught us also that the origin of form lies in the nucleus[313] even though its manifestation occurs elsewhere. The sequence of events which leads from the initial physiological differentiation to the final expression of almost infinite morphological variety in the tissues of any of the higher metazoa does not permit of any single or uniform explanation because a multiplicity of factors participate in the determination of the result, and their relative proportion differs from one instance to another. The situation however is this, that after division into two, four, eight, or sometimes more, cells which are morphologically identical, and as a rule spatially symmetrical, one or another cell comes to manifest a difference in reproductive rate leading to the quicker or slower multiplication of this part of the organism and ultimately to the appearance of morphological differentiations.

We can hardly avoid attributing the morphological differentiations which ultimately make their appearance to the physiological differentiations, expressed in varying

reproductive rates, which precede them. Since we do not know to what particular constituent of the nucleus division is in the first instance attributable, *i.e.*, which sets in train the complex sequence of events which constitutes mitosis, we cannot define the nuclear ratio, which determines the moment of cell division in terms of total nuclear mass or volume. This might be possible if the composition of nuclei were invariable, for even if the determining agent were to constitute only a minute fraction of the whole nucleus, it would in that event always constitute the same fraction, and therefore be proportionate to the nucleus as a whole. But in point of fact we know that the total volume of the nucleus is made up of many different components which are not always present in the same proportion to one another.[74] The proportion of nuclear sap, in particular, increases throughout the period between nuclear divisions. Nevertheless, if the measurements of nuclear and cytoplasmic volume are made just before or just after nuclear division, when the nuclei contain maximal or minimal proportions of nuclear sap, we may legitimately suppose that a general, although not necessarily an exact correspondence will obtain between the morphological ratio of nuclear to cytoplasmic volume at division, and the chemical ratio, which is the mass or proportion (*i.e.*, concentration) of a particular constituent or group of constituents of the nucleus, existing at the moment of division.

Conklin, who has carefully measured the ratio of nucleus to plasma at corresponding periods of the nuclear cycle, and at various stages of embryonic development,[74] finds that "when cell division takes place at regular intervals the kernplasma-relation is fairly constant; when it takes place at irregular intervals this ratio is variable. The longer the resting period the larger the nucleus becomes, and in extremely long resting periods the greater part of the plasma may be taken up into the nucleus." Hertwig[183,185] has also shown that an increase of nuclear

mass leads to a slowing of divisions and it is significant that he obtains this effect as a result of prolonged overfeeding in infusoria, a measure which, by affording conditions more readily permitting the attainment of large nuclear ratios, would enable the survival of the types possessing them, which would otherwise be eliminated by their failure to multiply. Conklin regards the large nuclear volume in such cases as the result rather than the cause of the slow rate of cell division, and this view is also in accord with the hypothesis which we have outlined. The relation of these results to the subsequent differentiation in development is indicated by Conklin in the following words:—"In the different blastomeres of this egg[c] there is no constant nuclear-plasmic ratio or kernplasma-norm. However in different eggs corresponding blastomeres have the same kernplasma relation, when measured at corresponding stages. The volumes of the protoplasm and of the nucleus show little variation in any given blastomere, and the kernplasma relation of each of the blastomeres named.....is practically the same in all eggs. Since many of these blastomeres are peculiar in oöplasmic constitution and prospective significance, it is not improbable that the peculiarities in their kernplasma relation may be the result of differentiations already present in the blastomeres."[74]

Where a similar morphological outcome occurs, therefore, similar divisional rates and kernplasma ratios are found, but where dissimilar morphological consequences are subsequently to ensue, even although morphological dissimilarities are not as yet made objectively manifest, there are found dissimilar divisional rates and kernplasma ratios which foreshadow the ultimate morphological outcome.

The mutual arrangement of similar cells, as D. W. Thompson has so beautifully shown in his work on *Growth and Form*,[490] is determined by very simple forces.

[c] *Crepidula plana.*

As soon as varying divisional rates begin to impose deformations upon the initially symmetrical assemblage, however, these forces, while remaining operative, are supplemented by others, of a more complex character. Deformation of a symmetrical assemblage of cells involves inequalities of environment. The consumption of nutrients and the production of waste products is no longer symmetrically distributed. Thus while all of the cells in a developing organism may still at this stage be morphologically similar, as in a culture of infusoria, yet their physical juxtaposition combined with the underlying physiological differentiation resulting in varying multiplicative rates, has set up conditions which invite or compel the assumption of differing forms and functions by the constituent cells of the community. Guided always by the graded variations of multiplicative rate which are unfolded by the nuclei and constitute the basis of inheritance, the resultant cells are compelled by the inequalities of their environment to display differing reactions and adjustments.

In some instances, as Loeb has shown,[235,236,241] tropistic reactions initiate a directional distribution of certain cell types to the exclusion of others, and thus the beginnings of a definitely oriented structure are laid down. In other instances the response to mechanical conditions, as of gravity or tension, or to localized inequalities in the availability of nutrients (induced by the deformations of the originally symmetrical assemblage), leads to the assumption of differing physiological habitudes which are reflected in the structure of the elements affected. In all cases, probably, the metabolic gradient which Child has shown to occur in axial arrangements of cells[69] tends to preserve an orderly extension of the directional impulses which are thus initially acquired. The morphological differentiation of an organized multicellular complex may thus be conceived as the outcome of physiological

differentiations among what are, as yet, morphologically similar units.

The existence of physiological differentiation in an unorganized community, such as a colony of infusoria, does not, as we have seen, lead of necessity to any very pronounced morphological differentiation, although minor morphological variations such as that of size may also occur in the various physiological types. But in a community of mutually adherent cells, such as that which constitutes the embryo of a developing metazoön, these differentiations must inevitably lead, through inequalities of growth, to deformation of the initially symmetrical assemblage, and these, in turn, to inequalities of condition which become at every successive stage more and more pronounced and eventually compel corresponding modifications of the form and nutrition of the cells. Physiological differentiation is not therefore the direct cause of morphological differentiation, but it leads the organism into a path which can have no other outcome, by successive adaptations to environment; a degree of morphological complexity is thus attained which is limited only by the degree and graduation of physiological differentiation which is permitted by the constitution of the germinal nucleus.

It follows from this conception of morphological differentiation that it originates indirectly out of changes in multiplicative rate which arise from internal modifications of nuclear equilibrium or of the interdependence of nucleus and cytoplasm, in either case leading to successive graded reductions of the interdivisional periods. Now whatever the modifications may be which lead to this result there must of necessity be some limit which they cannot transcend and every step towards this limit reduces the further potentialities of differentiation. It must be for this reason that the early blastomeres of many forms after separation from one another are still able individually to give rise to complete embryos, while at

later stages of development this becomes impossible[311] and in its place the cells retain merely a partial regenerative ability which diminishes progressively as differentiation becomes more and more extensive. The ability to reproduce the whole organism evidently rests upon the ability of the nucleus to furnish the whole gamut of graded kernplasma ratios which normally arise during the development of that organism. The loss of one of these steps, after a certain degree of physiological differentiation has occurred, restricts the morphogenetic potentiality of the differentiated cell to something short of that required for the production of the whole organism.

We have regarded the kernplasma-relation as the type of the nuclear changes which determine physiological differentiation, simply because, as we have seen, any other internal modification permitting multiplication in the presence of otherwise inhibitive accumulations of autocatalyst must abbreviate the interdivisional period exactly in the same way as a reduction of the kernplasma-relations. But if we go further and assume that this is not merely an illustration of one method of accomplishing this end, but the actual method which is employed, then successive differentiations would imply successive losses of substance from nuclei. Thus a differentiated nucleus would contain some nuclear material in less concentration than the undifferentiated germinal nucleus. It has been pointed out by Wilson,[523] p. 425 and ff., that loss of power to reproduce the whole organism is in fact associated with loss of nuclear chromatin. We need not for this reason assume that the nuclear chromatin is the substance of which the mass or concentration determines the moment of nuclear division, but we must obviously conclude that this substance accompanies, if it is not identical with the nuclear chromatin. Now the chromosome theory of inheritance compels us to reach the same conclusion in regard to hereditary qualities which, in turn, are manifested by an orderly succession of morphological differentia-

tions. Starting from totally different data and phenomena, therefore, the study of genetics and the analysis of growth converge and unite in attributing to the chromatin elements of the nucleus the origin of those graded variations which underlie the unfolding of the morphological complexities of the completely developed organism.

3. Senescence.—The overwhelming force of suggestion conveyed by the inevitability of the occurrence of death in man himself and in all those forms with which the generality of mankind are familiar, has not been without its effect upon the mental bias of the biologists themselves; so that until a comparatively recent period the phenomena of senescence were passed over as the inevitable outcome of life itself, as if they were implied in the very conception of life.

It is to Weismann that we owe the recognition, now general among biologists, that potentially immortal forms of life exist. In those unicellular forms which propagate themselves by fission the totality of the parent is included in the offspring who merely add to the living material thus handed on to them. The propagative powers of such forms seem to be potentially unlimited, provided only that they are not compelled to inhabit and share a limited volume of nutrient medium.

It is true that the observations of Maupas[278] tended to show that unlimited propagation of unicellular animals by fission is not possible without the occasional stimulus, as he believed it to be, of conjugation. The investigations of Jennings, however, have shown that conjugation in infusoria, so far from constituting a stimulus, actually delays subsequent reproduction.[208] The significance of conjugation for these animals lies, he believes, in the amphimixis thereby achieved and the greater variability of the offspring, enabling them to adapt themselves to a greater variety of environmental circumstances. The process of conjugation in higher plants and animals accomplishes two totally different purposes. It conveys to

the egg the hereditary characteristics of both parents which are thereafter redistributed among the germ cells of the offspring in varying permutations and combinations, giving rise to a variability not otherwise attainable. At the same time it constitutes a powerful stimulus to the development of the ovum upon which the higher forms have in general become so dependent that natural parthenogenesis is no longer possible in the majority of them. The discovery by Loeb of artificial means whereby this stimulus may be communicated to the egg[242] inaugurated a new epoch in our understanding of development and inheritance by demonstrating that the two phenomena, that of growth-stimulation and that of conveyance of hereditary qualities, are completely separable even in forms in which they normally occur together. In the infusoria, Jennings believes, these two functions are normally separated. The stimulus necessary to accomplish cell multiplication is produced by every individual. Conjugation in these forms accomplishes merely a partial exchange of hereditary qualities.

The periods of diminished reproductive capacity in infusorial cultures which have been noted by Maupas and many subsequent investigators, have been shown by Woodruff[528] to be due to monotony of the environment and possibly to the gradual exhaustion of necessary food constituents. Another factor, hitherto overlooked, is the density of the population of the parent culture from which individuals are procured to inaugurate a new culture. We have seen that individuals isolated from an old and densely populated culture exhibit a prolonged "lag-period" and if this is included in the estimation of division-time, the multiplicative rate of individuals isolated from old cultures must appear to be retarded. This culture will in a given period be very likely to produce fewer individuals than the parent culture and an individual reisolated from it will exhibit a briefer "lag"

and therefore higher apparent rate of multiplication than the previous culture. In this way irregular rhythms of reproductive rate may be established[410] such as have been observed by many investigators,[53,205,529] but they are actually attributable to variations of the environmental conditions at the time that new individuals are isolated. The individuals inhabiting an old culture medium exhibit senescence in the sense that they have temporarily lost the power of reproducing themselves in the medium which they inhabit and transfer to a new medium accomplishes "rejuvenescence" by raising the nutrient level so far as to permit the necessary nuclear synthesis for division to occur, when the issuance of the accumulated autocatalyst from the nucleus confers a youthful character upon the cell by re-establishing the autokinetic phase.

With constant renewal of the nutrient medium, therefore, an indefinite continuance of the autokinetic phase is theoretically possible and, in fact, Woodruff has been able by frequent renewal and variation of the nutrient medium, to propagate infusoria for over two thousand generations without the occurrence of conjugation or any permanent loss of reproductive capacity. The potential immortality of these forms may be considered, therefore, to have been experimentally established.

But the potentiality of unlimited reproduction by fission is now recognized not to be confined to unicellular organisms. The Jensen tumor has now been propagated by transplantation for so many generations that the duration of its life has already exceeded by many multiples the life duration of the mice which are its hosts. The propagation of living tissues **in vitro** has revealed unlimited potentiality of self-maintenance and reproduction in a variety of the specialized tissues of the vertebrata. Indeed we may now venture to surmise that so far from

death being the necessary outcome of life, potential immortality with unlimited capacity for reproduction is a universal characteristic of living matter.

In every instance of sustained reproductive capacity and indefinite duration of existence, however, one essential must be realized, and that is frequent renewal of the pericellular fluid.

According to Weismann, the death of higher organisms is due to the incompleteness of the allotment of germ plasm which is parcelled out to the somatic cells of the adult, which limits their powers of reproduction. The germ plasm, on the contrary, which is handed on from generation to generation of gametes without any diminution, is both potentially and actually immortal, forming a continuous living bridge which links one transient generation to the next.

This view met at once with the difficulty constituted by the regenerative powers which are so widely displayed by living tissues. The difficulty was temporarily overcome by the invention of a supplementary parcel of determinants solely designated for the purpose of enabling regeneration to occur. But the fundamental assumption that the reproductive capacity of somatic tissue is ultimately limited has now been shown to be incorrect, through the **in vitro** experiments to which reference has been made, and with the rejection of this assumption the theory itself must necessarily be discarded.

A variant of Weismann's hypothesis from the standpoint of energy-transformations rather than consumption of materials has been put forward by Rubner,[435] to the effect that living substance is only capable of undergoing a limited number of metabolic processes before it wears out and breaks down. Among mammals, however, man is distinguished by an exceptionally high and prolonged energy output, the total expenditure of calories per kilo-

gram of body weight during life being three or four times as great as in any other animal. A theory which encounters such a remarkable exception among the few examples hitherto subjected to investigation, obviously cannot be universal. Moreover, it breaks down for exactly the same reasons as Weismann's hypothesis. The discovery of the unlimited multiplicative capacity of differentiated tissues destroys the foundation of all hypotheses which relate senescence to the "using up" of any of the factors transmitted to the offspring by the germ-cell.

According to Minot, senescence begins in the earliest stages of development,[304] and he avers in fact, and Child for different reasons inclines to the same opinion,[69] p. 275, that the rate of senescence is highest in youth and lowest in advanced life. This opinion originates in a curious interpretation of the relationship of senescence to the rate of growth. If growth be regarded from a chemical point of view as a complex synthesis of new materials, the only way to compare its rate at different periods with that of any other chemical process is to measure from time to time the mass of living material which has been produced, and if we do this then, as we have seen, we find that growth occurs in successive cycles, in any one of which the rate of growth increases at first and subsequently decreases with age, as an autocatalyzed reaction increases and then decreases in velocity with the lapse of time. Minot, however, views the rate of growth quite differently. Unconsciously assuming (what obviously has to be proved) that the "normal" rate of growth is always proportionate to the existing mass of living material, he divides the rate of growth by the weight, thus obtaining the increment per unit of time per unit mass of tissue. In other words he determines $\frac{dx}{dt} \cdot \frac{1}{x}$. He finds that this diminishes throughout the duration of life in

the manner indicated in the accompanying figure (Fig. 25). He now identifies this diminution of rate of growth per unit mass with senescence, and naturally infers that the rate of senescence is at a maximum in youth, a conclusion which appears so artificial and so flagrantly opposed to experience that the validity of the premises from which it is deduced must be carefully scrutinized.

In the first place, as we have seen, the assumption was made that normal growth "ought" to be proportional to

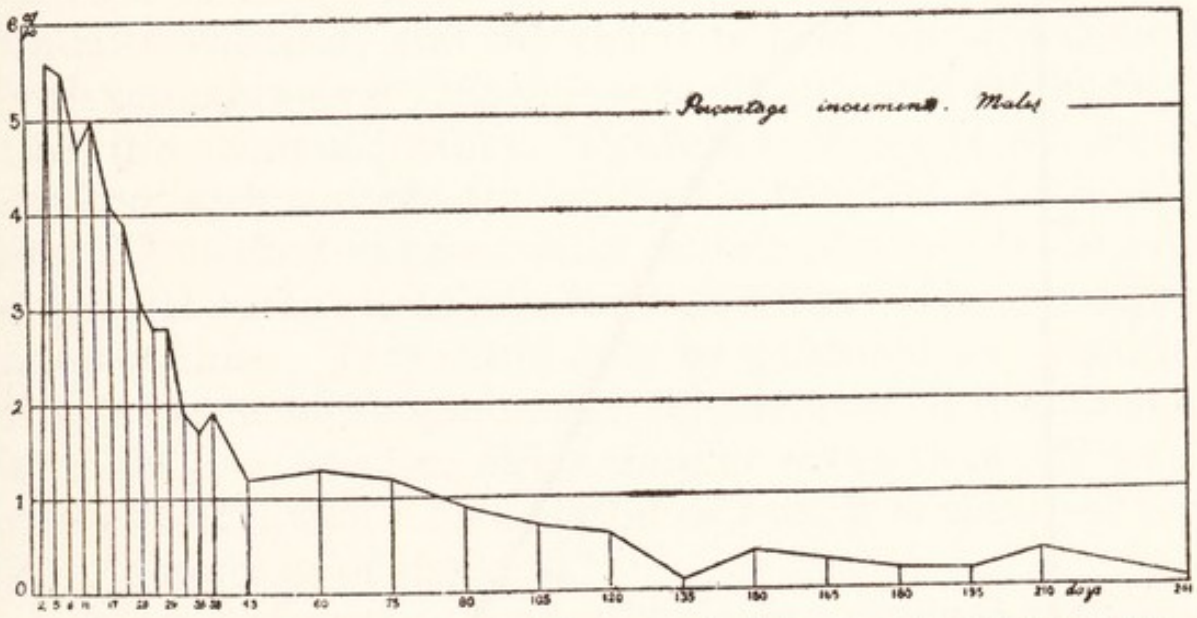


FIG. 25.—Ratio of velocity of growth to mean weight in guinea-pigs of various ages. (After Minot.)

the existing mass of living material. This is not true in the majority of chemical processes, and it is only true in an autocatalytic process if the products of the reaction, other than the autocatalyst itself, are removed as rapidly as they are formed. In a living organism, of which the parts remain contiguous, this removal does not occur and hence, as growth proceeds, the rate of growth must depart more and more widely from direct proportionality to the mass of the product. In fact the ratio $\frac{dx}{dt} \cdot \frac{1}{x}$ is proportional to $K(a-x)$ and would therefore, if but a single cycle were concerned, decrease in proportion to an exponential function of the time, at first rapidly and later very slowly. The accompanying

figure (Fig. 26) shows how this function diminishes in an autocatalyzed reaction. Its resemblance to the curve obtained by Minot is obvious. What Minot's curve does reveal, therefore, is that the retardative effect of its products is exerted in the earliest stages of growth, but

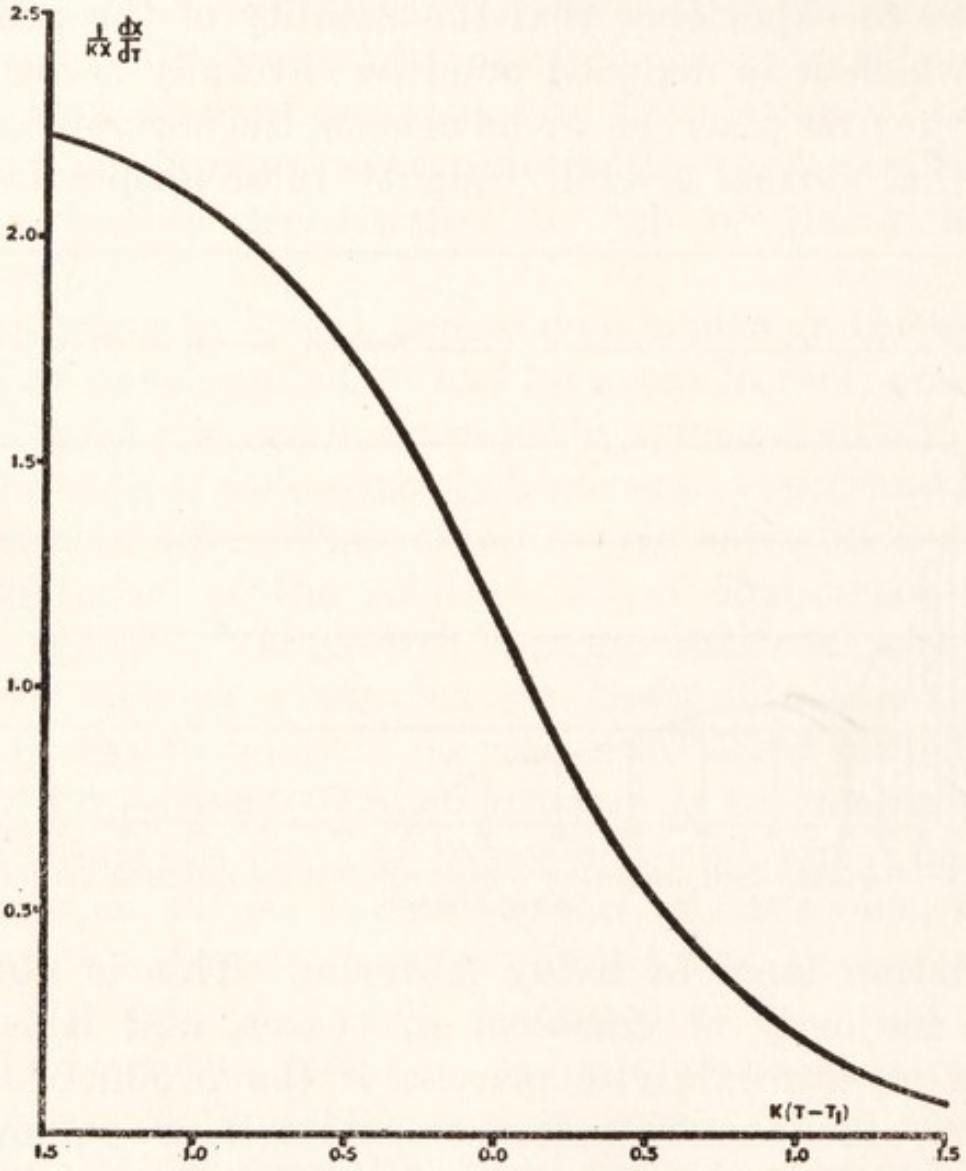


FIG. 26.—Ratio of velocity to extent of transformation at various periods in an autocatalyzed monomolecular reaction.

to call this senescence involves discarding the ordinary significance of the term which is better reserved for the phenomenon of progressively diminishing growth velocity which constitutes the autostatic phase of growth.

The mathematical basis of Minot's method of computing growth-rates has been criticised in the following words by D. W. Thompson[490] p. 72; "When we plot

actual length against time, we have a perfectly definite thing. When we differentiate this $\frac{L}{T}$ we have $\frac{dL}{dT}$ which is (of course) velocity; and from this, by a second differentiation, we obtain $\frac{d^2L}{dT^2}$, that is to say, the acceleration.

"But when you take percentages of y, you are determining $\frac{dy}{y}$, and when you plot this against dx, you have $\frac{dy}{dx} \cdot \frac{1}{y}$; that is to say, you are multiplying the thing you wish to represent by another quantity which is itself continually varying; and the result is that you are dealing with something very much less easily grasped by the mind than the original factors. Professor Minot is, of course, dealing with a perfectly legitimate function of x and y; and his method is practically tantamount to plotting log y against x, that is to say, the logarithm of the increment against time. This could only be defended and justified if it led to some simple result, for instance, if it gave us a straight line, or some other simpler curve than our usual curves of growth. As a matter of fact, it is manifest that it does nothing of the kind."

It was, of course, perfectly clearly realized by Minot that the **measurement** of senescence constitutes in itself no interpretation of its **origin**. He referred senescence, in fact, to an increasing differentiation of tissues leading to the acquirement of an over-proportion of cytoplasmic elements. Child, on the other hand, comes extremely close to anticipating the view of senescence about to be expressed, when he attributes the effects of differentiation to the comparative stability of differentiated tissue and the consequent absence of rejuvenescence which is the result of reproduction,[69] p. 269.

Before proceeding to the consideration of the view of senescence which is suggested by our present knowledge of the mechanism of growth, certain other theories of senescence must be briefly alluded to.

If we were to view "natural death" as the termination of existence due to the uniform senescent atrophy of all the structures of the organism, leading to a uniform depression and ultimately simultaneous extinction of all its functions, then "natural death" in all probability never occurs. In a world in which the organism could be completely shielded from fortuitous strains and the invasive activities of parasitic organisms, the phenomenon of "natural death" might conceivably occur. In actuality death is always in some measure accidental. Thus an aged individual contracts pneumonia and succumbs to its effects. Death is, in its immediate origin, attributable in this event to the invasion of parasites which are accidentally encountered. The same encounter, however, may have occurred repeatedly in the life of the individual, without his ever having been conscious even of indisposition. Why has the present encounter a different issue? Simply because the underlying senescence of his tissues has rendered them more vulnerable. His defensive mechanisms have been weakened, and had not this encounter decided the outcome, then another and possibly different encounter would have had a like result. Similarly when rupture of a cerebral vessel due to arteriosclerosis occurs, the existence of all parts of the body may be terminated by this "accidental" event, but its ultimate origin is the general senescence of the tissues, of which arteriosclerosis is merely one particular manifestation.

Salimbeni and Gery[437] have made a minute study of the tissues of a woman of ninety-three who died in consequence of an acute illness of only thirty-six hours duration. An unusual opportunity was then presented of ascertaining the condition of the organs of a very aged person without confusion by special injuries due to a protracted illness. They found a great number of abnormalities, including wide-spread sclerosis, leucocytic infiltrations, hypoplasia of the glandular tissues and calcification of the vessels, choroid plexuses and spinal cord.

The suprarenal glands were, however, hypertrophied. Any one of these abnormalities might have led, directly or indirectly, to the ultimate death of the subject, and while in any possible outcome death might be attributed to the particular lesion immediately responsible, yet in fact this would but have determined the mode of death, and mortality was actually ordained by the underlying senescence to which all of the lesions were ultimately attributable.

It is necessary to bear these facts in mind, for many hypotheses have been advanced, more particularly by clinicians, which attribute death in old age to particular lesions rather than to the underlying senescence from which these lesions have originated. As Child has stated,[69] p. 286: "So far as they have turned their attention to senescence, the anatomists, histologists and pathologists have often failed to recognize what the study of the lower organisms forces us to admit as a fact, *viz.*: that senescence is merely one aspect of development, and have confined their attention to, and based their theories upon, the morphological changes which occur in later life, and particularly in what we are accustomed to call old age."

Thus Muhlmann regards "physiological death" as attributable to the breakdown of nervous tissues, especially in the brain,[321,322] while Lorand[257] attributes physiological death to the failure of one or more of the organs of internal secretion, and Savill[438] refers it to the progressive loss of elasticity of the arteries, with or without renal complication, leading to hypertension and thus indirectly to the injury of many organs. But any part of a circle may, by similar reasoning, be made to appear the origin of the whole. Such hypotheses may be dismissed from consideration, for the reason that they offer only a specialized explanation of a perfectly general phenomenon which is displayed by all cell-communities which inhabit a restricted volume of nutrient medium. A phenomenon so

universal as senescence cannot admit of one explanation in the *Vertebrata* and a totally different explanation in the *Cœlenterata,* or in the higher plants. The phenomena to which senescence is ascribed by these authors must arise from some much more general underlying process, and although in the particular type (humanity) contemplated by the framers of these hypotheses, the factors enumerated by them may actually be responsible for the death of senescent individuals, yet they are not in themselves causes, but, on the contrary, consequences of senescence itself.

The hypothesis of Metchnikoff, however, calls for more especial consideration, because of the biological view of senescence which it implies.[301] Two phenomena especially impressed him in the senescence of the higher animals, namely, the arteriosclerosis which ultimately involves an important proportion of the blood vessels, and greatly injures their function, and in the second place, the leucocytic infiltration which his own discoveries have led us to interpret as an effort to remove decadent tissues. The latter he regarded as a necessary process, but its occurrence would manifestly be hastened by any circumstances injurious to the tissues, while arteriosclerosis is a condition the onset of which is notoriously hastened by many poisons, for example alcohol, or the toxins of syphilis. He was thus led to look for an origin of poisons within the bodies of the higher organisms, and the large intestine naturally suggested itself, being a seat of putrefactive changes which result in the production of substances of known toxicity, such as phenol and indole, which are excreted in the urine and must therefore be absorbed into the blood stream. He was under the impression, which recent results of Koessler have shown may have been mistaken, [169,220] that the substances produced during fermentation by acidophilous bacteria are less harmful than those produced during putrefaction, and thus he recommended the ingestion of milk-souring bacteria as a means

of implanting acidophilous bacteria in the large intestine and diminishing putrefaction. Results of unquestionable value have attended this procedure in certain cases, but the precise origin of the remedial effect in those cases in which it has occurred is still very much in doubt. There is little reason to believe that the flora of the intestine can be substantially modified in this way,[d] and still less for believing that the ultimate result of such a modification would be desirable.[182]

This suggestion of Metchnikoff has been widely interpreted by the general public as a proposal aiming towards the prevention of senescence, but, actually, its intention was only to delay senescence which, he believed, occurs prematurely in man and many animals owing to their possession of a capacious large intestine in which putrefaction is extensive. Natural senescence he appears to have regarded as an essential outcome of life, and his view of natural death does not appear to have differed very widely from that of Weismann, who considered that the limitation of life-duration is an adaptation which has arisen by the action of natural selection, because continued life of the individual after the reproductive period is a "senseless luxury" for the species. In other words he did not attempt to interpret the mechanism of natural senescence but simply to account for what he deemed its premature occurrence in man, before he had run the full course of his "orthobiosis."[302] The extreme longevity of man, in comparison with other mammals, affords us little ground, however, for crediting the view that our senescence is unduly hastened.

Of recent years much has been heard concerning the "rejuvenescence" which follows the hypertrophy of the interstitial tissue of the testes[e] consequent upon suppression of the gametogenic function by ligation of the sper-

[d] Unless aided by other dietetic procedures, such as ingestion of indigestible carbohydrates.

[e] The evidence of similar hypertrophy of female interstitial tissue is much less extensive.

matic ducts. A careful scrutiny of the data presented by Steinach, however, indicates no evidence whatever of rejuvenescence in animals so treated, in the sense of delay or overcoming of senescence.[472] In fact the author of the discovery himself advances no such claim. In the one instance in which the duration of life of an operated rat is specifically mentioned it did not exceed that of other, untreated animals. The effect appears rather to be of a stimulative character, possibly an acceleration of metabolism consequent upon synchronous stimulation of several endocrine organs, and it may very well be questioned whether such stimulation, in a senescent and exhausted organism, may not rather be a disadvantage than otherwise. The evidence upon which Steinach appears chiefly to depend is a reawakening of the sexual instinct, a form of "rejuvenation" which is far from desirable in the aged. As might be expected, the outcome of the operation, in some instances, has been disastrous.[299]

In the section of this chapter dealing with the retardative influences in growth (Section 1) it has been pointed out that the nutrient level in the medium inhabited by the cells of the higher metazoa is approximately constant from birth to old age. For communities of physiologically similar cells, therefore, the availability of nutrients does not alter with the age of the community, and an adult or non-multiplying cell-community does not cease to produce new cells because the old cells are receiving insufficient nourishment to support the nuclear synthesis which occurs in a younger community. Nevertheless, the nutrient level does indirectly determine the adult dimensions of a cell community, simply because, at a given nutrient level or value of a, the velocity of the forward reaction of nuclear synthesis does not sufficiently exceed that of the reverse reaction to enable the critical mass of the particular nuclear constituent which determines cell division to be attained. Physiological differentiation, or step-by-step lowering of the nuclear ratio,

renders nutrients available for nuclear synthesis which are useless to cells possessing higher nuclear-cytoplasmic ratios, because in them nuclear synthesis is inhibited by their inclusion of autocatalyst. Thus in competition for place and nutriment the ontogenetically younger cells have an overwhelming advantage over the ontogenetically older cells.

The handicap thus suffered by the ontogenetically older cells may operate to bring about their destruction in several ways. In the first place all living tissues are doubtless subject to injury from time to time in the performance of their functions or in the course of resistance to disease. This injury is repaired by regeneration, that is by reproduction so far as possible of the cells which have undergone destruction. Now the reconstruction of cells of high nuclear value, after extensive differentiation of the organism has occurred and a high concentration of autocatalyst has accumulated in the pericellular fluids, may become impossible, owing to inability of the cells to attain the nuclear ratio necessary to constitute their type. The injured cells, if they are replaced at all, must be replaced by cells of a more differentiated type which may not be able to discharge the functions of the cells which they have displaced. Such a change would not proceed far in any organ of fundamental physiological importance before it would indirectly lead to the injury of cells in other tissues, owing to the mutual interdependence of all the tissues of the body. This secondary injury would for the same reason be repaired in such fashion as to involve, probably, some further imperfection of function, and so the impairment of the whole organism would proceed at an accelerated rate. It is, indeed, a matter of common observation in Pathology that injury to tissues tends to result in their replacement by more differentiated tissue, and the impairment of function which is thus occasioned is very frequently one of the most serious consequences of disease. Hence the analogy which

Metchnikoff drew between the effect of poisons upon tissues and the effect of senescence itself, was a perfectly valid one, and the changes imposed upon tissue by the repair of damages which have been inflicted by the toxins of disease constitute in fact an accelerated senescence. It is only a matter of doubt whether toxins of intestinal origin play so predominant a part in this process as Metchnikoff supposed.

A more sustained effect of differentiation upon the community is, however, that which arises from the relative reduction of nutrient level for ontogenetically older cells which is brought about by the proliferation of more differentiated tissues. This effect may be more easily pictured if we imagine a community of physiologically similar cells, as for example a culture of infusoria, inhabiting a culture medium into which a flow of nutrients occurs, so as to maintain a certain nutrient level a. Nuclear synthesis is proceeding in each cell and is tending towards the equilibrium expressed in the formula:—

$$x_{ex} + x_{end} = \frac{k_1}{k_2} a \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (58)$$

in which x_{ex} is employed to designate the proportion of the nuclear autocatalyst included within the nucleus at the moment of the preceding division, and x_{end} that which has been subsequently synthesized. Just before this equilibrium has been attained we may suppose that one cell acquires the ability to divide at the nuclear ratio which has by then been attained, while the other cells of the community remain unable to divide. The dividing cell is "rejuvenated" by its division, that is, it discharges its accumulation of autocatalyst and reverts to an earlier phase of the autocatalytic curve. Nuclear synthesis proceeds more rapidly and the lower nuclear ratio which is requisite for the division of this type is repeatedly attained. An energetic nuclear synthesis is thus inaugurated in a community in which it was formerly occurring at a much retarded velocity.

Meanwhile the inflow of nutrients is maintained at a steady level by the mechanism which we have assumed[f]. But the population of the culture, prior to the appearance of the new type, represented (or was approaching) the maximum attainable nuclear synthesis for the given nutrient level. The inflow of nutrients which sufficed to maintain this equilibrium is now in part deflected to maintain a more rapid synthesis in other cells, and the former nuclear equilibrium can no longer be upheld. The accumulated autocatalyst in the older type of cell forces the reversion of nuclear synthesis until the velocity of the reverse reaction falls again to equality with the forward reaction, now diminished in velocity by the relative fall of nutrient level. A new nuclear equilibrium is established in the old type of cell, approximating towards that which will ultimately be attained by the newer type of cell. The older type of cell has been compelled to reduce its nuclear ratio. If this reduction should be inconsistent with the maintenance of function the old type of cell would have to disappear and give place to the new.[g] In a multicellular organism, however, the old type of cell has assumed certain functions upon the execution of which the organism has come to depend. The decadence of these cells must therefore ultimately involve the mortality of the whole community.

We have in fact in senescence an outcome of overpopulation which exemplifies the law of Malthus in the cellular domain. The effect might be likened to the consequences of introducing into a country inhabited by a race whose standard of life is high, members of a rapidly-propagating race whose requirements are small. No matter what positive increase of nutrient inflow (*i.e.*, production of food) might occur, the new type of popula-

[f] This mechanism is represented in mammals by the reciprocal activities of the assimilative, endocrine and excretory organs.

[g] Even if maintenance of their existence were still possible, it is readily conceivable that their functional efficiency might be seriously impaired by the inadequacy of their nuclear contents.

tion multiplying more rapidly than the old must inevitably increase until its rate of consumption equals the rate of production; but the rate of production will then be equalled to the consumption of the average, and the average is constituted now of a majority of the type which synthesizes protoplasm economically. The old type was not exclusively considered in establishing the rate of production. If it had been then the new type would have found nutrients in excess of its needs and multiplied until that excess no longer existed. But what is excess for the low-living type is inanition for the high-living type. Either the old type of individual must reduce its needs, or, if its functional constitution prohibits this, it must succumb and give place to the new.

The occurrence of a struggle for existence among the various cells and tissues of multicellular organisms has been especially emphasized by Roux,[431] but its importance in the interpretation of development appears in the first instance to have been indicated by G. H. Lewes,[229] p. 105. The application of this conception to the interpretation of senescence has previously been indicated by Mühlmann,[321,322] who has actually attributed senescence to the starvation of physiologically indispensable cells. The fact has hitherto been overlooked, however, that it is not actual deficiency of nutrients which determines the fate of the less differentiated cells, but the concurrent rise of autocatalyst level which renders their utilization for nuclear synthesis impossible. Thus in the midst of abundance of foodstuffs for the manufacture of nuclear constituents, and while such manufacture is actually proceeding in more differentiated cells, the less differentiated cells are actually diminishing their nuclear content through inability to maintain the rate of synthesis which is demanded by the excessive rate of reversion. This rate of reversion is never attained in the more differentiated cells because they undergo division first. Now there is only one ultimate equilibrium possible for all the cells in the organ-

ism, so long as we suppose that nutrients are accessible to them all and that the nutrient level is the same for them all, and that is the equilibrium expressed in the equation :–

$$x_{ex} + x_{end} = \frac{k_1}{k_2} a \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (59)$$

and the various tissues which are able to arrive at this equilibrium will differ from one another only in the relative proportions of x_{ex} and x_{end} which they contain.[h] This equilibrium must ultimately be determined by the most differentiated type of cell, which must obviously continue to multiply until an equilibrium is reached which lies below its own nuclear ratio at division. But this equilibrium may lie far below the nuclear ratio previously attained by ontogenetically older cells and these must undergo nuclear degeneration until the equilibrium now imposed upon them is attained. Starvation will affect the cells similarly but with this difference, that a much larger proportion of cell types is now undergoing degeneration simultaneously, and as the more differentiated tissues have less nuclear material to spare so also they may be expected to exhibit impairment of function more quickly than the cells which contain greater abundance of nuclear constituents. Even in starvation, however, the nutrient level is never zero[i] and hence cells possessed of very low nuclear ratios may actually multiply at the expense of the other tissues of the organism, as when a wound heals or regeneration occurs in a starving animal. That senescence may nevertheless be promoted by starvation in so far as it affects nuclear metabolism is illustrated by the fact that inanition accelerates the involution of an ontogenetically primitive tissue—the thymus[211,314] and also

[h] Specific differences of the ratio $\frac{k_1}{k_2}$ would of course permit a variety of simultaneous equilibria. Such may possibly occur in different species or individuals. Even if they occur in one and the same individual, however, the principles enunciated would obviously apply to each of the groups of equilibria thus constituted.

[i] The return of nutrients to the pericellular fluids by reversion of protoplasmic synthesis ensures this.

results in the production of hypotypical ovaries.[256] The usual effect of inanition, however, for reasons which will be discussed later (Chapter IX) is the reverse of the effect of accumulation of nuclear autocatalyst.

It has elsewhere been suggested that senescence in the metazoa is attributable to the appropriation of a progressively increasing share of foodstuffs by tissues of purely structural significance, the sclerous or supporting tissues.[409] These tissues, owing to their abnormal composition, are relatively expensive to maintain. In the manufacture of a molecule of the protein comprising connective tissue, several molecules of the more "normal" or parenchymatous type of protein must be sacrificed to provide the excess of one kind of amino-acid or another required for the synthesis of connective tissue protein. An excess of unutilizable amino-acids is at the same time rejected, and thus even a low rate of an anabolism or repair in such tissues may result in the consumption of a disproportionate share of nutrients.

While this hypothesis must doubtless embody a part of the truth, it suffers from the unsatisfactory feature that no reason is assigned for the progressive accumulation of sclerous tissues which is admittedly an accompaniment of old age. Moreover, there is some doubt whether it is applicable to all the various forms of life in which senescence actually occurs. The considerations which have been set forth above enable us to conceive a more general mechanism and therefore a more universal theory of senescence. In the competition for place and nourishment which occurs during the development and differentiation of the higher animals, the more highly differentiated tissues constantly tend to render conditions not only more unsuitable than ever for the multiplication of less differentiated tissues, but finally, through their contribution to the general reduction of the nuclear equilibrium, unsuitable even for their maintenance. So soon as the stage is reached at which conditions cease to be adequate

for the maintenance of some tissue which carries out an essential function for the community, the ultimate fate of that community is assured. In the light of this conception the accumulation of sclerous tissues with advancing age is seen to be merely a special instance of a general phenomenon, common to all cell communities which inhabit a restricted volume of nutrient medium.

In short, a community of cells inhabiting a circumscribed nutrient medium is continually struggling to accomplish the impossible task of adapting itself to conditions which by the very process of adaptation, are automatically rendered more and more unfit for the perpetuation of the community. Senescence is therefore the necessary outcome of differentiation.

By a very different route, we have thus been led to a theory of senescence which is practically identical with that which has been advanced by Child. According to this investigator "it is the repeated process of reproduction, rather than their low degree of differentiation which prevents progressive race senescence and death in the protozoa. Each division brings about some degree of rejuvenescence, which may balance the senescence during the interval between divisions,"[69] p. 442.

The effect of differentiation in promoting senescence is, as we have seen, due to the fact that it inhibits nuclear synthesis in less differentiated cells, and thus renders cell division impossible.

Evidently, if this be the case, then the more rapid the accretion of tissue late in development, and therefore of the highly differentiated type, the shorter will be the duration of life of the whole community. Now in the course of observations upon the growth and longevity of large numbers of white mice, upon which they have been engaged for a number of years, Robertson and Ray have found that "in any given group of animals, those which live longest represent a relatively stable subgroup, highly resistant to external disturbing factors, displaying

subnormal variability and a more or less marked, but not invariable, tendency to early overgrowth and relative paucity of tissue-accretion in late life. The short-lived animals, on the contrary, are relatively unstable, sensitive to external disturbing factors, supernormally variable, and, as a rule but not invariably, display relatively deficient early growth and a tendency to rapid but unstable accretions of tissue in late life."[422] The corre-

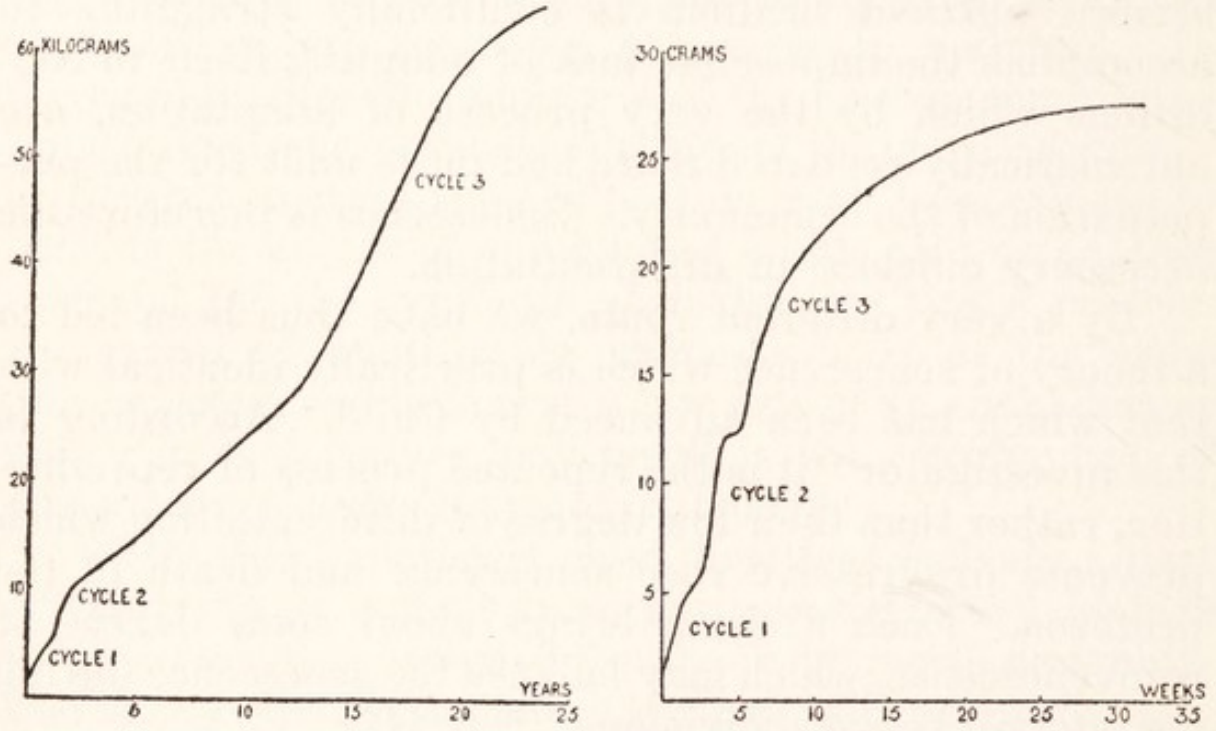


FIG. 27.—Comparison of the curve of growth of man (left) with that of the mouse (right).

spondence of these observations with the indications of the hypothesis outlined above is manifest.

Among the mammals, man is distinguished by his extraordinary longevity. His growth curve in comparison with those of other mammals also displays the peculiarity that the third growth-cycle is extremely slow to develop, its onset is greatly delayed, and when the time-units are adjusted to a like scale, the velocity of this last growth-cycle is greatly inferior in man to its velocity in, for example, the mouse,[409] p. 473 (see also Figure 27). This implies relative invariability of the cells composing our tissues, and hence delay of the appearance of highly differentiated types. The less differentiated cells possessed

of higher nuclear ratios are therefore granted a longer lease of life.

It has been shown by Child,[69] p. 281, that nervous (cellular) tissues have an exceptionally high metabolic rate, and the extreme sensitiveness of cerebral activity to lack of oxygen affords ample confirmation of his estimates. This, and the high degree of resistance to inanition which is displayed by nervous tissues, points to their possession of high nuclear ratios, and indeed the histological appearances bear direct testimony to this fact. The cellular elements of the nervous system are therefore but little differentiated in the sense in which we have employed the term, and they represent an ontogenetically primitive type[j]. Now Friedenthal has pointed out[140] that the ratio of brain weight to body weight, or to the two-thirds power of the body weight[k], which he terms the "cephalization-factor," varies from one species of animal or bird to another in extremely close correspondence with the maximal attainable duration of life. The following are among the figures which he cites in support of this thesis[l]:

The various estimates of maximal life-duration can only be regarded, excepting in the case of the mouse, as very hazardous approximations, since but little exact information is available even in the case of man, and, with the exception of the mouse, the data for other species are even more scanty. The mean duration of life would be a far better standard of comparison than the maximal duration of life, since the magnitude of the latter estimate may be so greatly affected by a single exceptional observation. On the other hand, statistical estimates of the

[j] The irreplaceability of nerve cells which have been destroyed by injury[38] further confirms this view.

[k] Friedenthal considers that the primarily functional elements of an animal, as contrasted to the constituents of primarily structural significance, vary as the two-thirds power of the body weight. This assumption received support from the investigations of Dreyer and co-workers.[100]

[l] The maximal life-duration of the mouse estimated from the statistical observations of Robertson and Ray, has been added to the table.

TABLE XLIII

MAMMALS

Species	Cephalization-factor	Maximal life-duration (according to Hausmann) in years
Man	2.67 to 2.81	80 to 150
Elephant	1.24 to 1.34	90 to 100
Anthropoid Apes	0.76 to 0.65	—
Horse	0.43 to 0.57	45
Deer	0.40 to 0.50	30
Bears	0.36 to 0.50	50
Dogs	0.34 to 0.51	15 to 20
Cats	0.29 to 0.34	20
Oxen, Giraffes, Antelopes	0.30 to 0.40	30
Squirrels	0.16 to 0.20	6
Insectivora	0.06 to 0.18	6 to 10
Mice	0.04	3

BIRDS

Species	Cephalization-factor	Maximal life-duration (according to Hausmann) in years
Carrion Crow	0.168	100 (?)
Parrots	0.147 to 0.177	100 (?)
Alpine Crow	0.114	50
Buzzard	0.11	—
Owl	0.113	—
Finch	0.086	8
Sparrow	0.086	—
Duck	0.0731	—
Snipe	0.0585	—
Quail	0.0495	—
Heron	0.0459	15
Pheasant	0.0343	15
Fowls	0.0249	10 to 20
Ostrich	0.0195	—

average duration of life are lacking, save for man and mice, and even the estimates for man which are available include accidental deaths and deaths from epidemic infections. However, notwithstanding the approximate character of the estimates, they afford clear evidence of a tendency for longevity to be associated with a high degree

of development of the nervous system. If the nervous system may be taken as indicative of the proportion of relatively primitive tissues in the body, these results lend further support to the view that the relative longevity of man is due to the comparative abundance of undifferentiated tissue which enters his composition.[m]

[m] This is not inconsistent with the view, which has been expressed elsewhere,[409] p. 518 that the nervous system may affect growth in a special fashion, namely, through the high proportion of cholesterol which it contains. The effect of cholesterol upon the growth of animals probably arises from delay or prevention of differentiation [418] and in this way the nervous system may indirectly promote a high proportion of undifferentiated tissues in the adult.

CHAPTER VIII

DIFFERENTIATION AND DEVELOPMENT

1. **Differentiation and the Partition of Nutrients.**—Reverting to the equations which were set forth in Chapter I, it is evident that the velocity of the forward reaction of nuclear synthesis is determined by three factors, namely, in the first place, the specific reaction-velocity of the process, k_1, in the second place, the concentration of the autocatalyst, which is proportional to x, and in the third place, the concentration of the raw materials or substrates of nuclear synthesis.

The reverse, or degenerative reaction, which, as in all chemical reactions, is proceeding simultaneously with the forward reaction, is determined by only two factors, namely, in the first place, the specific reaction-velocity k_2, and in the second place, the mass of the included nuclear catalyst.

The net rate of nuclear synthesis is the excess of the forward reaction-velocity over the reverse reaction-velocity, and this becomes zero, and nuclear synthesis ceases, when the product k_1ax becomes equal to the product k_2x^2. Now this will obviously be deferred, if the other factors are not simultaneously modified, by any increase in the value of a, that is, in the concentration of the available (nuclear) nutrients. The condition of multiplicative stasis, therefore, which is imposed by excess of autocatalyst, is relative to a given abundance of nutrients, and may be interrupted by their increase, or replaced by actual degeneration in consequence of their decrease.

The arrest of growth with the attainment of maximum population in a culture of unicellular organisms, or of adult dimensions in a higher animal, is not due to lack of sufficient nutriment to support nuclear synthesis, but it

is due to lack of the nutrients necessary to maintain such a velocity of synthesis as to overcome the high rate of degenerative change which is compelled by the accumulation of autocatalyst. At any prevailing "nutrient level" in the pericellular fluids, therefore, a definite nuclear equilibrium is ultimately attained, but at a different nutrient level this equilibrium is displaced. It is to this factor, no doubt, that we must attribute the increased dimensions of animals or man which occurs, up to a certain limit, in consequence of improved hygienic conditions or greater abundance or variety of the dietary (*vide* Chapter II). That a limit is set which may not be surpassed by the administration of any abundance of food is due to the fact that the reverse reaction of nuclear synthesis increases in velocity as the **square** of the concentration of the autocatalyst, whereas the velocity of the forward reaction only increases as the **first power** of the "nutrient level." Hence the effect of accumulating autocatalyst rapidly overwhelms the effect of increasing nutrient level and enforces a new condition of stasis in consequence of a relatively slight increase of bodily dimensions.

At any intermediate stage of development certain cells will have attained a condition of stasis, while others, in consequence of their lower nuclear ratios or "kernplasma-relations" are still enabled to sustain nuclear synthesis because they are still able to divide and shed their accumulations of autocatalyst into the pericellular medium. It must be recalled that every cell owes its content of autocatalyst to two sources. It has received an **exogenous** supply at the moment of the preceding division from the accumulations in the pericellular fluid.[a] This bears no necessary proportion to the mass of nuclear (chromatin) materials within the cell. It is a single, soluble and diffusible constituent of the nucleus which is distributable

[a] Or, what comes to the same thing, it has retained a proportion of its previously accumulated endogenous supply which corresponded to the exogenous "autocatalyst level" at the moment that the division occurred.

between the medium and the nucleus whenever the nuclear membrane is disrupted. The morphological elements of the nucleus may correspond to a higher or a lower autocatalyst concentration than that which prevails the moment after division has been accomplished. In the autokinetic phase of any growth-cycle the former of these alternatives will prevail, in the autostatic phase, the latter. Immediately after reformation of the nuclear membrane synthesis results in the formation of an endogenous supply of autocatalyst, which is retained within the nucleus and must bear some definite proportion, probably linear, to the mass of the morphological nuclear elements produced in the same time. If we designate these two fractions of the autocatalyst content by the symbols x_{ex} and x_{end} we see that the differential equation of autocatalysis within each individual nucleus must be within :—

$$\frac{dx}{dt} = k_1 a\,(x_{ex} + x_{end}) - k_2\,(x_{\epsilon} + x_{end})^2 \quad . \quad . \quad . \quad . \quad . \quad (60)$$

Equilibrium, *i.e.*, zero net velocity of this reaction, will occur when the positive and negative terms on the right hand side of this equation become equal to one another. Dividing by the common factor $(x_{ex} + x_{end})$ we see that this will occur when :—

$$x_{ex} + x_{end} = \frac{k_1}{k_2} a \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (61)$$

From this it is at once evident that if x_{ex} be large then x_{end} must be small and *vice versa*. Hence, if the accumulation of autocatalyst in the pericellular fluid is considerable only a small endogenous supply of autocatalyst can be produced before equilibrium is imposed upon the reaction of nuclear synthesis, and if this does not lead to cell division then no further multiplication of this type of tissue is possible. But if the nuclear mass (kernplasma-relation) necessary for division of this particular type of cell is below the equilibrium value, then the endogenous accumulation of autocatalyst can escape into the pericellular fluid so that renewed synthesis of nuclear material be-

comes possible. Cells possessed of low nuclear ratios will therefore continue to synthesize nucleus and cytoplasm in the presence of accumulations of autocatalyst which impose stasis upon less differentiated cells.

Now in any group of parallel reactions, that is of reactions which are occurring simultaneously and consuming the same raw materials, each substrate which enters into the reactions is shared between them in proportion to the velocity with which they occur. The various reactions proceed at their own independent rates, and if the quantity of materials available for transformation were unlimited, each reaction, or, in the present case, nuclear syntheses in each of the various types of tissue, would go forward at the same speed as it would if the other nuclear synthesis were not occurring simultaneously. Hence, those tissues in which nuclear synthesis is still proceeding rapidly, in consequence of their ability to divide, must consume the lion's share of the available nutrients and in consequence reduce the average nutrient level for tissues which are already adult. It is for this reason that tissue-repair will occur after injury even in a starving animal,[69] p. 156. Everything contributes to favor those tissues in which mitosis is still proceeding, and this effect must be exaggerated in proportion to the accumulation of autocatalyst and consequent increase of the velocity of nuclear catabolism.

It might be imagined that it would be a simple matter to establish this mode of origin of differentiation by the mere measurement of nuclear and cytoplasmic volumes at successive intervals of development, or in the various types of tissue produced. Except when the differences are very large, however, no information can be looked for from such measurements. As the investigations of Conklin have shown, the nucleus contains many constituents in variable proportion, and we do not know to which of these the occurrence of cell-division is attributable. Nor can we expect to be able to follow up the reduction of nuclear ratios by a quantitative measure of hereditary

(genetic) potentialities of the differentiated cells, if such were possible. We know that a marked reduction of nuclear ratios results in loss of hereditary potentialities, and we may safely assume that this loss is the usual consequence of every degree of differentiation, but we cannot assert that it is invariably so. There might, for example in the nucleus of the ovum, be an excess or duplication of certain genetic elements which might be dropped in subsequent differentiations without entailing absence of any of the genetic factors of the organism. A double dose of the factor which determines the presence of an hereditary quality in the offspring does not as a rule lead to a doubling of the quality. Up to a certain point, therefore, a cell containing reduplications of certain genetic factors might produce several successively differentiated generations of offspring before total loss of any genetic factor need occur.

2. **Differentiation and Metabolic Rate.**—As the more differentiated tissues in their turn become condemned to stasis, through the overtaking of the velocity of nuclear anabolism by the velocity of nuclear catabolism, their condition is substantially different from that of the ontogenetically older tissues. The older and less (physiologically) differentiated tissues were fixed at a time when the pericellular concentration of autocatalyst was relatively low, and in consequence of this the initial store of autocatalyst which they received at the moment of the last nuclear division was relatively small. At equilibrium, therefore, the endogenous proportion of autocatalyst must be relatively large and the nuclear mass correspondingly so. At the same time, however, these cells were characterized by inability to divide until the nuclear mass had attained a high level,[b] and failure to divide again was imposed by the fact that this level could not be attained

[b] That is to say, the mass of that constituent which determines the moment of division. As we have seen in Chapter IV, general considerations indicate that, at the moment immediately preceding or succeeding division, this must usually be proportionate to the morphological nuclear mass.

before the velocity of reversion overtook that of synthesis. At a high nuclear level, therefore, these cells remain, approximating towards, but not attaining the critical "kernplasma ratio" at which they would divide.

The ontogenetically younger cells are stabilized at a time when the pericellular concentration of autocatalyst is relatively high, and their initial store of autocatalyst is correspondingly large, their endogenous proportion correspondingly small. By so much the sooner, therefore, is the kernplasma-relation attained, or, failing that, stasis imposed by the accelerated velocity of nuclear reversion. These cells are characterized by their ability to divide at a relatively low nuclear mass, and when developmental stasis is imposed by their failure to attain even this nuclear level, it is evident that the level which is actually attained must be below that which was attained by the nuclei of the ontogenetically older tissues.

Hence, as one tissue after another becomes stabilized during the process of autocatalyst accumulation which limits the dimensions of every cellular community, it bears the date of its stabilization upon the dynamic equilibria within the nuclei. These are set at a high level in the older cells, at a lower level in the younger cells, and the descent of level stands in proportion to their ontogenetic youthfulness.

If it were possible to correlate the dynamic equilibria in the nuclei with any of the measurable metabolic phenomena of the cell, then we should expect these to be serially ordered, also, in proportion to their age. If, for example, we were justified in assuming that a large nuclear ratio leads to a high rate of oxidation in the cell, then the metabolic rate of tissues should stand in inverse proportion to their ontogenetic youthfulness, and therefore, in general, to the degree of differentiation which they display.

Now this ontogenetic gradient of metabolic rates is precisely what Child has found to obtain and to char-

acterize the development of metazoa.[69] The effect is to leave in our tissues a trail which retraces in metabolic gradients the developmental history of our bodies. The ontogenetically older and less differentiated cells which bear within them greater suppressed potentialities of regeneration have high metabolic rates. The ontogenetically younger and more differentiated cells display lower metabolic rates. In organisms of simple structure this results in an axiate metabolic gradient, highest at the apical or head end, which is formed first, and descending posteriorly. In branched organisms metabolic gradients are found in each of the several axes and it may be inferred that similar gradients exist in the vertebrata, but that the primitive orientation is excessively deformed owing to the multiplicity of axes, and the variety of tissues involved in development. In certain situations, however, for example the intestine, the primitive orientation of metabolic gradients along anatomical axes still admits of recognition in the adult.[12,13]

The assumed proportionality between the oxidative activity of cells and the proportion of nuclear material[c] which they contain therefore leads us to infer the existence of developmental gradients of metabolism which have actually been observed. The question presents itself whether any more direct evidence exists in favor of this assumption, which, in fact, is far from being wholly gratuitous.

It has been shown by Loeb in animals[242] and by Osterhout in plants[360] that the nucleus is the seat of the most energetic oxidations in the cell. Moreover, the oxidative activity of the egg cell is greatly increased by fertilization,[248] possibly in consequence of the synthesis of nuclear materials which is thereby initiated. It has, indeed, been contended by Warburg[507,508] that the in-

[c] Of that specific kind which determines the moment of nuclear division and which appears usually to bear a relation to the chromatin constituents of the nucleus (see above).

creased energy of oxidation which succeeds fertilization is confined to the surface of the ovum. This conclusion he bases upon the following facts, (*a*) that sodium hydroxide does not penetrate the egg but gives rise to increased oxidation, while ammonium hydroxide, which does penetrate the egg, does not increase oxidation. (*b*) That certain narcotics, for example, phenylurethane, suppress nuclear division without diminishing the oxidative rate. (*c*) And that polyspermia accelerates the division-rate without increasing the rate of oxidation.[507] Loeb and Wasteneys, however, have shown that the concentration of sodium hydroxide which suffices to increase the oxidations of the egg, is so toxic as to altogether inhibit subsequent development, and that non-injurious concentrations of all bases are without demonstrable effect upon the rate of oxidation.[246,249] The action of narcotics, or the occurrence of polyspermia, also involves very fundamental interference with the physiological economy of the cell which might readily lead to aberrations of the mechanism of nuclear division. Under such circumstances nuclear division-rate may not bear its normal relation to nuclear synthesis, and no evidence is therefore afforded by Warburg's experiments to the effect that nuclear synthesis may occur without increase of oxidations.

On the other hand there is much evidence to indicate that the manufacture of nucleic acid itself is an oxidative synthesis.[238] If any increase of nucleic acid occurs within the nucleus itself during the interdivisional periods, then the necessary phosphorus must be derived from phospholipins, or other organic compounds of phosphorus, since phosphates have been shown by Macallum to be absent from the nucleus.[263] Now, the investigations of Miescher[303] have shown that the energetic synthesis of nuclear materials which occurs during spermatogenesis in the salmon, involves a consumption of phospholipins, presumably to provide the phosphorus required for the synthesis of nucleic acid. If the carbohydrate radicle

of nucleic acid is similarly derived from phospholipins, then extensive oxidation of fatty acid radicles must necessarily accompany the process. In agreement with this view the investigations of Plimmer and Scott[378] and Tangl and von Mituch[487] have shown that the lipins in the yolk of a hen's egg are the source of the energy which is consumed in building up the tissues of the embryo.

But, on the other hand, Bohr and Hasselbalch have shown that the energy of development in mammals is derived from carbohydrates.[37] A possible solution of this apparent contradiction is indicated by the fact that in the early stages of the development of certain forms, particularly the silkworm[491] and the salamander,[165] lipins are actually synthesized from carbohydrates or from glucoproteins, the phosphoric acid component being derived from phosphates contained in the cytoplasm.[364,365] The abundance of phospholipins in embryonic tissues is commented upon by all observers, and their significance in relation to nuclear synthesis can hardly be questioned, but the possibility is indicated by the experiments of Tichomiroff and Gortner that one part of the cell may be engaged in performing the synthesis of lipins from carbohydrates for subsequent or simultaneous employment by another part of the cell–the nucleus–as a substrate for nuclear synthesis. We cannot, in fact, differentiate clearly between the activities which underlie synthesis of cytoplasm and those which accompany the synthesis of nuclear materials. It has, however, been shown that the lecithin content of fertilized sea-urchin eggs diminishes progressively during the earliest stages of development, when nuclear volume is increasing rapidly and cytoplasmic volume but little,[425] while Paul and Sharpe[368] have shown that during the period leading up to moulting in *Crustacea,* while mitoses are occurring rapidly without any increase in the size of the animals, the phospholipin content decreases progressively and to a very marked extent.

Quite apart, however, from the question of the mode of origin of the carbohydrate radicle, the purine and pyrimidine bases in nucleic acid, are probably derived from constituent amino-acids of protein,[d] or from the nitrogenous bases of the phospholipin, by processes which in either alternative must involve oxidation. The essentiality of oxygen for development has repeatedly been demonstrated.[239]

While the nucleus is not necessarily, or even probably, the only site of oxidations in the cell, it is evidently the seat of an important proportion of them and, as Osterhout has shown in his investigations cited above, the nuclear oxidations are more energetic and intense than those which occur elsewhere. Dependence of total oxidative activity upon the proportion of nuclear materials in a cell is therefore probable, and as kernplasma ratios diminish so might we expect oxidative activity to sink. There is no need to assume that the autocatalyst of nuclear synthesis is actually an oxidizing agent. We have seen that the endogenous fraction of the nuclear autocatalyst, although possibly but a minute part of the nucleus, nevertheless stands in approximate proportion to the total mass of nuclear (or at least chromatin) material. This is implied in the fact that as the chromatin material decreases so also does the potentiality of further differentiation.[523] Other constituents of the nucleus may, and probably do, stand in similar proportion to the whole, and therefore also to the endogenous portion of the autocatalyst. An energetic oxidizing enzyme is undoubtedly one of the essential constituents of the nucleus. Its general proportionality to the nuclear cytoplasmic ratio is therefore to be anticipated.

A leading feature of development is the progressive decrease of metabolic activity which attends it.[69,262,271] From maturity to old age the calorific output also steadily

[d] *Vide* the importance of arginine and histidine in the synthesis of purines, Chapter V. See also Burian and Schur.[51]

diminishes, the total reduction of output per square metre of surface being, according to Du Bois[109] about thirteen per cent. by eighty years of age in men. In the light of the above considerations this must be regarded as the necessary consequence of progressive differentiation and diminution of kernplasma ratios. Collectively, therefore, the organism as a whole displays in time the phenomenon which is evidenced by its axially oriented tissues in space, namely, the inferior metabolic rate of the ontogenetically youthful and therefore more highly differentiated cells.

3. Regeneration and Development.—The power of regenerating lost parts is possessed in some measure by all living organisms. It may be confined to restoration of continuity of surface, as in the repair of a superficial lesion in a higher animal, or the repair of the lesion created by removal of a limb without restoration of the skeletal structures, or it may extend so far as to permit the regeneration of the whole animal from a mere fragment, as in the case of *Planaria*.

A phenomenon so universally characteristic of living matter must arise from some fundamental property of life. It must be implied in the mechanism which maintains and induces multiplication of the cell. Any adequate theory of growth, therefore, must embrace the phenomenon of regeneration not merely as an accident, but as an essential consequence of the mechanism which induces growth.

It was this difficulty which confronted Weismann in the application of his hypothesis of the germ-plasm control of inheritance and development. His view, which in its most essential aspects has received very strong support from the results of modern genetic investigation,[313] was that the nuclear chromatin of the ovum contains a vast number, corresponding to the ultimate number of cells in the organism, of irreducible fragments or "determinants" which become sorted out into smaller and smaller parcels at each successive cell division "un-

til finally only one kind of determinant is contained in the cell, *viz.:* that which has to determine it,"[513] p. 63. This marks the culmination of development, and the phenomenon of regeneration, resulting in the production, in some instances, of a vast variety of cell-types from cells presumed to contain the determinant of but one cell or type of cell constituted a formidable impeachment of the hypothesis. Weismann's attempt to overcome this difficulty betrayed a fundamental weakness of the hypothesis, and, had it but been interpreted aright, would have pointed the way to the modification which was requisite to make his hypothesis acceptable. He postulated the highly artificial assumption that the power of regeneration, notwithstanding the universality of its appearance, is actually a secondary adaptation, produced by selection, in the particular part of the body which is most subject to injury or loss (*loc. cit.*, Chapter II). The cells composing these tissues were supposed to inherit, not merely the determinants governing their form, but also a special parcel of determinants which is employed only in the case of need for the purposes of regeneration. One would have to suppose, in consequence, that every cell of *Planaria* contains an almost complete parcel of determinants, since an extremely small fragment is able to produce the whole animal,[e] and if this be granted then the validity or necessity of the former assumption falls under very grave suspicion. For if, in these instances, the cell is able to carry along with it a parcel of determinants representative of almost every cell-type in the body and yet ceases to multiply when the adult structure and dimensions have been attained, why, then, should not this be the case in other organisms? The very fact, that adult dimensions are approximately uniform, which led to the assumption that they are attained when the determinants are finally sorted

[e] There is actually a lower limit of size below which pieces are unable to regenerate the whole animal, but this limit is so low and the situation of the piece which may regenerate the whole is so varied that the argument is obviously valid.

out into individual cells, is seen not to be inconsistent with the fact that these cells after all contain a great multiplicity of determinants which only become effective under specific conditions attending injury. Lack of regenerative ability in other instances may therefore be just as readily attributed to absence of the conditions requisite to excite regeneration, as to variations of the ability of the cells to regenerate, consequent upon absence of the necessary determinants.[f]

Of course it might be argued, and this was in fact implied in Weismann's hypothesis, that the regenerative parcel of determinants is treated quite differently from the normally morphogenetic parcel, being handed on unaltered from cell to cell, while the morphogenetic parcel is undergoing devolution into smaller and smaller parcels until the irreducible fragment of germ-plasm is attained and development ceases. Only injury and the necessity for repair will open the regenerative parcel. But if this were so then the actual phenomena of regeneration would become more difficult than ever to interpret, for as Pflüger has pointed out[373] we would then expect every injury of whatsoever extent to result in the reproduction of the whole organ injured. For example, if the leg of a salamander is cut off at any level, the parts which have been lost are regenerated.[458] If the whole leg has been removed it is the whole leg which is restored, and if but a part of the leg be removed that part only is regenerated. But if the regenerative parcel of determinants is handed on unaltered from cell to cell in the original development of the leg, why does not the whole leg develop in response to each and every injury? We come back again to variations in the exciting causes of regeneration and then the proof of internal determination vanishes, or if, to save the hypothesis, we assume that the regenerative parcel

[f] Weismann's theory of regeneration has also been very severely criticized from another aspect. It has been conclusively demonstrated that the power of regeneration in any part bears no relationship to its liability to injury,[311] Chap. V.

of determinants dwindles *pari passu* with the developmental parcel, then why are its morphogenetic potentialities not exhausted in the process in the same way that the morphogenetic potentialities of the developmental parcel have been exhausted? And how shall we account for such a phenomenon as the regeneration of an antenna in the situation of an eye,[179] or the production of a lens from an iris?[526]

Difficulties of this character have led to the general abandonment of Weismann's hypothesis in the form which it assumed in the hands of its author. Nevertheless, the impression daily grows stronger and more broadly founded upon fact, that an essential verity underlay the hypothesis. Modern genetic investigation attributes more and more definitely to the nucleus of the fertilized egg the totality of the characteristics of the offspring. Originally resting upon no more extensive foundation than the disproportionality of nucleus to cytoplasm in the spermatozoön, coupled with the observed fact that male and female parents are equally potent in the transmissal of hereditary characters to the offspring, this generalization is now established so securely that we are enabled to map the individual chromosomes comprising the nuclei of the germ-cells into areas which convey the separate characteristics of the individuals which are destined to develop from them.[313] We are led to suspect that the difficulties encountered by Weismann's hypothesis had their origin in some invalid assumption which was superadded to a perfectly valid principle.

It is a fact of capital importance in this connection that although regenerative ability varies greatly in different species, the regenerative power of the tissues of any given species diminishes with their differentiation. In other words, the more highly differentiated the tissue the less is the variety of tissues to which it can give rise when injury calls forth repair. Thus the early blastomeres of the developing egg are individually capable, when

separated from one another, of giving rise to the whole embryo,[105,432] although had they remained in normal juxtaposition with the others they would only have given rise each to a fraction of the embryo corresponding to their position in the cluster. The period of development at which this ability is lost varies in different species. Thus in the sea-urchin it is retained until the 8- or even 16-cell stage of development,[104,237] in *Amphioxus* it is retained only until the 4-cell stage.[522] It is present for one or two cell divisions in the blastomeres of a great variety of forms,[311] pp. 236 and ff., and it is probable that the production of "identical" twins by higher animals is attributable to the separation of the first two cleavage cells with the production of a complete embryo from each.[240]

Proceeding further in the ontogenetic history of the organism, bisected sea-urchin blastulæ also possess the power of producing whole embryos, although one-half of the great number of cells produced by subdivision of the ovum has been removed from each of the regenerating fragments.[101] Once gastrulation has begun, however, the bisected embryo is no longer able to produce the whole unless the archenteron is included, and even in this latter case, if the terminal pouches have been removed by the section the transected archenteron heals over, but new pouches are not produced (Fig. 28). With each successive stage of differentiation the regenerative potentialities of the tissues become more restricted. **There is thus a gradient of regenerative abilities which corresponds in its distribution in time to the gradient of kernplasma-relations which descends from the fertilized ovum to the fully adult organism.** In some cases this gradient is very flat, and the tissues of the adult retain extensive regenerative potentialities. In others it is steep and the adult retains only a restricted ability to replace lost parts.

Facts such as these lead to the conclusion thus summarized by Driesch that "There are more morphogenetic possibilities contained in each embryonic part

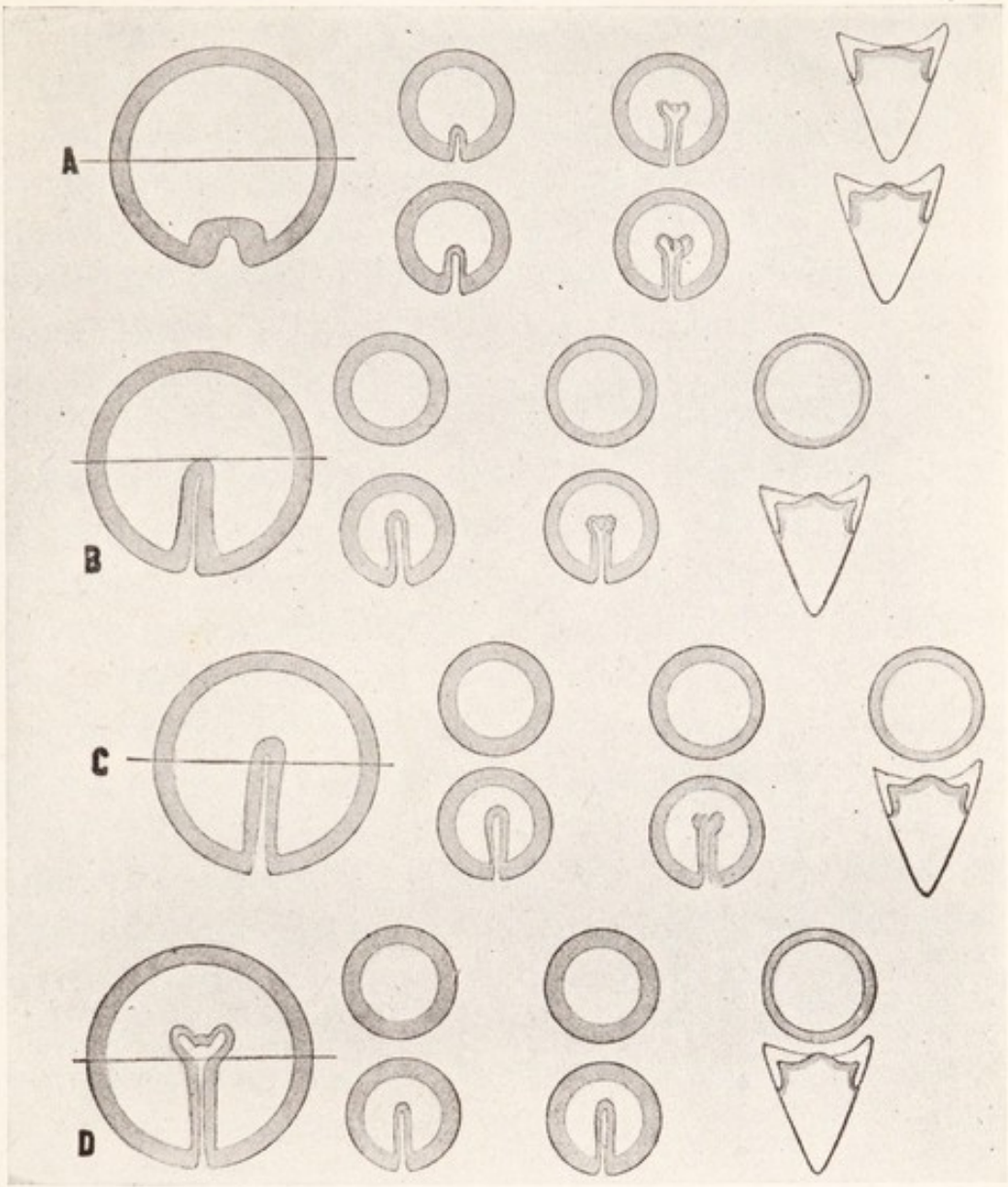


FIG. 28.—A. Blastula of sea-urchin beginning to gastrulate. Cut in half as indicated by line. Two rows of figures to right show development of upper and lower halves. B. Later gastrula cut in half. Two rows of figures to right show later development. C. End of gastrulation process. Embryo cut in half. Two rows of figures to right show later stages of each half. D. Formation of endodermal pouches from inner end of archenteron. Embryo cut in two. Two rows of figures to right show later stages. (After Morgan.)

than are actually realized in a special morphogenetic case,"[105] p. 77, and of course they are inconsistent with the view of Weismann that the initially complete germ-plasm is sorted out into qualitatively different determining parts at each successive cell division. For a time all cells in the developing embryo remain equipotent, or "totipotent"[311] to produce the whole of the cells composing the organism, and although while ontogeny proceeds a reduction of this "totipotence" occurs, it appears, in the light of all the evidence now available, to be quantitative rather than qualitative.

Driesch has indeed assumed that these facts render impossible any physico-chemical interpretation of the phenomena of development and he assumes the existence of an "entelechy" or self-producing force which shapes the organism towards a preintended form notwithstanding injury or deformation. This force would appear to be dissipated by its own performance in a manner strikingly similar to the dissipation of energy or the consumption of material in a chemical reaction, for each successive phase of development lessens, in his view, the latent possibilities of the resulting cells. This theory in its narrow application anticipated the wider theory of Bergson and shares in common with its successor the difficulty that while it rejects physico-chemical causation, it must utilize the nomenclature and conceptions of physics and chemistry to define the metaphysical entity which it postulates.

The real difficulty which has been responsible for leading us into this quandary has been the lack of any clear conception of the nature of the factors which determine the ultimate dimensions of an organism. Two separate problems were in fact attacked at once by Weismann because he supposed that they were inseparable, the problem, namely, of growth, and that of inheritance. If one and the same mechanism is responsible for both of these phenomena, then, he inferred, the process which determines the hereditary characters of an organism must

also be one which determines the cessation of its development. A mechanism must be devised which, like the wound-up mainspring of a clock, will "run down" when its round has been accomplished. If indivisible units determine the characteristics of cells, then cessation of development must occur when this indivisible unit has become isolated, and hence the parcelling out of the germplasm, a process which ends only when it has reached the ultimate stage of distribution at which each cell contains but one indivisible determinant.

But there was no necessity after all, to postulate this identity of the growth-limiting mechanism with the mechanism which determines inherited characters. It is true that the totality of inherited characters is only revealed in the adult, so that full development is essential to display them, because this is the only method we have of defining the characters of an organism. We cannot define or recognize those which it cannot display. Were growth not arrested we cannot say that other inherited characters might not appear, and, in fact, phenomena suggestive of such a possibility are not infrequently observed.[g]

We have supposed that the differentiation which results in the development of the varied tissues of the adult is accomplished by the step-by-step diminution of the ratio of the nuclear constituent which determines the moment of cell-division to the total volume of the cell at division. The new cell by virtue of its differing nuclear ratio reacts in a characteristically different manner from its progenitor to its environmental conditions and hence produces ultimately a different somatic unit. If the successive steps are small the variety of tissues will be great, if the steps are large, the variety of tissues will be small. Each cell is potentially able to reproduce itself indefinitely and

[g] For example, when senescence of the adult female enforces degenerative atrophy of the female generative organs, secondary sexual characters of the male frequently make their appearance. The administration of thyroid tissue induces metamorphoses in Axolotl which do not normally occur.

also all the types more differentiated than itself. It is not the indivisible unit of germ-plasm which has determined the character of the cell, but the residue after certain units have been abstracted.[h]

In Weismann's view "Ontogeny, or the development of the individual, depends.....on a series of gradual qualitative changes in the nuclear substance of the egg-cell." If we but substitute the word "quantitative" for "qualitative" in this statement we have an amended form of the hypothesis which is simultaneously reconcilable with the facts of development and those of regeneration.

A fundamental difficulty which confronted Weismann's hypothesis arose from the fact that nuclear division is almost always symmetrical, the two parts of the nucleus and its constituent chromosomes being substantially equal,[311] p. 256. Division occurs at differing nuclear volumes which decrease as development proceeds,[74] but the nuclear material existing at the moment of nuclear division is shared equally between the daughter-cells. At the end of development many cells remain possessed of very large nuclei, in fact these are probably the cells which attain developmental stasis first. If, therefore, the cell form is determined at the end of ontogeny by the presence of a single determinant, that determinant cannot by any means be identical with the chromatin substance of the nucleus. Weismann was, in fact, compelled to assume that the greater part of the nucleus is not involved in the transmission of hereditary characters. Yet the testimony of modern genetic investigation clearly points to the chromatin elements of the nucleus as the transmitters of hereditary characteristics. The hypothesis which we have outlined overcomes this difficulty. The residue of nuclear material at the conclusion of ontogeny carries with it all the potentialities which the cell could realize if it were not for the contiguity of other cells sharing the same fluid medium which condemns the cell to developmental stasis

[h] Through failure to reproduce them before cell-division occurred.

through inability to attain the nuclear ratio which is essential for division. Let this obstacle be removed, and, as the in vitro cultivation of tissues has demonstrated, virtually unlimited reproduction is possible.

So long as the nuclear mass remains constant or does not fall below a certain level at the moment of division, all of the cells share alike the potentialities of the parent-cell, and hence for a few divisions the blastomeres which are produced by cleavage of the egg display all the potentialities of the egg itself when they are isolated from the rest. So long as they remain in contact all the effects of mutual contiguity, namely mechanical stress, competition for nutrients and allelocatalytic effect combine to render the full realization of these potentialities impossible. At a later stage the potentialities of all the cells[i] are reduced, as the nuclear ratio is reduced, and production of the full embryo from a single cell is no longer possible. A group of these cells may, however, so far supplement each other's deficiencies as to enable production of a whole embryo from half a blastula. Physiological differentiation is as yet very slight and every cell contains nearly (but evidently not quite) a full complement of inheritance-bearing material. Directly a more decisive differentiation has occurred and invagination of the archenteron has proceeded a certain distance, the morphological equivalence of the different parts of the embryo has disappeared. The reduction of the nuclear ratio which is necessary to enable cell multiplication to continue, carries with it the penalty of lost potentiality. Not all of the hereditary character-determinants are still there, something is now lacking, and so, as with the unhappy owner of the *Peau de Chagrin,* the inevitable end approaches nearer with the satisfaction of every need.

The immense simplification which this view of development introduces into our conception of the mechanism of inheritance is manifest. Instead of a preformed archi-

[i] Or of the great majority.

tecture of determinants within the parent nucleus which reproduces in miniature the future structure of the organism, we have simply a number, which is not necessarily excessively great, of successive stages in the devolution of the nuclear ratio, each of which corresponds to a particular type of tissue. Each type of tissue is capable of giving rise to its own type indefinitely, but its capacity of self-propagation is limited by the allelocatalytic effect of the community. Each type of tissue carries also its own degree of instability of the nuclear ratio, and as a fresh downward step of the nuclear ratio is achieved, a fresh outburst of growth of the corresponding tissue will occur. The inheritance of a single additional step in this devolution or the acceleration or delay of any existing step will result in the appearance or disappearance of some corresponding characters of the adult, and the earlier in development such a change occurs the more profound will be its ultimate effect.

In a word, the germ-plasm has but to propagate itself in one cell after another, as it obviously does, and the mechanism which determines the moment of division will accomplish its devolution. The imperfect germ-plasm which remains can but perpetuate its kind and hence the next succeeding reduction of the nuclear ratio must accomplish a further stage of its devolution. The multiplication of each of the successive types of tissue thus produced is delimited by the allelocatalytic effect of the community.

Thus we inherit, not specific characters, but potentialities. The sum of the characters of the adult is a compromise between the diverse potentialities of its parts. Any alteration of the environmental factors may upset this compromise and give to this or that tissue an undue advantage in the outcome. It is to this that we must doubtless attribute the production of monstrosities through an alteration of the chemical environment in which development is occurring[478] and the regeneration of a diversity of

head-forms in *Planaria* when decapitated animals are acclimatized to conditions which cause "differential inhibition" of the growth of certain tissues.[70]

4. **Polarity in Morphogenesis.**—The vast majority of animals display a striking degree of symmetry, which is either radial or bilateral, and both plants and animals display polarity, that is to say a tendency to develop particular structures at one end, and other, quite different structures at the opposite end. The leaves and roots of plants, tentacles and stolons in hydroid polyps, head and tail in worms: these are illustrative of the same fundamental tendency.

This polarity is not merely exhibited by the animal as a whole but also in its parts. Thus, a planarian deprived of head and tail by transection at either end regenerates a head at the anterior end, and a tail at its posterior end, and this order is never reversed.[311] In some instances polarity is obviously determined by gravity,[436] but in the majority of instances in animals gravity cannot be the operative factor.

The attempt has been made to attribute the polarity of the adult to a fundamental polarity of protoplasm, starting with the egg.[102] Any such explanation is rendered invalid, however, by the heteromorphosis experiments of Loeb.[235,243] Furthermore, the cleavage planes of the early embryo may be displaced by compression, but the resultant individual displays normal polarity.[273]

A much more probable hypothesis is that proposed by Bonnet[39] and Sachs[436] and extensively applied to the interpretation of heteromorphosis by Loeb.[235] This hypothesis assumes the existence of formative materials or specific foodstuffs, each of which promotes the formation of a particular type of structure. Thus the growth of roots at the lower end of a cut stem of a plant is assumed to be due to a flow of root-forming substances to the base and of shoot-forming substances at the apex. The polarity may be reversed, after considerable delay, by inverting

the stem and the delay is attributable to the time necessary for the redistribution of the determining substances. If we suppose that the shoot-forming substance is specifically lighter than the root-forming substance we can

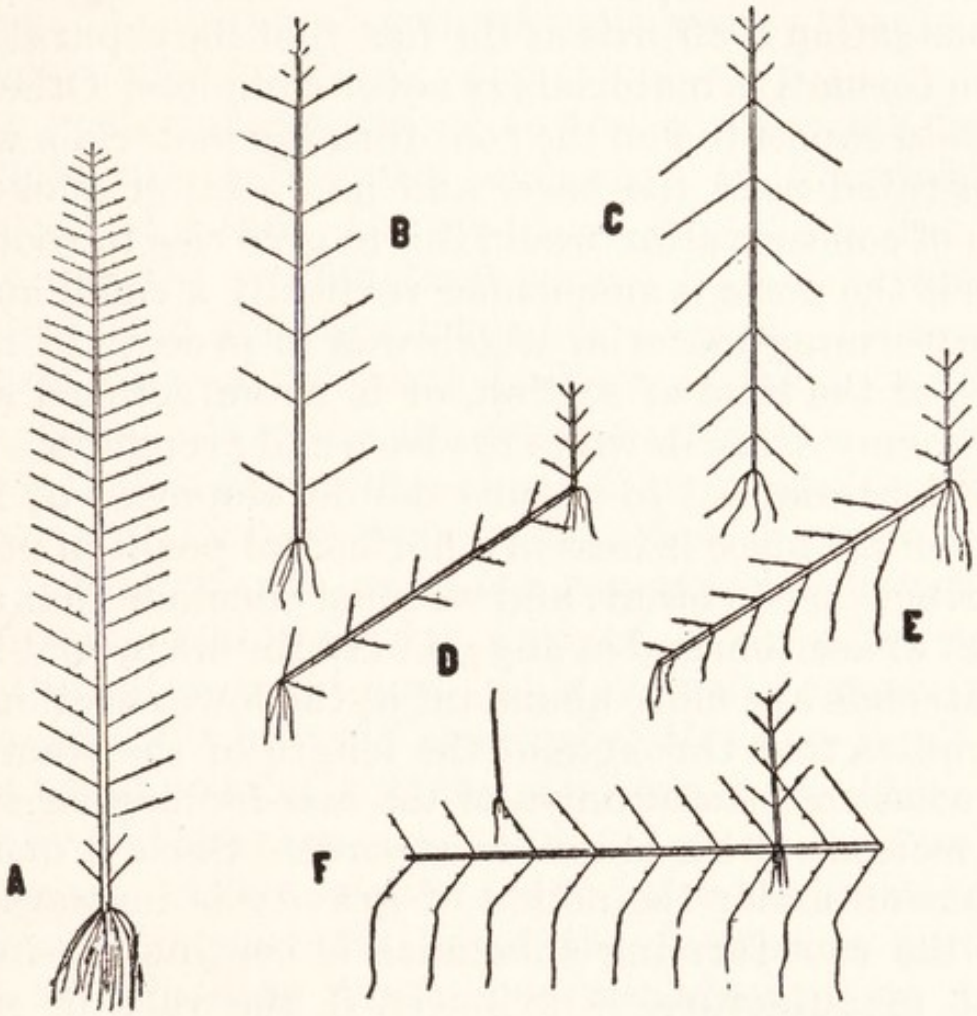


FIG. 29.—A. Normal stalk of *Antennularia antennina*. B. Piece regenerating in vertical, normal position. C. Piece regenerating in inverted position. D. Piece regenerating in inclined, vertical position. E. Piece regenerating in inclined, inverted position. F. Piece regenerating in horizontal position. (From Morgan, after Loeb.)

readily explain the polarity of plants and of such animals as *Antennularia* (Fig. 29).

The objection has been urged by Driesch[103] that when pieces of the stalk of *Antennularia* are placed horizontally, roots nevertheless form at what was previously the basal end. This observation, however, by no means conflicts with the hypothesis of formative materials which are influenced by gravity, for the horizontal position may be expected to exert no influence upon the longitudinal dis-

persal of the formative materials and their arrangement will therefore remain as it was at the moment of section, namely, root-forming materials in the lower and shoot-forming material in the upper part of the stem. The really significant aspect of this experiment is, however, the illustration it affords of the fact that the separation of the two formative materials is never complete. Otherwise we should expect to find the root-forming materials wholly concentrated near the base, and any section above this region of concentration would fail to give rise to roots. Of course if the piece is suspended vertically a small amount of root-forming material which was in process of manufacture at the time of section, or is manufactured subsequently, may drain down to the base and accumulate there in sufficient amount to ensure the development of roots. But when the piece is held in a horizontal position no such redistribution can occur, and we must conclude that at the moment of section, and at any point in the stem, root-forming materials are more abundant at the lower section, and this implies that throughout the length of the stem there is a gradient of concentration of the root-forming substance, which increases from above downwards. Such a mode of distribution under the action of gravity is inconceivable unless the root-forming substance is continually in process of manufacture. Obviously if the rate of manufacture were a little slower than the rate of settling by gravity the result of the drainage of the material from the apical end where it is undergoing manufacture would be to bring about just such a distribution or gradient as that which we have inferred.

There is, however, another difficulty confronting the theory of formative materials, and that is this:—When a stem is sectioned at any point the posterior cut-end of the anterior portion forms roots and the anterior cut-end of the posterior forms shoots. This is explicable where the distribution may be modified by gravity, as, for example, through drainage of root-forming materials away from

the anterior cut-end of the posterior piece, rendering the growth of roots impossible, while the root-forming materials are unable to escape from the posterior cut-end of the anterior portion. The problem assumes a different aspect, however, when, as in *Planaria,* the formation of anterior and posterior structures occurs while the pieces are in a horizontal position. The situation, then, is this:— At the posterior cut-end of the head piece tail-forming materials are sufficiently abundant to determine the formation of a tail. On the other side of the line which constitutes the section they cannot be appreciably less abundant, and yet here a head is formed. If we are to interpret this in the same fashion as the formation of roots and shoots in the cut stem of a plant, then we must suppose that in *Planaria,* as in all other animals exhibiting like polarity, there is a continuous flow of material from the front rearwards, or in the reverse direction, so that tail-forming materials are drained away, after section, from the anterior cut-end. It is difficult to conceive any mechanism which would accomplish this flow in all of the forms which exhibit polarity, and which would be in nowise affected by transverse section, **unless the flow is due to diffusion.** Again, we are led to infer the existence of a gradient of concentration of the formative materials, which is upheld by continuous manufacture on the one hand, and redistribution by diffusion, on the other.

But here another serious difficulty confronts us in the fact that diffusion only occurs from a region of high to one of low concentration. If tail-forming substances are continuously formed at the anterior end and redistributed towards the posterior end by diffusion, a diminishing gradient will be established, but the maximum concentration of tail-forming material will reside at the head-end. If the place of manufacture be the tail, and the flow is in the reverse direction, then the tail-forming materials will be increasing progressively at the anterior cut-end of the tail piece, which nevertheless forms a head, and tail-form-

ing materials during the same time will be progressively diminishing in concentration at the posterior cut-end of the head piece, which nevertheless forms a tail.

Let us now, without any preformed conceptions, consider what must be the nature of a growth-influencing substance which would bring about through its distribu-

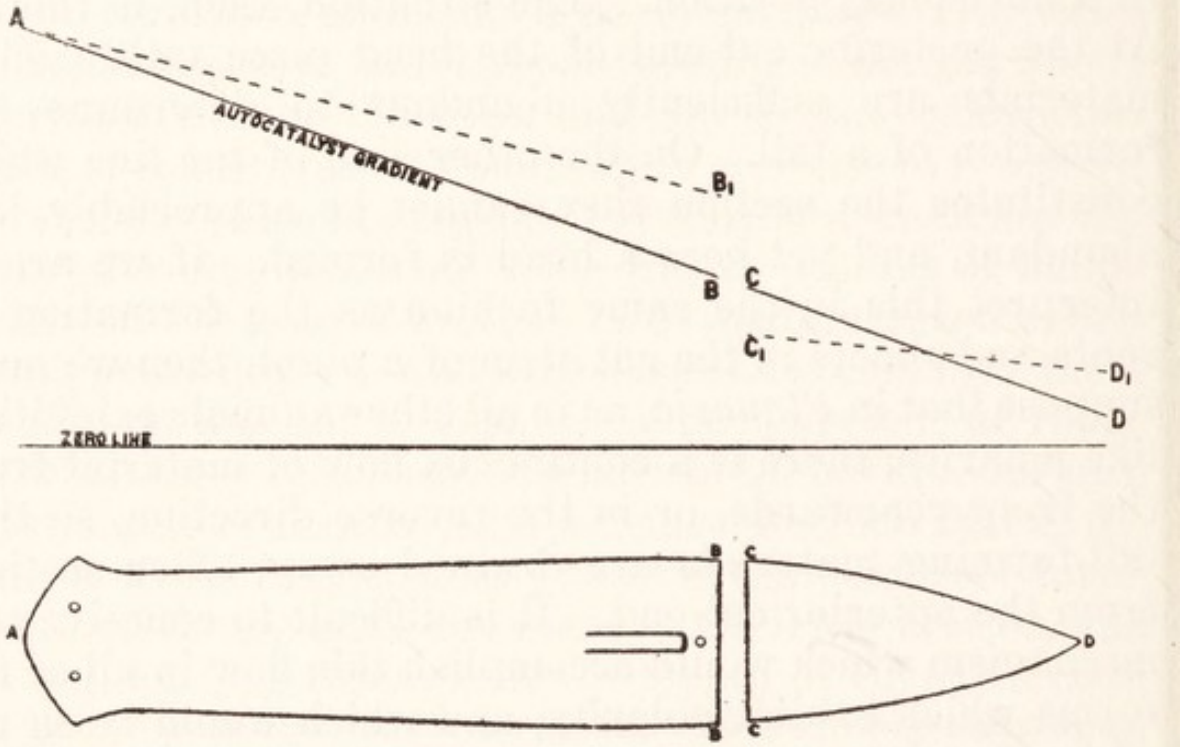


FIG. 30.—Showing the effect of section of a planarian upon the distribution of the autocatalyst of growth, originating chiefly at the head end. The full line indicates the normal distribution of autocatalyst; the dotted line, its distribution some time after section.

tion by diffusion, the experimental results which have been described.

In the accompanying figure (Fig. 30) let A – D represent a planarian of which A is the head end and D the posterior extremity. Consider that it has been transected, forming two cut-ends B – B and C – C. A substance capable of influencing growth in a way which has yet to be determined diffuses from the head end at which its concentration is maximal to the tail end at which its concentration is minimal. The gradient of concentrations is schematically represented by the lines *AB, CD*. The process of section has interrupted the continuity of the gradient, but the flow due to diffusion nevertheless continues in each

part. This will lead to a flattening of the gradient in the piece *CD*, indicated by the dotted line, and a consequent lowering of the concentration of the growth-influencing material at c – c. Simultaneously by continued production aided by diffusion, its concentration will rise at B – B. What is the experimental outcome? A tail grows at B – B and a head at c – c. If, therefore, the growth-influencing substance has any effect at all upon the growth of head tissue **this influence is inhibitory.** If the direction of the flow be conceived to be in the opposite direction, from tail to head, then the substance must be one which inhibits tail-growth. In either event the substance produced by any part which is capable of influencing regeneration in any other part, **is one which inhibits the growth of the part in which it is produced.**

That this is the true interpretation of the polarity which is displayed in regeneration has recently been demonstrated by Loeb.[245] The experiments were made on pieces of the stem of *Bryophyllum calycinum*. Each node of the stem of this plant has two leaves in an opposite position, and in the axil of each leaf there is a dormant bud capable of giving rise to a shoot. The line connecting two buds of one node is at right angles to the line connecting the two buds of the next node. The experiments are thus described by their author:—

"**Experiment i.** A piece of stem, containing six or more nodes is cut out from a plant, all the leaves are removed, and the piece is put into a horizontal position with the line connecting the two buds of the most apical node vertical. In this case both buds in the apical node may begin to grow, but as a rule only the upper bud will continue to grow, while the growth of the lower bud will soon stop altogether, or will be considerably retarded. None of the buds on the other nodes will grow out. Roots will grow chiefly on the underside of the stem, but in the last node and at the cut end they may form on the upper side as well as on the lower side of the stem.

"Experiment ii is the same as Experiment i, except that the upper apical bud is cut out. In this case the lower apical bud will grow rapidly, but in addition one or both of the buds of the node next to the apical will grow out. These buds never grow out where the upper apical bud is preserved and healthy.

"Experiment iii is the same as the previous experiment, except that the lower apical bud is removed, while the upper one is preserved. In this case the upper apical bud will grow out, but none of the others."

Loeb concludes that "all of these observations are intelligible if we assume that a bud which begins to grow produces and sends out inhibitory substances toward the base of the stem."[j] Further experiments showed that similar inhibitory effects are exerted by the leaves, while the growth of leaves actually promotes the growth of roots at the basal end.[244] Loeb further makes the exceedingly important suggestion that **the substance which inhibits shoot-formation may be identical with the substance which favors root-formation.**

Now we have seen that any community of cells in which nuclear synthesis has attained the autostatic phase the accumulation of the nuclear autocatalyst in the pericellular fluids progressively retards and finally suppresses nuclear synthesis. The same accumulation will, however, actually favor nuclear synthesis in cells which, by repeated divisions, are enabled to remain in the autokinetic phase. Referring to Equation 60, while x_{ex} is the same for both types of cell, x_{end} is large in the former kind and is kept small in the latter by repeated division. We know, furthermore, that the nuclear autocatalyst is diffusible. (From the existence of allelocatalytic effect.)

[j] It was also found that apical shoots will grow and inhibit the growth of lower shoots even after the lower shoots have begun growing, from which Loeb infers that the inhibitory substance is not distributed in an apical, but only in a basal direction. This is not inconsistent with our knowledge of diffusion in living organisms, which is frequently unidirectional, but the same effect could occur in another way, namely, by possession of lower kernplasma ratios by the cells of the more apical buds.

The nuclear autocatalyst therefore combines in itself all the properties necessary to bring about the effects observed by Loeb, and we have furthermore merely to assume the existence of varying degrees of differentiation in the cells composing the fragment of stem to understand how one and the same substance, namely, the nuclear autocatalyst, may inhibit the multiplication of one type of cell while actually promoting the multiplication of another.

This method of interpreting the phenomena obviates another difficulty which is encountered by the hypothesis of specific formative materials, and that is the great diversity of such agents which must otherwise be assumed.[311] As Morgan has pointed out, "In animals that regenerate laterally as well as anteriorly and posteriorly, we should be obliged to assume side-forming stuffs as well as head-forming and tail-forming stuffs; and since the kind of structures that are produced at the side are different at each level, we should be obliged to assume that there are many kinds of lateral stuffs." (*Loc. cit.*, p. 267.) Furthermore, each of these formative materials must flow in its specific direction and often in a contrary direction to the flow of other formative materials, so that for the polarity of the organism itself we have only substituted a polarity of circulations. The effects are explicable if we merely suppose the existence of a single growth-controlling substance, the nuclear autocatalyst, which inhibits or accelerates, on the one hand according to its own concentration, and on the other according to the nuclear ratio of the cells affected.

One illustration will suffice to show how the polarity of an animal may be established. We have seen in the preceding section that all of the cells composing sea-urchin blastulæ are equipotential in the sense that transection of the blastula in any meridian results in the reformation of a blastula, and the development of the whole embryo. But if the transection is not performed an invagination of cells

occurs at one pole, resulting in the formation of the archenteron, and when this invagination has proceeded a certain distance the parts of the gastrula are no longer equipotent. Regeneration of a whole embryo requires the presence of the archenteron.

Now to what is this invagination of cells actually attributable? Evidently to a more rapid multiplication of cells at this pole. If all of the cells composing a hollow sphere multiply with equal rapidity the sphere will merely become larger. If the cells at one part of the sphere multiply with decidedly disproportionate rapidity, the mass which results must either bulge or invaginate.[189] But, in terms of our hypothesis, superior multiplicative rate at given nutrient and autocatalyst levels implies a lower kernplasma-relation, so that these cells attain the critical nuclear ratio for division more rapidly than the other cells which compose the community. Up to this time any of the cells composing the blastula might possibly have been capable of giving rise to this new type of cell, or at all events any particular group of sufficient size would be likely to contain at least one cell capable of giving rise to this type. Even after invagination has begun another group may be separated from the blastula by transection which will still be capable of giving rise to an archenteron. But when the development of the archenteron has proceeded a certain distance, the situation is changed. The rapid production of new cells has resulted in a rise of the autocatalyst level[k] which delays the development of all the other cells in the community, through augmentation of the velocity of the reverse reaction of nuclear synthesis. So long as no divisions occur in the non-invaginating part of the blastula this effect will not be operative, but directly a division occurs the dividing cells will receive a sudden increase of exogenous autocatalyst which initially accelerates nuclear synthesis

[k] And also, possibly, a fall in the nutrient level, which would operate in the same sense.

but defers the attainment of their higher autocatalyst level. Hence a repetition of invagination cannot occur at other points in the blastula. If the non-invaginated part be now cut off, the delay of multiplication may be so great as to prevent sufficiently rapid development at any one area to cause invagination. Obviously the magnitude of the inhibitory effect will depend upon the space separating the kernplasma ratio of the two types of cells, upon the length of time that the autocatalyst has been accumulating, and upon the ability of the parent-cells to achieve the new type in a single step of nuclear devolution, or in a series of stages culminating in the typical archenteron-forming cells, and so the precise effect elicited will vary from one organism to another, but one characteristic will attach to all instances, and that is the existence of a gradient of autocatalyst concentrations due to the initial advantage gained by one of the formerly equipotent cells of the community. As to the causes which enable one or a few cells thus to gain the advantage, they may be so numerous that their combined effects are fortuitous in their incidence. But once the advantage is gained it is strengthened and perpetuated by the autocatalyst gradient, diminishing from the growing point outwards, which now becomes established.

As the gradient diminishes outwards its effect will vary, and in relatively elongate organisms may simultaneously result in the promotion of the growth of more distant cells which are yet in the autokinetic stage of autocatalysis, lack of any effect upon cells at an intermediate distance, and suppression of the development of the majority of cells in the immediate neighborhood. Each of these effects, in turn, may be simultaneously exhibited at a single level by cells possessed of differing nuclear ratios. Wherever promotion of growth occurs a fresh autocatalyst gradient will be established, exerting similar differential effects upon the growth of diverse tissues. Thus the animal assumes a multiple polarity, the gradients

of autocatalyst concentration being as numerous as the axes of growth, and the events happening in any part of the community exert an ultimate effect upon the development of the whole. We have, in fact, "a multiplicity of elements and the interpenetration of all by all."

5. **The Production of Gametes.**—The conception of the process of physiological differentiation which has been developed in the preceding pages, involves, as an essential outcome, the partial loss of the hereditary factors contained in the nuclei as development and differentiation proceed beyond a certain point:—partial loss, and not total as in Weismann's hypothesis, but such as to render impossible the reproduction of the entire organism by the most highly differentiated tissues.

Such a process would necessarily bring about, not only its own termination, but also the extinction of the species, unless some means were available to hand on the undiminished germ-plasm to the succeeding generation. It is evident how this might be accomplished, for the difficulty does not confront our hypothesis, as it confronted Weismann's, that the development of somatic tissues necessarily involves fragmentation of the germ-plasm. Somatic cells which have not exceeded a certain measure of differentiation may still contain germ-plasm which is capable of originating, by step-by-step diminution, all of the cell-types which ultimately comprise the adult organism. Any such cell is obviously a potential gamete irrespective of its situation in the organism, its morphological character which it has assumed in response to its environment, or its history in the development of the organism. Direct unbroken descent of the germ-plasm from generation to generation without participation in the formation of somatic tissues was an essential corollary of Weismann's hypothesis. When this corollary failed the hypothesis in its original form became untenable.

In general we should expect to find a fairly immediate descent of gametogenic tissue from the ovum, simply for

the reason that a prolonged developmental history would almost inevitably involve a degree of differentiation incompatible with reproduction of the entire organism. In animals indeed, the germ-path is usually a brief one and in certain cases, as in the classic instance of *Ascaris* discovered by Boveri,[44] the lineage of the germ-cells is so brief that a direct transmissal of unaltered germ-plasm from one generation to the next is readily susceptible of demonstration. Moreover, in this instance, the loss of nuclear material which accompanies the development of somatic tissues may directly be perceived. In many other animal forms the germ-path, although not so brief as in *Ascaris,* is relatively short in comparison with the developmental history of other tissues comprising the organism.[523]

In plants, however, different conditions prevail, for here we find gametogenic tissues among the most highly differentiated parts of the plant and the tissues themselves participate in the developmental history of the individual in such a way as to altogether preclude the idea that morphological differentiation is incompatible with the retention of complete reproductive capacity.[69]

Nevertheless we cannot in any way avoid the conclusion that wherever complete reproductive capacity is observed, there is situated a full complement of germplasm. The testimony of modern genetic research is absolutely clear upon this point, and we can no longer entertain any doubt that the developmental history of the organism is inscribed in the nucleus of the ovum.[313] To aver this is not to deny participation of the cytoplasm in the outcome, but of this we may be sure, that any chromosomal modification or defect will be reflected in a corresponding modification or defect of the development of the individual proceeding from that cell.

Even the brief developmental history of the gametogenic tissues in the animal arouses the suspicion that some measure of physiological differentiation may have oc-

curred. The prolonged developmental history in plants renders this conclusion inevitable. Objective evidence and theory concur in indicating that somatic differentiation involves a step-by-step diminution of nuclear content, in consequence of which the majority of somatic cells are no longer capable of reproducing the whole organism. The gamete, nevertheless, is found possessed of the full complement of nuclear substance and the gamete may spring, in plants at least, from relatively differentiated tissues.

We cannot suppose that the missing germ-plasm is reformed by the residue which remains. That would imply the ability of a fragment to restore the whole, a conclusion which genetic research has rendered untenable. There exists an irreducible minimum of chromatin material without which reproduction of the whole organism is impossible, for otherwise no cell would exist which could not reconstruct the whole organism. It is not in the somatic cells but in the gametes that the geneticist finds the chromosomal origin of the characters of the adult, and a multitude of observations unite in demonstrating that a loss of chromosomal material representing hereditary factors in the germ-cell can never be replaced at any stage in the ontogeny of the individual.

Still less can we suppose that the cytoplasm reforms the missing germ-plasm. The mere fact that the spermatozoön is equally potent with the ovum in securing the transmission of hereditary characters shows that the formative influence of cytoplasm, apart from included nuclear elements, is negligible. The type of structure which is assumed by any given cell may be, and indeed usually is, determined by the individual reaction of its cytoplasm to the environment, but the quality of the cytoplasm which directs its morphogenesis in response to external factors must be communicated to it by the nucleus, for otherwise the determinative rôle of the nucleus in inheritance would be incomprehensible.

Only one alternative appears to remain open to us and that is that the germ-plasm is still present in the gametogenic tissues even when these are highly differentiated. **The germ-plasm has become lost to the nucleus, but has not yet become lost to the cell.**

We have seen that the multiplication of cells in a community is terminated by the accumulation of the nuclear autocatalyst in the pericellular fluids, and its consequent accumulation in the nuclei. During the period of isolation of the nucleus from the pericellular media and the cytoplasm,[1] that is, between one nuclear division and the next, the nuclear autocatalyst undergoes a further increase *pari passu* with the increase of nuclear substance. The effect of these combined increments is to accelerate the attainment of nuclear equilibrium and ultimately this equilibrium anticipates the attainment of the nuclear ratio which is necessary for division. This outcome of stabilization can only be averted by a reduction of the nuclear ratio and this reduction entails such modification of the metabolism of the cell that its response to environmental factors is altered and the result is a greater or lesser measure of morphological differentiation.

We have advanced no hypothesis to account for this reduction, nor have we attempted to explain the nature of the relationship between cytoplasm and nucleus which determines that at the attainment of a certain proportion between the two, which differs in different tissues, nuclear division must occur. The data upon which any such explanation must be based are almost wholly lacking in our present state of knowledge. But it is clear that however the reduction of the necessary nuclear ratio may be achieved the result must be to enable cell-division, restora-

[1] This isolation is to be understood as relative, not absolute. Although the nuclear autocatalyst cannot traverse the nuclear membrane, nor, it appears, can inorganic salts,[263] the nuclear substrates, including probably amino-acids,[73] must evidently be able to penetrate the nuclear membrane, for otherwise nuclear synthesis within the nuclear membrane would be impossible.

tion of the autokinetic phase, and consequent nuclear growth which would otherwise have been impossible.

Now there is one way in which we can conceive a reduction of nuclear ratio to be brought about, which may not be that which is generally operative, but which can be actually seen to occur in certain instances (for example, the somatic cells in *Ascaris*), and serves to elucidate the power of the gametogenic tissues to retain a full complement of germ-plasm while maintaining their powers of multiplication at a high pericellular level of the nuclear autocatalyst. If a portion of the nuclear substance were to be extruded from the nucleus, and cease to be enveloped in the nuclear membrane, it is obvious that the advantages of a low nuclear ratio would be obtained without any necessary loss of germ-plasm from the cell. This extruded germ-plasm might be handed on unaltered to certain cells, in part only to others, and the former type of cell would remain a potential gamete.

It is true that this suggestion raises a number of questions which cannot at present be answered. We would wish to know how the cell thus constituted acquires the ability of dividing at the new nuclear ratio, and this is a question which our ignorance of the precise mechanism of cell division prevents us from answering.[m] If, indeed, the extruded nuclear material still plays its usual part in determining the moment of cell division, the occurrence of division at a reduced nuclear ratio is readily comprehensible in the first instance, but the divisions of the daughter-cells, at least of those which do not receive a full complement of germ-plasm, still remain to be accounted

[m] The hypothesis has been elsewhere propounded that cell division is due to a bipolar synthesis of nucleic acid from lecithin with resultant equatorial increase in the concentration of choline which reduces the equatorial surface tension.[391 393 394] This hypothesis, however, if we presume that it is correct, still leaves unanswered the question why the synthesis becomes bipolar at one moment in the history of the nucleus and not before. Contemporary investigations hold out the promise of an early solution of this problem,[234] and even at this stage we may confidently assume that the processes which determine cell-division are physical in character.

for. It must be considered unlikely that the extra-nuclear germ-plasm grows and reproduces itself as the intra-nuclear germ-plasm does.[n] All the available facts point to the nucleus as the sole locality of the synthesis of chromatin material, and, besides, indefinite reproduction of the extra-nuclear germ-plasm would again lead to every cell in the organism becoming a potential gamete. We are really at a loss here simply because we do not wholly comprehend the mechanism of cell-division. But it is clear that whatever the mechanism may be it is one that can operate at various nuclear ratios, and this fact, as we have seen, is all that is required to enable us to interpret the phenomena of differentiation and the ability of cell communities to continue the production of certain cell types at an autocatalyst level which prohibits the reproduction of others.

Now extrusion of a portion of the nuclear material, constituting the "polar bodies," is a normal episode in the maturation of the ovum; in this instance the extruded material is contributed by all parts of the nucleus in equal proportion, so that the genetic constitution of the remainder is unaltered. Analogous extrusion into the cytoplasm, rather than to the exterior of the cell, would explain the ability of certain gametogenic tissues to produce new cells at a high pericellular level of autocatalyst while retaining their genetic constitution unaltered. It is, indeed, the belief of Godlewski that such extrusion of nuclear material into the cytoplasm constitutes an essential factor in the maturation of the ovum, and he accounts for the large increase of nuclear material during the early divisions of the sea-urchin egg, without anything approaching proportionate increase of nucleic acid content,[277] by supposing that the nuclear material is reabsorbed in succes-

[n] Contribution of cytoplasm to inheritance, possibly through the agency of included nuclear elements, has, however, been occasionally observed especially in plants,[313] p. 219.

sive divisions, and becomes once more enveloped within the nuclear membranes.[161,162] The low autocatalyst level at this stage would obviously permit such a reabsorption without involving the cessation of cellular multiplication. The low metabolic rate in unfertilized ova also points to a low nuclear ratio,[247] while the great increase after fertilization points to a high nuclear ratio. On the other hand, fragmentation of the meganuclei and the dispersal of their substance into the cytoplasm occurs in infusoria as a consequence of starvation[214] when, of course, the tendency is to reduce the nuclear ratio in order to permit continued multiplication.

It may appear that this suggestion involves a gratuitous complication of the theory of growth which has been presented, merely in order to account for a special case. But it is perfectly evident from many points of view that the nuclear equilibria in gametes are of a peculiar nature, such as do not occur in other tissues. Thus the gametes of both sexes originate from the gametogenic tissues through a series of cell-divisions which are manifestly atypical and designed, in the case of the ovum, to reduce the nuclear-cytoplasmic ratio without proportionate reduction of genetic potentiality. The researches of Loeb have shown us that all that may be required in some instances to induce division of the ovum is a solution or admixture of certain of its constituents.[242] No other cells of the organism behave similarly, and a special mechanism must be invoked to explain their behavior, because it is in reality unique and must necessarily be so in order to secure the potentiality of reproduction in an organism already far advanced towards senescence.

Whatever may be the real nature of the factors which determine the peculiar behavior of gametes it is evident that the hypothesis of growth which has been presented in these pages avoids the main difficulty encountered by Weismann's hypothesis, for since in our view it is the remainder and not the broken off fragment of the germ-

plasm which determines the physiological (and ultimately morphological) character of cells—the possession of a sufficient remainder by certain cells to give rise to a large part, or even the whole of the organism, is readily conceivable. No special parcel of determinants need be assumed, conferring upon the cell its genetic, as contrasted to its somatic, characters. Our hypothesis ascribes the genetic and the somatic characters of the cell to one and the same origin, namely, the residue of germplasm which it has succeeded in retaining.

CHAPTER IX

THE NUTRIENT LEVEL IN RELATION TO GROWTH

1. Factors Which Determine the Nutrient Level.—We have stated that the nutrient level in the tissue fluids of the higher metazoa is approximately uniform throughout life. We infer this from actual comparison of the composition of fetal and new-born with adult blood in the mammal,[72,129,181,444 a] and from the nature of the mechanisms which we can perceive to be involved, and the success they achieve in maintaining the composition of adult blood unaltered despite very extreme environmental fluctuations.

The consequences of inanition are necessarily modified and in part determined by the existence of these mechanisms. Their nature is only incompletely understood in the higher *Vertebrata* and in the invertebrates, of course, they are for the most part unknown or merely inferred from morphological appearances. They involve, however, a coöperation of diverse tissues by means of which the organism is enabled to achieve a high degree of independence of the nutritional fluctuations in its environment. Now investigation has revealed the fact that the evolution of higher forms has been accompanied by a gradually increasing independence of the environment[178,264] which is achieved by the creation and maintenance within themselves of a suitable environment for their constituent cells. Thus fluctuations in the hydrogen ion concentration of the pericellular media are prevented by the presence of "buffer salts" in the blood (bicarbonates and phosphates) and the coördinated activity of the respiratory centre and the kidneys in the elimination of excess of volatile acid (CO_2) on the one hand, or of fixed

[a] Minor differences, *e.g.*, between the compositions of fetal and maternal blood, manifestly do not affect the general validity of the principle.

acids or bases on the other. In addition the process of deaminization which is normally succeeded by the transformation of ammonia into urea may be interrupted at the stage of ammonia production, the ammonia being then available for the neutralization of acids. The whole mechanism is automatic, each irregularity being cancelled by the reaction which it itself calls forth. No other type of mechanism, obviously, would be of any service to the organism.

In a similar fashion the higher metazoa have attained independence of variations of saline composition and osmotic pressure in the external environment.[264] This is achieved by the selective elimination of excessive constituents by the kidneys, and this function is exerted with such precision that, according to Macallum, the saline composition of the blood of the *Vertebrata* closely resembles the probable composition of sea-water at the time when the proto-vertebrate type first acquired a kidney. The lower animals display no such uniformity in the composition of their tissue fluids. The *Medusæ,* for example, contain salts in almost the same proportion that they occur in the sea-water which they inhabit. In plants, also, a considerable variation of mineral content occurs in different soils.

Again, in the homoiothermic animals, independence of temperature fluctuation has been attained by the co-operation of nervous, metabolic and vascular activities, and optimum conditions for enzyme activity are thereby ensured.[409]

By a process of the same character, but probably at an even earlier stage of evolution, the higher metazoa have acquired a considerable degree of independence of fluctuation in the supply of nutrients. Doubtless this was essential to enable the development of the higher and more delicately balanced functional activities, but, on the other hand, a considerable degree of differentiation and delegation of function has been requisite to enable the attain-

ment of the nutritive invariance now possessed by the higher *Vertebrata,* and one is led to conjecture whether the underlying impulse in the evolution of these forms has not quite as frequently been internal as external, originating in the advantages gained by an ever-increasing independence of short period fluctuations in the environment. We have seen, in the chapter which deals with senescence (Chapter VII), that the inevitable consequence of the acquirement of this fundamental measure of environmental invariance has been to result in the mortality of the individual, and to escape the consequences of this the mechanisms which ensure the discharge of gametes, or the severance of part of the tissues from the nutrient media of the parent, must necessarily have undergone parallel elaboration. In this sense, therefore, death is, as Weismann supposed,[510,511,512] an adaptation of advantage to the species, but the difficulty which confronted Weismann's hypothesis, that, namely, of explaining how death could ever be evolved by selection, is avoided when we realize that the mortality of the individual is an incidental and unavoidable outcome of the acquirement of a character which is of real temporary advantage to the individual, and permanent advantage to the race,[b] while its disadvantages to the individual do not at the same time constitute any disadvantage to the race.

The regulation of the nutrient level in the mammal begins at the assimilative surface, in the selective absorption of the products of digestion. The experiments of London[6,7] have shown that when proteins of abnormal amino-acid constitution are administered to animals the amino-acid which is present in unusual excess is rejected by the intestinal epithelium. Thus, in a dog fed with gliadin, a protein which contains an unusually high proportion of glutamic acid, it was found that after digestion had been proceeding for some time the duodenum con-

[b] Because the advantage to the individual endures sufficiently long to permit of reproduction.

tained 0.75 grams of tyrosine to 2.5 grams of glutamic acid, the jejunum contained only 1.1 grams of tyrosine per 20.9 grams of glutamic acid, and the ileum contained only a trace of tyrosine as contrasted with 33 grams of glutamic acid. The proportion of tyrosine to glutamic acid in the completely hydrolyzed protein is 1.2 to 43.7.[376] Evidently, therefore, tyrosine was absorbed from the intestine much more rapidly than glutamic acid, a large proportion of which must have escaped utilization and have been discharged in the fæces. It is for this reason that the utilization of protein in the diet is very defective if the protein is abnormal in its amino-acid constitution, and since the proteins of meat[c] approximate more closely to those in our tissues than proteins of vegetable origin, the nitrogen in vegetables is much less completely utilized than the nitrogen in meat. The following are figures obtained by Thomas.[262]

TABLE XLIV

BIOLOGICAL VALUES OF DIFFERENT PROTEINS, AS MEASURED BY THE PERCENTAGE OF QUANTITY OF BODY PROTEIN WHICH THEIR INGESTION WILL SPARE FROM LOSS

Ox meat	104	Yeast	71
Cow's milk	100	Casein	70
Fish	95	Nutrose	69
Rice	88	Spinach	64
Cauliflower	84	Peas	56
Crab meat	79	Wheat flour	40
Potatoes	79	Cornmeal	30

The utilization of protein is much improved after a period of starvation,[198] so that the non-assimilation of excessive amino-acids is not due merely to a consistent inability of intestinal epithelium to absorb any other than a definite admixture of amino-acids, corresponding to the average composition of the tissue proteins. On the contrary it is due to active selection which is modifiable in response to the requirements of the tissues.

Similar selection is exercised towards the fats and is similarly modifiable by starvation or even by deprival of

[c] Including, of course, glandular tissues.

fat. Thus, on a normal mixed diet Munk found[324] that in a subject fed with no other that mutton fat, the chyle-fat, after absorption, had a lower melting point than pure mutton fat. In other words, glycerides of oleic acid had been added to the fat from the tissues during the process of absorption. Similarly when the subject received no other fat than cetyl palmitate which melts at 55° C., the chyle-fat was found to melt at 36° C., and to consist of a mixture of glyceryl tripalmitate and glycerides of oleic acid to the extent of fourteen per cent. of the mixture. The fats may also be modified in the opposite direction, and the proportion of oleic acid glycerides reduced during their transmission through intestinal epithelium. Thus when cod liver oil, which contains a great excess of unsaturated fatty acids, is administered to dogs, the iodine number of the fats after absorption is less than that of the fat in the food.

On the other hand during starvation the fat ingested is laid up in the tissue fat-depôts almost unaltered. Fatty acids which are quite foreign to the normal tissues of the animal may in this way be introduced into the fat reserves and the fat of a dog may be converted into mutton fat by previous starvation followed by a diet in which the only fat is mutton tallow (Munk).

In the case of carbohydrates the various monosaccharides which result from digestion appear not to be selectively absorbed, but rather to be transformed to the single circulating monosaccharide glucose. Thus the ingestion of lactose or cane-sugar must lead to the appearance of galactose and lævulose respectively in the intestine, yet these carbohydrates are normally utilized and contribute to the formation of glycogen in the liver, from which we might infer that they enter the circulation and are distributed to the tissues in the altered form of glucose. No other carbohydrate than glucose has as yet been certainly detected in the blood, although their occasional presence therein may be inferred from the fact that ex-

cessive ingestion of either cane-sugar or lactose may lead to the appearance of traces of the unaltered disaccharide together with more considerable quantities of its constituent monosaccharides in the urine. The seat of the transformation of galactose or lævulose into glucose is not certainly known, for the fact that monosaccharides are absorbed into the portal circulation leaves open the alternative that the transformation may occur in the liver. Recent investigations suggest that important stereochemical changes may be induced in sugars by the intestinal juices,[188] but the possibility of their occurrence in other tissues is shown by the fact that mammary gland tissue transforms glucose into galactose, and excision of the mammary glands in a lactating animal leads to glycosuria, if any sugar appears at all in the urine, and not to lactosuria or galactosuria.[275] On the other hand sudden cessation of lactation, with the mammary glands intact, often leads to the transient occurrence of lactose in the urine.

The selective activities of the intestinal epithelium, aided possibly by the liver, contribute therefore to reduce fluctuations in the quality of the dietary, and the quantity of individual constituents. There is no doubt that some measure of similar selection is exerted by all cells, so that this represents no special mechanism created *de novo* in the vertebrate, but rather an adaptation of a ready-made property of protoplasm in general. The particular feature of the process which at first sight calls for special interpretation is the modifiability of the selective activity by the needs of the tissues, but when we recollect that the intestinal epithelium shares in the needs and shortcomings of the body as a whole, through the medium of the circulation, this adjustment no longer appears so extraordinary.

The act of feeding in the higher animals is intermittent, and this results in the pouring of superabundance of elementary foodstuffs into the blood at certain times, and absence of assimilation at others. Yet the composition of

the blood alters comparatively little during assimilation, although, of course, the rise in fat, glucose and amino-acid content is sufficiently definite to be appreciable. The absorbed foodstuffs, however, rapidly disappear from the blood, while the fat and carbohydrate reserves of the body in the fatty tissues on the one hand, and the liver and the muscles on the other, undergo concurrent increase. In the case of the proteins, or the amino-acids resulting from their digestion, it was for long supposed that no storage occurred, but we now know, by direct analysis,[500,501,502,503] that storage of amino-acids occurs to a definite extent in all tissues but that each tissue has a "saturation" limit of amino-acid content, characteristic of that species of tissue, which cannot be exceeded. Moreover, the storage is very transient, destruction by deaminization occurs from the beginning, and the tissue soon becomes "desaturated" again unless fresh income of amino-acids is provided by assimilation. The rate of desaturation is also characteristic of the type of tissue and is highest in the liver.

Now this storage of reserve food materials for each of the three main types of foodstuffs, and to some extent it appears for the accessory foods as well, is a reversible phenomenon. That is, as the tissues draw upon the blood for their current supplies, the reserves are discharged into the blood from the various storage depots, to replace the current expenditure. This process may be illustrated by the sequence of events which accompanies muscular exercise. The energy of the contracting muscle is furnished by the oxidation of carbohydrate which is provided in the first instance by the depolymerization of the glycogen reserves in the muscles. The equilibrium between the glucose in the blood and glycogen in the muscles, which may be represented by the equation:—

$$n\mathrm{C_6H_{12}O_6} \rightleftarrows (\mathrm{C_6H_{10}O_5})_n + n\mathrm{H_2O}$$

is thereby displaced and the reaction now proceeds from left to right to restore it. This involves the abstraction of

glucose from the blood, which, in turn, recoups itself by the depolymerization of the glycogen reserves in the liver. Throughout this process the manifest tendency is to maintain a certain minimum of glucose in the blood. The "nutrient level" in the blood, in other words, determines and is determined by the equilibria which lead to the storage of reserves.

In the special case which is constituted by the amino-acids the storage capacity of the tissues is very limited and a special mechanism is required to dispose of amino-acids which are absorbed in excess of this capacity. This is provided by the "specific dynamic action" of the proteins,[433] or, as Lusk has shown,[261] of certain amino-acids which they contain. Thus if a starving animal is losing a certain amount of tissue-protein daily, the administration of this amount *per diem* does not suffice to balance the nitrogenous input and output. On the contrary, on increasing the nitrogenous input an increase of nitrogenous output also occurs and the nitrogenous balance remains negative. A further increase of nitrogenous input calls forth a still greater metabolism of protein until, on an exclusively protein diet, a balance between intake and output is attained with an output of nitrogen no less than three and one-half times that which is observed in the starving animal.[489] In man the quantity of protein thus required to obtain nitrogenous equilibrium is greater than he can conveniently consume, and, even when nitrogenous equilibrium has been attained, the carbon balance remains negative, since not only the nitrogenous metabolism, but the metabolism of fats and carbohydrates as well is stimulated by protein. The effect of protein, therefore, is to greatly increase the heat-evolution of the body, and the replacement of fat or carbohydrate by protein in a diet which is just sufficient to maintain equilibrium results in rendering the diet inadequate to replenish current expenditure.

It has been shown by Folin that there are two distinct types of nitrogenous metabolism in the body.[138,139] The one, which he believes to be endogenous, or expressive of the actual breakdown of tissue, gives rise to creatinine and "neutral" sulphur compounds (from cystine), while the other, or exogenous type of metabolism, yields urea and sulphates. These differences are exemplified in the following table, contrasting the urinary output on high and low protein diets:—

TABLE XLV

	High protein diet	Low protein diet
Volume of urine....	1170 c.c.	385 c.c.
Total nitrogen	16.80 grams	3.6 grams
Urea nitrogen	14.70 "	2.2 "
Creatinine nitrogen .	0.58 "	0.6 "
Total sulphur (SO_3)	3.64 "	0.76 "
Inorganic SO_3	3.27 "	0.46 "
Ethereal SO_3	0.19 "	0.10 "
Neutral SO_3	0.18 "	0.20 "

It will be observed that the reduction of the 24-hour nitrogenous output from 16.80 grams to 3.6 grams resulted in a reduction of urea output from 14.70 grams to 2.2 grams, and of inorganic sulphate from 3.27 to 0.46 grams. But the creatinine and neutral sulphur output did not diminish at all. These latter constituents must arise, therefore, from some steady source of supply, comparatively little influenced by the protein intake, while urea and sulphates must be produced concurrently with the stimulation of metabolism which arises when the protein intake is increased.

Now the source of the urea which is manufactured by the liver is ammonia, derived from the deaminization of amino-acids in accordance with the equation:—

$$HOOC\ R\ NH_2 + H_2O = HOOC\ R\ OH + NH_3$$

so that we may infer **that the exogenous metabolism of amino-acids is initiated by their deaminization.** The rate of deaminization is increased by protein assimilation so that this

process constitutes the first stage of the "overflow" mechanism, whereby the presence of excess of amino-acids in the blood during assimilation is prevented, and a steady level of amino-acid content of the blood maintained, despite the low storage-capacity of the tissues for excess of these food elements.

The process of deaminization, however, although greatly reducible in extent, is never reduced to zero throughout life, so that urea constitutes a large proportion of the urinary output of nitrogen even in extreme starvation.[33] This may mean that a part of the endogenous metabolism also produces ammonia, but it is more probable that actual breakdown of tissue protein occurs only in special situations, except in response to a decided fall in the amino-acid content of the blood and consequent desaturation of the tissue reserves.[343] Thus we have seen in Chapter V that maintenance, but not growth, is possible upon a diet which contains no lysine. Spontaneous destruction of the majority of tissue protein molecules would certainly lead to destruction of this amino-acid radicle as well as others unless, indeed, it is employed over and over again in the reconstitution of tissue protein, which would imply a special mechanism arresting the deaminization of this amino-acid while others are undergoing deaminization alongside it. The origin of the urinary urea in starvation thus appears to reside in an unrestrainable continuance of the mechanism which normally guards the tissues from undesirable excess of amino-acids in the pericellular fluids. This leads, in starvation, to ultimate depletion of the circulating amino-acids and their replacement from the tissue reserves, and these, in turn, must be replaced by the actual breakdown of cytoplasm, which in normal nutritive circumstances would be averted by the assimilation of a fresh supply of amino-acids. Under these circumstances lysine probably shares in the destruction which other amino-acids undergo.

The utility of this mechanism to the organism is not at first sight evident, but it becomes so when we consider that the objective of living organisms is not the maintenance of the individual, but growth and reproduction of the species. Both of these objectives require motility as the means of their attainment and the preservation of motility[d] implies the consumption of energy. For the furnishing of this energy the carbohydrate and fat reserves are first drawn upon, but as they decrease in starvation the structural elements of the body, the tissue proteins, now afford the only possible source of energy. They must be kept available, therefore, for this ultimate need, and the process of deaminization furnishes hydroxy fatty acids which are readily available sources of energy, A reduction of deaminization to zero in starvation would mean that the structural elements of the body, even those least essential, would be locked up beyond recall, and inaccessible to the organism even in the extremity of starvation.

The nature of the mechanism which controls the deaminization of amino-acids is as yet unknown, but there is much evidence to connect the thyroid with the process.[e] In the first place the administration of thyroid tissue causes an extraordinary rise of nitrogenous output[386] while metabolism in general is at extremely low level in diseases arising out of thyroid hypofunction, such as myxœdema. In the second place Kendall has shown[215,217] that the active constituent of the thyroid, thyroxin, or tri-iodo-indole propionic acid, enters into combination with amino-acids by either its carboxyl or its amino groups, so that it is capable of entering into intermediate combination with amino-acids as we would expect it to do if it were capable of catalyzing reactions into which the amino-acids enter. Furthermore, while thyroxin itself, when injected in-

[d] And, in warm-blooded animals, the preservation of bodily temperature as well.

[e] We are not here concerned with the question whether the deaminization is a primary effect of the thyroid autocoid, or only a secondary consequence of increased oxidation.

travenously, causes little disturbance of the organism, thyroxin injected together with amino-acids gives rise to serious symptoms of intoxication which resemble those which follow continued administration of excess of thyroid tissue.[216] Apparently, therefore, thyroxin has a special relation to amino-acid metabolism and excessively rapid deaminization is attended by danger of intoxication.

The circulating nutrient fluid of the *Vertebrata,* the blood, may therefore be conceived as a reservoir of foodstuffs which is maintained at a constant level by three main types of process. An **inflow** due to the assimilative activities of the digestive tract, an **outflow** into the tissues originating in their needs for growth, repair or maintenance, and an **overflow** which is secured by storage in the case of fats and carbohydrates, and by deaminization in the case of proteins. Thus any lack of equivalence between inflow and outflow is offset by the overflow. As in the case of the other instances of environmental invariance discussed above, uniformity of the nutrient environment is achieved by a series of dynamic equilibria involving the coördinated participation of many tissues. Its success is of course dependent upon the ultimate satisfaction of appetite by food, but even at the point of death from starvation the blood is still found to contain elementary nutrient constituents.[509]

2. **Inanition and Growth.**—The facts which have been enumerated in the preceding chapters have shown us that the elementary nutritive requirements are diverse and that, in consequence, there must be as many species of inanition as there are irreplaceable necessities in the dietary. From this point of view, therefore, the "deficiency diseases," which originate in deprival of one or more of the accessory food factors, are so many varieties of inanition. In the interpretation of the mechanisms of growth, however, very valuable information has been obtained from the effects of total deprival of food. Now we have seen that animals are able not merely to maintain

themselves, but to grow to full adult dimensions on a diet which contains only protein, salts, and the accessory food factors, with, of course, the addition of water.[355,356] Total deprival of food, therefore, constitutes deprival of protein, salts, and accessory food factors, with deficiency of calories.

Of these deficiencies it appears probable that the deficiency of protein is responsible for the majority of immediate effects. The effects of deprival of the accessory food factors are only evidenced after a certain lapse of time, relatively brief in the case of the water-soluble accessory, but still appreciable. The effects of total deprival of food are, however, evidenced at once by loss of weight and an alteration in the relative proportion of the nitrogenous constituents of the urine. Deficiency of calories doubtless aids the effect of deprival of protein by throwing upon the tissue proteins the burden of supplying the energy-needs of the body, as well as replacing the daily wastage of amino-acids through deaminization. No change in the fundamental character of the effects occurs during prolonged starvation[33] until immediately before death, when a sharp rise of nitrogen elimination heralds the fatal outcome.[538] At no time are symptoms displayed which recall the effects of deprival of the accessory food factors, and if lack of these plays any part in contributing to the result, it appears that it is a minor one. For the purposes of our discussion, therefore, we may regard total deprival of food as amounting to the deprival of protein (*i.e.*, amino-acids) and calories. The concurrent deficiency of mineral salts may contribute to the acidosis of starvation through lack of inorganic bases,[488] but this owes its origin in the main to the fact that the energy of the body in starvation is derived solely from the oxidation of proteins and fats of which the former yield sulphuric acid (from cystine) while the latter, in the absence of carbohydrates from the diet, are imperfectly oxidized and produce oxybutyric acid.[212]

Now upon what structures might deprival of protein be expected to press most hardly? We have seen that the limited capacity of cells for the storage of amino-acids results, when calories are insufficient in the diet, in the destruction of tissue protein to provide the energy expenditure of the organism. Those structures, therefore, which consist of protein will be sacrificed, and protein constitutes the structural basis of cytoplasm. It is even open to question whether the nuclei of cells contain any protein at all.[5] The experiments of Abderhalden and Kashiwado are not quite conclusive upon this point. They state that the thymus nuclei which they prepared by digestion of thymus tissue in some instances had fragments of cytoplasm still adherent to them, and the nuclei yielded a small amount of protein. This protein might have been derived from the adherent shreds of cytoplasm, or from the nuclear membranes. In any case the quantity was very small and the nucleo-proteins which may readily be extracted from a variety of tissues may quite as likely be derived from cytoplasm as from nuclei. However this may be, protein is not the quantitatively leading constituent of nuclei as it is of cytoplasm, and for this reason we should expect the cytoplasm of cells to be diminished by starvation.

To a less extent the nuclei must suffer as well, through reduction of the nutrient level of those foodstuffs which are requisite for the synthesis of nuclear constituents. The cytoplasm proteins, however, suffer not merely from the fall of nutrient level due to deprival of the foodstuffs necessary for their manufacture, but also from the further fall of nutrient level due to the demand upon the proteins for energy. The cytoplasmic proteins, therefore, sustain a double demand and the nuclei only one. We should expect in consequence to find starvation leading to a reduction of both cytoplasm and nucleus, but to a disproportionately large reduction of cytoplasm.

Now it is the unanimous testimony of investigators that this anticipation is borne out by facts. The earliest observations appear to have been those of Leonard in 1887,[228] who ascertained that the cytoplasm of the liver cells in the frog becomes very greatly diminished during the winter hibernation, and increases again in spring with the taking of food. The following are the measurements which she records:—

TABLE XLVI

Month	Average diameter of the cells in μ
November	29.2
December	16.2
April	12.0
June	17.2
July	27.4

When we consider that the volume of the cell decreases as the cube of the diameter and that the diameter had decreased by the end of winter to considerably less than half the summer diameter, it is evident that no less than nine-tenths of the total volume of the cell had been lost by the end of the period of hibernation. This extraordinary decrease of protoplasm was entirely suffered by the cytoplasm, and the nuclei, so far from decreasing in volume during the period of hibernation, actually underwent a slight increase. This latter, however, may possibly have been a secondary phenomenon due to mitosis occurring in some of the cells, because the minimum nuclear volume did not alter appreciably throughout the year.

The liver is a storehouse of reserve food material in the form of glycogen and is also a laboratory for the manufacture of an important adjuvant to digestion, bile, and for the transformation of ammonia, resulting from deaminization, into urea. These profound effects of starvation upon liver cytoplasm therefore, contrasting so strongly with the comparative lack of effect upon the nuclei, might perhaps be regarded as arising solely from the consump-

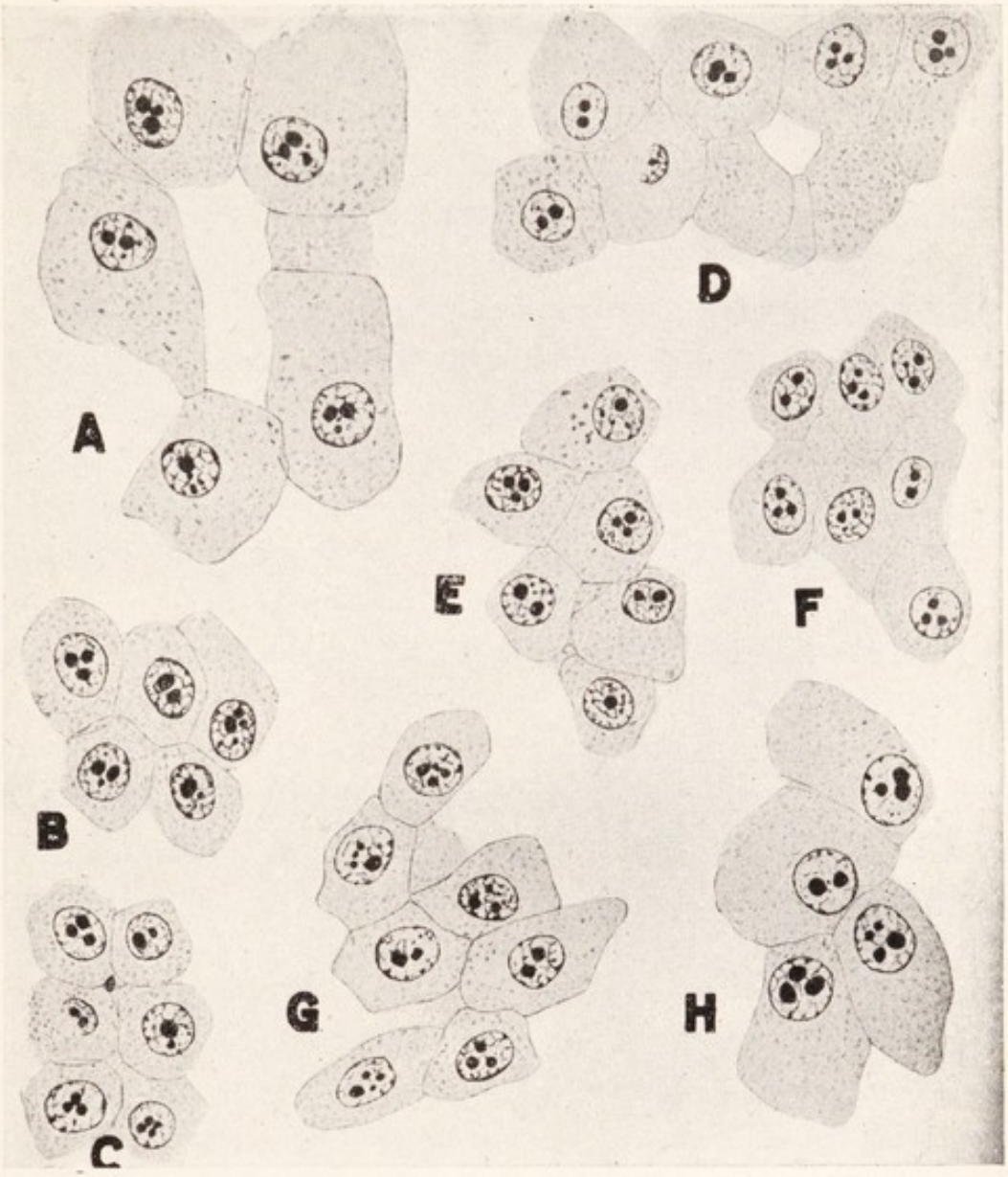


FIG. 31.—A. A group of liver cells from a normal salamander (*Diemyctylus viridescens*). B. A group of liver cells from a small salamander starved one month. C. Same from a small salamander starved two months. D, E, and F. Groups of liver cells of large salamanders starved respectively one month, two months and three months. G. A group of liver cells of a salamander starved three and a half months and then fed for four days. H. Same from a salamander starved three and a half months and then fed for eight days. (After Morgulis.)

tion of glycogen and the arrest of digestive and excretory activities. Very numerous subsequent investigations have shown, however, that this is not the case, and that the phenomenon is a perfectly general one which occurs in all the types and tissues which have been examined histologically after subjection to starvation. (Fig. 31.)

Thus Morpurgo[318,319] confirmed Leonard's observations two years later and added to them the fact that similar cytoplasmic reduction occurs in the cells of the pancreas, while the nuclei are not reduced. These observations were made upon starved pigeons, so that they showed that the effect is not peculiar to the "physiological" starvation which is endured in hibernation and that it occurs in diverse species. Carlier added further confirmation in 1892,[57] working with the liver of the hibernating hedgehog. Lukianov[260] subsequently showed that the pronounced decrease of cytoplasmic volume in the liver of the starving rat is also accompanied by a slight decrease of the volume of the nuclei.

Citron[71] has shown that the ectoderm cells of cœlenterates undergo reduction of size during starvation, and Child,[69] p. 155, has shown that the reduction of size of planaria during starvation is due to the reduction of the size rather than of the number of the constituent cells.

Very extensive investigations on cell-size and nuclear-size in starvation have been carried out by Morgulis.[314,315] He finds that in the salamander starvation results in decrease of cell-size and that the greater proportion of this decrease occurs in the cytoplasm. The volume of the cells decreases more rapidly than the body weights, so that an actual increase of the number of cells must accompany starvation, the significance of which fact will be apparent later. In the albino rat he finds a similar decrease of cell-size in the liver and pancreas and also a decrease in the size of the nuclei. In this case the number of cells diminishes, so that the volume of the cells decreases less rapidly than the body weight.

It should be recollected that the observed decrease of volume of the cytoplasm during starvation, great as it is, affords only a partial picture of the actual loss, for the true loss of weight has been shown by Morgulis to be partly masked by the imbibition of water,[316] and Aron has shown that the calorific value of tissues decreases during insufficient feeding, owing to a decrease of their organic content.[20,21,22]

Morgulis, Howe and Hawk[317] found a decrease of cytoplasm in a great many tissues of dogs which had been subjected to prolonged starvation. Frequently the cell outlines become obscured, resulting in the formation of a syncytium. The cell boundaries are rapidly reformed around the intact nuclei when food is readmitted.

The time-relations of inanition have been carefully studied by A. G. Mayer in a cœlenterate, *Cassiopea xamachana*. He finds that this animal loses weight during starvation according to the law:—

$$y = W\,(1-a)^x \quad \ldots\ldots\ldots\ldots \quad (62)$$

where y is the weight after x days of starvation, W is the weight at the beginning of the period of starvation, and a is the fraction of the existing weight which is lost in a unit of starvation-time (one day). The following table shows a satisfactory correspondence of theory and observation:—

TABLE XLVII

Table for $y = 100\,(1 - 0.0567)^x$

Days of starvation	Observed average weight	Calculated weight
0	100	100
2	88.5	88.97
4	77.82	79.16
6	71.35	70.43
9	59.73	59.11
12	50.44	49.61
15	42.08	41.64
19	32.9	32.9

The accompanying curve (Fig. 32) is constructed from these data.

We may write Mayer's formula in the following way:—

$$\log y = x \log (1 - a) + \log u \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (63)$$

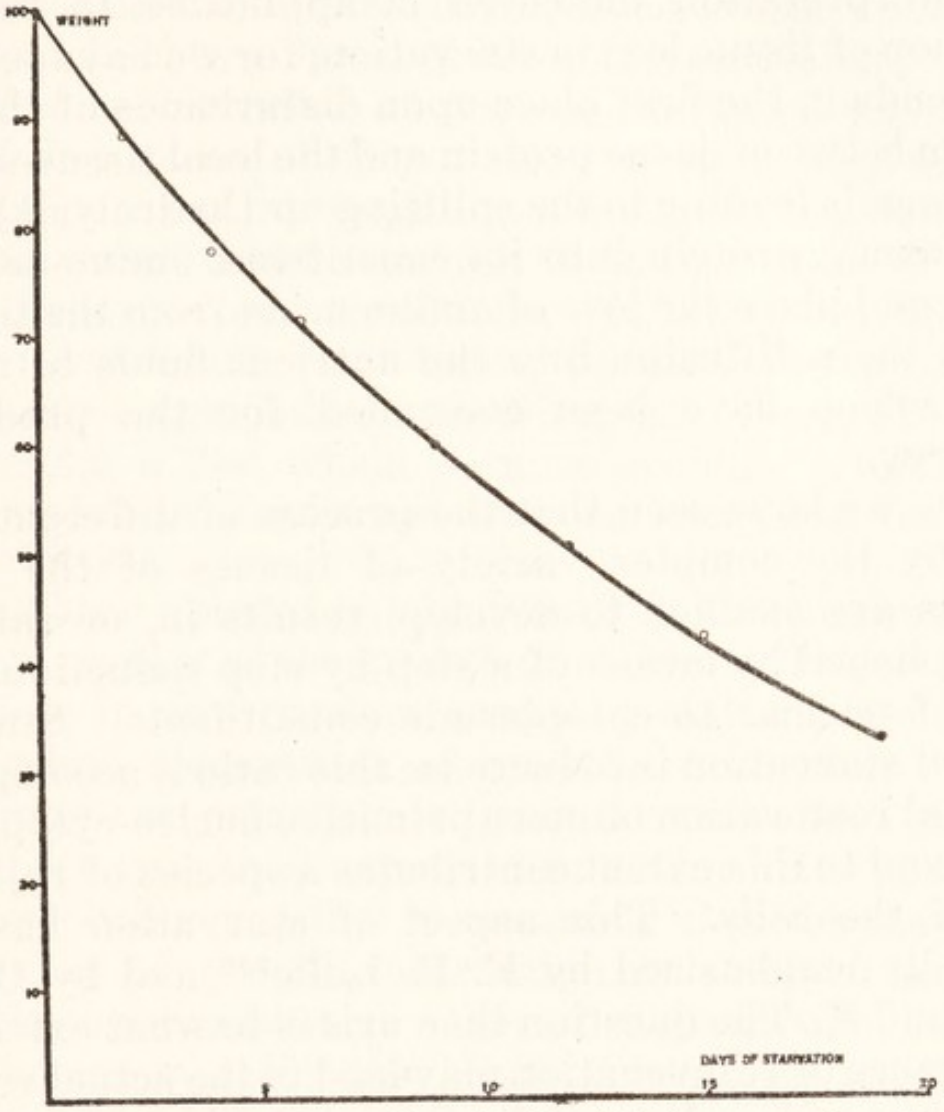


FIG. 32. Curve of inanition, showing loss of weight of *Cassiopea xamachana* during starvation. Curve calculated from formula of Mayer. The observed weights are indicated by the small circles. (Measurements by Mayer.)

Whence by differentiation

$$\frac{dy}{dx} = y \log (1 - a) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (64)$$

So that the rate of loss of tissue is proportional to the mass which is yet retained. This is identical with Wilhelmy's law, which implies a chemical process in which but one molecular species is undergoing decrease of con-

centration, as, for example, the hydrolysis of a protein. The same law, however, applies to those reactions which depend upon the rate of diffusion of a dissolved substance away from the surface separating two phases, as for example, when zinc is suspended in acid.[329] In either event the interpretation indicated is applicable to the phenomenon of tissue loss in starvation, for we have seen that it depends in the first place upon disturbance of the equilibrium between tissue protein and the local tissue-store of amino-acids leading to the splitting up (hydrolysis) of the cytoplasmic protein into its constituent amino-acids. In the second place the loss of amino-acids from the tissue is due to their diffusion into the nutrient fluids to replace those which have been consumed for the production of energy.

Now we have seen that the process of differentiation, whereby the complex variety of tissues of the higher animals are enabled to develop, results in, or rather is accomplished by means of a step-by-step reduction of the ratio of nuclear to cytoplasmic constituents. Since the effect of starvation is to increase this ratio it accomplishes a partial restoration of more primitive nucleo-cytoplasmic ratios and to this extent contributes a species of rejuvenation of the cells. This aspect of starvation has been especially emphasized by F. R. Lillie[232] and by Child,[69] p. 155 and ff. The question then arises to what extent this appearance of rejuvenation may lead to the actual restoration of an earlier phase of developmental history.

It has been observed by Morgulis[314] that there is a great acceleration of growth-rate in salamanders after a period of inanition. This may be most strikingly demonstrated by comparing the proportion of the food intake which is converted into protoplasm before and after a period of inanition. If at a certain period of growth the proportion of food converted into protoplasm was 26 per cent., after starvation and the readmission of food it rose to as high as 73 per cent. He states that "it is possible

that the reduction in size of the cells, or rather the diminished ratio between cell and nucleus, has something to do with the observed processes of intense growth, and that the rejuvenescence of the organism is analogous to the condition in the embryo, where the cell-body is likewise small in relation to its nucleus.'' He cites results obtained as early as 1885 by a Russian investigator, Kahan,[213] who found that rabbits after 17 days of inanition, when they had suffered a loss of 31 per cent. of their body-weight, gained 56 per cent. in weight on a diet even less sufficient than that which could maintain them in a state of equilibrium under normal conditions. In other words, what was formerly a diet which barely permitted maintenance becomes after a period of starvation, and even at the same body weight, a diet which permits growth. **The rate of cellular anabolism is enhanced after starvation and the readmission of food.**

It has been pointed out by Boas,[36] in the course of his statistical studies of the growth of children, that retarded individuals in any random group of children generally exhibit a late acceleration of growth. ''In other words, that among the retarded individuals the whole energy required for growth is expended in a very brief period.'' We are probably justified in assuming that the majority of instances of growth-retardation in children are attributable to some form or other of malnutrition, so that the phenomenon noticed by Boas reveals the same tendency in children that other observers have encountered in animals. Stewart[476] has also shown that when food is readmitted to starved rats, growth is more rapid than in younger animals of the same weight which have not been subjected to starvation. The result is that the stunted rats are able, after readmission of food, to overtake the full-fed controls before the end of the normal growth period. In the same year it was shown that similar phenomena are exhibited by mice which have been subjected to inanition,[397] p. 83.

A very striking instance of this general tendency is afforded by the compensatory overgrowth which succeeds the post-natal loss of weight in infants.[397] It is a familiar fact that for a variable period, in normal cases not exceeding one week, the majority of infants lose weight after birth. This is a phenomenon which is very generally observed after the birth of animals.[362] In the case of man it is usually attributed to the fact that for the first few days after birth the child frequently receives little or no nourishment.[49,517] That this probably is not the only factor involved, however, is shown by the fact that the post-natal loss of weight is usually much greater when the child is excessively large, and also by the fact that it occurs even in young guinea-pigs, which are born in such an adult condition that they are capable of feeding themselves entirely by the fourth day after birth. It must be admitted, however, that the power of these animals to nourish themselves at such an early date assists to overcome a large part of the post-natal retardation of growth, for the retardation is relatively slight in guinea-pigs and frequently leads to actual loss of weight only in the male.[384]

In connection with the investigation of the birth-weight of South Australian infants, the results of which have been enumerated in Chapter II, a considerable number of data were obtained concerning the weights of the children at the end of the first and second weeks succeeding birth.[396] We have seen that the extra-uterine curve of growth is continued smoothly backwards into the intra-uterine period (indicated by the weights of prematurely delivered children) so that the post-natal loss of weight does not arise from a delay or regression of growth, independently of birth, occurring at a certain stage of development. If this were the case then in children born late it should occur before birth, and in those born early some corresponding time after birth. There is, on the contrary, no correlation between the length of the period of gestation and the extent or duration of the post-natal loss of

weight. There is, indeed, a certain tendency for infants born at the late periods to lose more heavily than those born after briefer periods of gestation, but this is attributable to the fact that the infants born at the later periods are heavier, and therefore larger, than the infants born at the earlier periods.

The following tables exhibit the relationship between the post-natal loss of weight and the weight of the infant at birth, all infants weighing, for example, between 115 and 125 ounces at birth, being regarded as having weighed 120 ounces at birth. Infants of which the weight at birth fell upon a limiting weight separating two weight-classes (*e.g.*, 115 ounces) are included in both classes (*e.g.*, the 110-ounce and 120-ounce classes).

TABLE XLVIII

MALES

Weight at birth in ounces	Number of infants	Weight at one week after birth	Loss (−) or gain (+) in ounces
80	1	91	+ 11
90	3	86	— 4
100	6	104	+ 4
110	10	108	— 2
120	12	117	— 3
130	9	128	— 2
140	12	132	— 8
150	3	144	— 6
160	3	150	— 10
170	1	160	— 10

TABLE XLIX

FEMALES

Weight at birth in ounces	Number of infants	Weight at one week after birth	Loss (−) or gain (+) in ounces
80	3	77	— 3
90	5	94	+ 4
100	7	105	+ 5
110	17	108	— 2
120	19	118	— 2
130	17	125	— 5
140	6	129	— 11
150	2	138	— 12

It will be seen that there is a very clear correlation between the magnitude of the post-natal loss of weight and the weight (and therefore, presumably the size) of the

infant at birth. The infants weighing over 130 ounces at birth suffer especially severely, while infants weighing less than 110 ounces at birth, so far from losing weight during the first week after birth, frequently gain considerably. From this it would appear legitimate to infer that mechanical shock during delivery is an important factor in determining the post-natal loss of weight.

The average weight at one week after birth of all the male infants born after periods of gestation lying between 245 and 325 days[f] was 121.4 ounces; the average weight of South Australian male infants at birth is 127.3 ounces.[g] Hence the average observed loss of weight during the first week after birth of South Australian male infants is 5.9 ounces, or 4.6 per cent. of their weight at birth.

The average weight at one week after birth of all the female infants born after periods of gestation lying between 235 and 325 days was 115.0 ounces; the average weight of South Australian female infants at birth is 121.2 ounces. Hence the average observed loss of weight during the first week after birth of South Australian female infants is 6.2 ounces, or 5.4 per cent. of their weight at birth.

From these figures it would appear that the growth of female infants is more retarded by birth than that of male infants. That this is not really the case, however, is shown by the following considerations.

It must be recollected that the above figures do not represent the whole of the loss of weight due to birth. At the time of birth the infant is growing rapidly, and if it were not for the shock, nutritional and mechanical, of birth, the infant would increase considerably in weight during the first week of post-natal life. The observed loss of weight is therefore not the actual loss of weight. We may estimate the actual loss of weight to within a very close approximation by the following method:—

[f] Thus excluding those periods of gestation which departed so widely from the mean as to be probably pathological or otherwise abnormal in origin.

[g] See Chapter II, Section 3.

We have seen (Chapter II) that the latter part of the pre-natal and the first 9 months of the post-natal growth of South Australian male infants may be represented very accurately by the formula:—

$$\log \frac{x}{341.5 - x} = 0.136\,(t - 1.66) \quad . \quad . \quad . \quad . \quad . \quad . \quad (65)$$

where x is the weight of the infant in ounces, and t is the time, measured in months of 30 days, which has elapsed since birth. Now at one week $t = \frac{7}{30} = 0.23$. Hence after putting $t = 0.23$ in the above formula we can estimate what South Australian male infants would weigh at one week after birth, if it were not for the retardation of growth and loss of weight due to birth. In this way we find that at one week after birth South Australian male infants **should** weigh 133.1 ounces. They actually do weigh, as we have seen, 121.4 ounces. The **actual** loss due to the nutritional and mechanical shock of birth is therefore 11.7 ounces, or 9.2 per cent. of the weight at birth.

The formula which similarly represents the growth of South Australian female infants is:—

$$\log \frac{x}{350 - x} = 0.111\,(t - 2.47) \quad . \quad . \quad . \quad . \quad . \quad . \quad (66)$$

and from it we find that at one week after birth South Australian females **should** weigh 126.2 ounces. They actually do weigh 115.0 ounces. The **actual** loss of weight due to birth is therefore 11.2 ounces, which is 9.2 per cent. of the weight at birth. Hence males and females suffer an **equal** retardation of growth as a result of the nutritional and mechanical shock of birth.

During the second week of post-natal life there is a marked gain in weight, which not only makes good the loss of weight during the first week, but considerably exceeds it. The average weight at two weeks after birth of the male infants born after periods of gestation lying between 245 and 325 days was 130.6 ounces; hence (*cf.* above) the average gain over the weight at birth at the end of two

weeks of post-natal life is 3.3 ounces. The average weight at two weeks after birth of all female infants born after periods of gestation lying between 235 and 325 days was 124.2 ounces, the average gain over the weight at birth being 3.1 ounces.

These gains, however, although they more than serve to make up the **observed** losses of weight due to birth, do not entirely compensate for the **actual** loss of weight due to birth (*i.e.*, the observed loss plus the **gain** in weight which the infant would have displayed had it not been for the retardation due to growth). This may be shown by putting $t = 0.47$ (= two weeks) in the formulæ above, and computing with their aid the weights which the infants should display at two weeks after birth. In this way we find that at two weeks after birth, South Australian male infants **should** weigh 139.3 ounces, while they actually **do** weigh 130.6 ounces. Hence the retardation of growth due to birth is, after two weeks of extra-uterine growth, 8.7 ounces, or 6.8 per cent. of the weight at birth. Similarly we find that at two weeks after birth South Australian female infants **should** weigh 131.2 ounces, whereas they actually **do** weigh 124.2 ounces. Hence the retardation of growth due to birth is, after two weeks of extra-uterine growth, 7.0 ounces, or 5.8 per cent. of the weight at birth.

Although the gain in weight during the second week of extra-uterine life is not sufficient to entirely make up the loss due to birth it will be noted that it **partially** does so, for the loss of weight in males due to birth is 11.7 ounces at one week and only 8.7 ounces at two weeks. In other words, the effect of birth does not result in a **permanent** subnormality in the weight of the infant, because the loss of weight due to birth is made up by a **compensatory overgrowth** (*i.e.*, growth in excess of the normal increment corresponding to the given age or weight) which amounts during the second week, in males, to 3.0 ounces. This compensatory overgrowth is even more pronounced in females, amounting to no less than 4.2 ounces during the

second week of extra-uterine life. Evidently females recover more rapidly than males from the inhibitive effects of birth upon their growth, a fact which is doubtless correlated with the greater tolerance of adverse conditions and the lesser variability of the post-natal loss which female infants display.

The compensatory acceleration of growth during the second week may be shown in another way. From the above equations it may readily be computed that between the end of the first and the end of the second weeks of extra-uterine life the increment of weight in males, if their growth proceeded at the normal velocity indicated by preceding and succeeding weights, should be 6.2 ounces. The observed increment is no less than 9.2 ounces. Similarly the increment during the second week in females should be 5.0 ounces and actually is 9.2 ounces. Evidently the rate of growth in males is accelerated 48 per cent. and that of females no less than 84 per cent.

By the end of the first month of extra-uterine life this compensatory process has entirely made good the loss of weight due to birth, so that at one month the observed weight of male infants is "normal," that is to say, lies exactly upon the continuous curved line which represents preceding and succeeding growth. In the case of South Australian female infants there would even appear to be over-compensation, since the observed mean weight at one month is decidedly "supernormal."

"From these facts it would appear that the normal weight of a growing organism at any given age represents a true dynamic equilibrium, any disturbance of which is rectified by internal regulation, just as a gyroscope restores equilibrium to a mass which has been displaced from its normal position through the action of an external force" (*loc. cit.*, p. 82). The importance of this mechanism in promoting recovery from the effects of disease must also be very great.

The experiments of Osborn and Mendel[338,342,346] have shown that the capacity for restitutive growth is maintained throughout life. "The capacity to grow is not lost with age, but only by the exercise of this fundamental property of animal organisms." [338] The stimulation of growth-rate due to partial starvation[445] was shown by comparison of the rates of growth of rats which had been stunted by diets of varying types of inadequacy[h] and of younger animals of the same weight. Thus the daily increments during resumed growth of stunted rats after 100 days may equal 4 per cent., whereas, normally they rarely exceed 1 per cent. In connection with these experiments the suggestion was put forward by Osborn and Mendel that particular cells, possibly those of certain endocrine glands, may advance in their development more nearly normally than the great mass of the tissues and thus come to exert "an undue stimulus upon the energy transformations leading to growth." Their own experiments had, however, shown that sexual maturity in stunted rats occurs not at the age but at the weight at which it would normally occur,[342] so that the "puberty gland" or interstitial tissue of the gametogenic organs, at all events, does not develop very much in advance of the rest of the organism.

This suggestion has been carefully investigated by Jackson, who examined the thyroids, parathyroids and hypophyses of starved rats. No evidence was found in favor of the view that the accelerated growth following periods of suppression may be due to the continuation of the development of the ductless glands.[204] On the contrary, exactly the same changes were found to occur, during starvation, in the cells of these glands, as in the cells of the other tissues and organs of the body. The nuclei become relatively larger and richer in chromatin, while the cytoplasm becomes small in amount and undifferentiated in structure. The cells thus approximate towards the condi-

[h] All involving, however, inadequacy of amino-acid intake.

tion pertaining to an earlier stage of development. In the parenchyma of the *pars* anterior of the hypophysis the nuclei form 34 per cent. of the total cell-volume in the new born, decreasing to 24 per cent. at three weeks, and 20 per cent. at ten weeks (adult relation). During inanition the loss is relatively greater in cytoplasm, so that the nucleus comes to form 26 to 28 per cent. of the cell-volume in young animals held at the maintenance level of nutrition, and 23 to 26 per cent. of the cell-volume in adults with acute or chronic inanition. He attributes the acceleration of growth after readmission of food, to the enhanced proportion of nucleus to cytoplasm, and the relatively youthful character which is thus induced in the cells. We have seen (Chapter VIII) that there is much evidence to show that the rate of metabolism in a cell is proportionate to its nuclear (chromatin) content. The effect of starvation being to reduce the ratio of cytoplasm to nucleus the metabolic rate per unit of weight should thereby be increased. Now, as a matter of fact, it has been shown by Child that the metabolic rate, which decreases during inanition,[69] increases upon readmission of food to a level considerably exceeding the pre-starvation level. "The rate of production of carbon dioxide in the starved, reduced animal (*Planaria*) is practically equal to that of the young, growing animal of the same size, and this rate is much higher per unit of body-weight than that in large, old animals." (*Loc. cit.*, p. 161.) This more rapid utilization of food must lead, of course, to a more rapid synthesis of protoplasm than that which would correspond to the age of the starved animal.

Another way of viewing the matter leads us to the same conclusion. If we suppose, as indeed we can hardly avoid doing, that the nucleus plays some part in assisting the manufacture of cytoplasm, during the normal growth of an animal the nucleus and cytoplasm are increasing simultaneously and the rate of synthesis of cytoplasm is never what it might be if the nucleus could get ahead of it.

But this is exactly what is accomplished by starvation. The cell is reduced to the size of a young cell, but is provided with an almost fully developed nucleus. Hence it acquires cytoplasm more rapidly than a young cell of the same size.

From the same point of view we can interpret the differential effects of starvation upon the loss of weight of various tissues and organs. The loss of weight which occurs in starvation is by no means uniformly distributed throughout the body. The following table displays the loss of substance, in percentage of the normal weight, of the various tissues of cats after death from inanition:—[505]

TABLE L

Tissue or organ	Loss of weight per cent.
Fat	97
Spleen	67
Liver	54
Testes	40
Muscles	31
Kidneys	26
Skin	21
Intestine	18
Lungs	18
Pancreas	17
Bones	14
Heart	3
Central nervous system	3

It will be observed that those organs which are most essential to the preservation of existence are those which suffer least extensively from the deprivation of food. This must be due to some definite peculiarity of the metabolism of those tissues which are able to maintain their weight under these adverse circumstances. The nature of this peculiarity may easily be inferred from the fact that the speed of metabolism is exceptionally great in just those tissues, the heart and nervous system, which most successfully resist the disintegrating effects of inanition. Thus the heart is constantly engaged in transforming large amounts of potential energy into mechanical work

and yet it carries within itself an extraordinarily small reserve of energy-yielding materials. The glycogen-content of the muscular tissues of the heart, instead of being exceptionally high, is, as a matter of fact, no higher than that of the skeletal muscles, which are only contracted intermittently. The heart must thus depend, for the maintenance of its exertions, upon the direct and unceasing withdrawal of nutrient materials from the circulating fluids. In so doing it is forced to compete with all the other tissues of the body, and yet it does so with so much success that whereas the majority of the other tissues lose a very considerable part of their weight, the heart maintains the integrity of its substance until death is imminent. This implies that the rate of utilization of nutrients by the heart must greatly exceed that of the other tissues, so that foodstuffs are appropriated by it in advance of the ability of other tissues to consume them.

The high metabolic rate of the central nervous system may be inferred from the fact that its consumption of oxygen is exceptionally great. The first effect of deprivation of oxygen is to arrest the higher activities of the central nervous system[459] and those substances which paralyze the oxidizing enzymes, such as cyanides, arrest the activities of the central nervous system before any other tissue is affected to a comparable degree. The intensity of oxidations in the central nervous system testifies to the rapidity of the destruction of its constituents. The fact that it maintains its integrity even in starvation, therefore, implies a proportionate rapidity of reconstruction.

The synthesis of the various tissues of the body from the foodstuffs which are contained in the circulating fluids may be regarded as the result of a multitude of parallel reactions, all consuming similar substrates, although not in identical amounts and proportions. Now in any group of parallel reactions, that is, of reactions which are occurring simultaneously and consuming the same raw materials, each substrate which enters into the reactions is

shared between them in proportion to the velocity with which they occur. The various reactions proceed at their own independent rates, and, if the quantity of materials available for transformation were unlimited, each reaction, or the synthesis of each particular kind and type of tissue, would go forward at the same speed as it would if the other tissue-syntheses were not occurring simultaneously. The nutrient level of the pericellular fluids is, however, not unlimited, but adjusted, as we have seen, by a dynamic equilibrium, to the average needs of the body as a whole. In the competition for these materials, therefore, the most specifically rapid syntheses will have a decided advantage over the specifically slower syntheses, and when the nutrient level sinks below the normal, as in starvation, the more rapidly metabolizing tissues will maintain their integrity for relatively prolonged periods at the expense of the more slowly metabolizing tissues.

Starvation, therefore, tends to rejuvenate the organism in two ways; in the first place by reducing the ratio of cytoplasm to nucleus to a level characteristic of an earlier stage of development, and in the second place by preferentially diminishing the tissues of low metabolic rate, and thus leaving a greater proportion of tissues of high metabolic rate, which are usually tissues of a less (physiologically) differentiated character, that is to say, tissues of an ontogenetically early type.[1]

But there is yet another way in which we may suppose that inanition assists in development upon the readmission of food. We have seen that cell-division occurs when the ratio of nucleus to cytoplasm attains a certain critical value, characteristic of the particular type of tissue, and that the successive differentiations which occur in ontogeny owe their origin to the step-by-step diminution of this critical ratio, each new ratio conferring upon the cells a new physiological type which modifies their reactions to

[1] And, for this reason, they form a large proportion of the most physiologically essential organs.

their environment and the character of the syntheses which proceed within them. In this way morphological differentiation arises in consequence of an underlying physiological differentiation.

The ratio which compels division is normally approached by an increase of nuclear material, but in starvation it may also be attained by the diminution of cytoplasm. We cannot of course assume *apriori* that this will likewise cause division to occur, but there is experimental evidence to show that it may do so in fact. Thus Gaglio[155] found mitoses occurring in the nuclei of the muscles of starved frogs. Morpurgo[319] found that mitoses continued to occur even in acute inanition in the tissues of pigeons. Hofmeister[190] found mitoses in the adenoid tissues of the alimentary canal of cats even after 17 days of starvation. A particularly significant observation is that of Jonson,[211] who found mitoses reduced in number but still occurring in the thymus during starvation, notwithstanding the fact that the involution of this tissue is greatly accelerated by inanition. Morgulis has found nuclei dividing mitotically in salamanders at every stage of inanition.[314] Jackson was able to suppress mitoses by starvation in the *partes* nervosa and intermedia of the hypophysis of the rat, but in the *pars* anterior they still occur occasionally even in rats nearly dead from inanition.[204] Upon readmission of food the mitoses in the *pars* anterior corresponded roughly to the number found in younger animals of the same weight.

In these several different ways, therefore, inanition accomplishes a measure of rejuvenation of the multicellular animals. This arises in consequence, as we have seen, of the operations of the mechanism which maintains a relative invariability of the nutrient level during normal alimentation. The continuance of its activities, during inanition, sets the current of nutrients in the direction opposite to normal from the tissues to the blood, and since the cytoplasm suffers a double drain, to supply its own

substrates and also the fuel required to furnish the calorific output of the body, the cytoplasmic constituents of the cell are reduced more rapidly than the nuclear constituents and the cells return to the nuclear-cytoplasmic ratio of a less differentiated type.

But the rejuvenescence is apparent and not real, for it must not be forgotten that the nuclei are also, although in less measure, suffering loss of substance through the fall of their nutrient level. Certain cells, those namely of the older ontogenetic types, which were barely able to maintain the nuclear level necessary for the preservation of their physiological character and function, must now find it impossible to continue their existence. Hence, for example, involution of the thymus is accelerated by inanition. Should any of these cells undergo division through reduction of their cytoplasm and consequent relative enhancement of the nuclear ratio, their condition becomes more hopeless than ever, for the attainment of the high nuclear ratios characteristic of such cells is impossible at the autocatalyst-level which has increased since this type of cell first arose in the processes of development. In general, however, those cells which will be induced to divide most readily by reduction of their cytoplasm will be those which were upon the verge of dividing before inanition commenced, *i.e.*, the cells most recently produced, and hence the general effect of the mitoses will be to enhance the relative number of (physiologically) differentiated cells. It is true that morphological differentiation may be diminished by the destruction of cytoplasmic structures, but that is soon restored by the readmission of food, and when cytoplasmic equilibrium has once more been regained the organism is older to the extent that the autocatalyst-level has risen through the occurrence of mitoses, and to the extent that cells of the ontogenetically older types, now irreplaceable, have been lost by their failure to maintain their nuclei during the period of inanition. If mitoses could, indeed, be totally suppressed

at a nutrient level which permitted maintenance of the existing cells, then a different result would be obtained, for at this level existence could then be indefinitely prolonged without development and without regression. But we have seen that in the tissues of the vertebrate animals mitoses occur at every stage of inanition and even when the animal is upon the verge of death. In *Planaria* it may be otherwise, perhaps because the number of physiological types (*i.e.*, differing nuclear ratios) is smaller in these relatively simple animals, for Child has been able, by supplying incompletely developed planaria with just sufficient nutriment to support existence without permitting growth, to maintain them in this condition for a period corresponding to several generations of the animals which were allowed to develop normally,[69] p. 167.

In general terms, therefore, the immediate effect of inanition upon the balance of the tissues, is the opposite of the effect of the accumulation of autocatalyst in the pericellular fluids (see Chapter VII). But the condition of a starved animal differs from that of a young animal of the same age in that the autocatalyst-level in its pericellular fluids is not less than that corresponding to its actual age. It is indirectly for this very reason that the restitution of lost cytoplasm is so extremely rapid upon the readmission of food. When food has been readmitted and cytoplasmic equilibrium restored, the animal is older to the extent that mitoses have occurred and the larger nuclear ratios have been unable to maintain themselves.[j] Rejuvenation is transient, and the ultimate effect of inanition is increased differentiation and a loss of primitive cells. It is for this

[j] The excretion of uric acid, the end-product of the purine metabolism in man, continues and even rises during starvation.[33] It is probable that this is derived from nuclei which have been destroyed by the fall of nutrient level, although it is difficult to be sure of this because certain nucleic acids (guanylic acid and inosinic acid) probably occur in extra-nuclear situations. However, we should expect the proportion of cells which succumb, either to cytoplasmic or to nuclear losses, to increase progressively during starvation. The premortal rise of nitrogen output probably originates in this manner.

reason that after repeated or over-prolonged inanition the normal adult dimensions can never be attained, despite the accelerated growth-rate upon readmission of food.[315] The increased rate of growth in these animals is offset by the diminution of its extent. Of like origin is the production of hypotypical ovaries by inanition, in which the graafian follicles undergo premature atresia and the ova fail to mature.[256]

3. **The Energy-equivalent of Growth.**—The storing-up of tissue-substance which is possessed of a definite calorific value necessarily results in the retention by the growing animal of a proportion of the energy-value of its food. Furthermore, a considerable proportion of the heat-value of the diet, varying with age and the rate of growth, is additionally consumed in the performance of the functions of the animal, and especially in the maintenance of motility and the dissipation of heat. In the case of the proteins the deaminization of amino-acids proceeds, as we have seen, at a rate which is comparatively unaffected by the occurrence or non-occurrence of growth. Hence we find that in any given species of animal, the slower the accretion of tissue, the greater the energy which is consumed per kilo of tissue built up, since the parallel reactions which determine the performance of function and the maintenance of the nutrient level are in such a case proceeding for a longer time. The following are illustrative results which were obtained by Aron:—[21,22]

TABLE LI

GROWTH OF DOGS

Animal number	Calories consumed in fifty days	Increase in weight in fifty days	Calories consumed per gram of tissue increase
B	19,950	1,570	12.7
C	13,925	1,000	13.9
VIII	9,500	780	16.4
XII	10,750	838	15.6

From these results it is clear, as Aron points out, that an animal gaining 1000 grams during fifty days needs fewer calories for this gain than one which gains 1000 grams in one hundred days, the reason being, as indicated above, that in the first instance the animal needs to be "maintained" for only one-half as long as in the latter. The following are comparable observations made by Aron upon Filipino children:—

TABLE LII

MARIA INOCENCIA

From week	To week	Number of days	Increase in grams			Calories	
			From	To	Per day	Per day	Per kilo
21	26	35	3500	3600	3	350–375	100–105
26	31	31	3650	4225	17	450	115–120
31	35	28	4225	4811	21	500	125

TABLE LIII

MIGUELO PRIEGA

From week	To week	Number of days	Increase in grams			Calories	
			From	To	Per day	Per day	Per kilo
4	9	35	3550	4175	17	350–400	100
9	13	28	4175	4850	24	450–475	105

Hence during the entire period of the investigation Maria Inocencia increased in weight at the rate of 14 grams per day, and consumed a daily average of 450 calories. Miguelo Priega, on the other hand, increased in weight at the rate of 21 grams per day and consumed about the same number of calories per day and a considerably smaller number per kilo.

According to Rubner[434] the energy consumed per kilo in doubling the birth-weight of animals is always very nearly the same, excepting in the case of man, namely

about 4,000 calories. The following data are presented by Rubner in support of his thesis:—

TABLE LIV

ENERGY-CONSUMPTION PER KILO IN DOUBLING THE BIRTH-WEIGHT

Species	Energy-consumption
Horse	4,512
Cow	4,243
Sheep	3,926
Hog	3,754
Dog	4,304
Cat	4,554
Rabbit	5,066
Man	28,864

The generalized form of this relationship would be:—

$$\frac{E}{x} = a \log x + b \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (67)$$

where E is the energy-consumption, x the weight of the animal, and a and b are constants which are independent of the species (except man). Doubling of the weight would obviously always add an equal amount to the quotient $\frac{E}{x}$, that is, to the total energy-consumption per kilo. This leads to the differential equation:—

$$\frac{dE}{dx} = \frac{E}{x} + a \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (68)$$

which means that the consumption of energy per unit mass of tissue accretion increases in proportion to the energy which has already been consumed in reaching the weight to which the new unit of tissue is added. This obviously corresponds in a general way with the facts ascertained by Aron. It is certain, however, that Rubner's law is not a generally applicable law of growth, because not only does it fail to apply to man, among the very small number of species investigated, but also, unless some factor varying with the time be superadded to Equation 68 it would lead to the absurd conclusion that an adult animal ceases

to consume any energy at all. This follows from the fact that when x reaches a constant value, as at the attainment of adult weight, then, from the equation, E must also be constant. But since E is the total energy expenditure from birth, it cannot remain constant unless energy expenditure ceases altogether. Rubner's law, therefore, only partially expresses the facts, and is at variance with his further generalization, that the energy-consumption of animals per kilo for the duration of life after the attainment of maturity is approximately the same for all species, man, again, affording an exception to the rule (see Chapter VII).

CHAPTER X

HYPER-DIFFERENTIATION; CANCER

CANCER is a disease of growth which, as we now know, arises from a disorder of function, and not through the direct agency of any specific infecting organism. It is usually, but not invariably, a disease of old age, and the casual relationship of its incidence to chronic local irritative stimuli may now be regarded as having been established beyond any question, since it has been experimentally induced in animals by agencies belonging to this category. Thus the tendency to develop cancer of the skin on the part of those whose skin is constantly contaminated by soot or tar has long been recognized, and in recent years cancer of the skin has been induced experimentally in mice by applications of tar.[325,495,532,533] According to Ross[428,429] there are specific substances ("*auxetics*") in tar which stimulate the proliferation of cells, but while this may doubtless be so, the diversity of the irritative agencies which are known to be capable of giving rise to cancer is so great as to discourage the idea that a specific explanation is required in the case of tar. Thus, cancer of the bladder is stated to be exceptionally prevalent among workers in aniline dye factories, presumably because irritative substances are excreted in the urine and during their stay in the bladder give rise to chronic irritative conditions which subsequently culminate in cancer. Cancer of the stomach has been experimentally induced in rats by the ingestion of cockroaches which are infested with a worm (*Spiroptera*) which attacks the tissues of the stomach in the rat. As a consequence of the irritative changes thus induced, cancer frequently supervenes.[130,131,132,133,134] The classic instance of the hill-dwellers in India, who carry fire boxes strapped about

their bodies and develop cancer of the skin in what is otherwise a very rare situation, namely the surface of the abdomen,[326] affords a striking example of the importance of irritative reactions in determining the place of incidence of cancer. With the aid of tethelin, which stimulates the growth of cancer cells, it has been found possible to produce cancer experimentally in rats by inducing chronic inflammatory reactions.[127] Scar-tissues and slowly-healing lesions are fruitful sources of cancer in clinical experience. The variety of the irritative agencies which may thus serve to initiate cancer, demonstrates that the processes of repair are exceptionally favorable to the appearance of this type of cell.

On the other hand, the tendency to develop cancer in response to prevalent irritative stimuli has also been shown to be hereditary.[452,453,460] It can be bred into or out of a strain, so that the incidence of a particular type of tumor may be increased or decreased by selection or by hybridization. A peculiar feature of this inheritance, however, lies in the fact that the susceptibility of particular tissues to either spontaneous primary, metastatic or inoculated tumors is inherited[137] and the susceptibility of one type of tissue is by no means necessarily linked with the susceptibility of another.[454] Thus in any given strain metastatic tumors (if there are any) tend to occur in those organs in which the primary tumors of that strain occur, and this tendency is transmitted from generation to generation, being evidenced by primary tumors now in the one organ and now in another, but confining itself to the given group of organs. It appears to be indifferent whether the tumor is primary or metastatic, the hereditary condition of the organ rendering it susceptible to both. Since the tumor tendency may be transmitted by an animal which never develops a tumor itself[454] the cancer characteristic would appear to be recessive.

The type of tumor (sarcoma or carcinoma) would also appear to be inherited together with the susceptibility of

the tissues in which it occurs. The following examples are adduced by Slye:—A parent female with a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver and a secondary sarcoma of the kidney, can transmit to the strains derived from her a tendency to:—

1. Carcinoma of the mammary gland.
2. Sarcoma of the mammary gland.
3. Adenoma of the mammary gland.
4. Carcinoma of the liver.
5. Sarcoma of the liver.
6. Adenoma of the liver.
7. Carcinoma of the kidney.
8. Sarcoma of the kidney.
9. Adenoma of the kidney, or any combination of these nine.

If she is mated with a male, either himself developing a lung or mediastinal tumor or else heterozygous to these locations of tumor, *i.e.*, transmitting but not displaying the susceptibility, the resulting strains will show in addition:—

10. Carcinoma of the lung.
11. Sarcoma of the lung.
12. Adenoma of the lung.
13. Carcinoma of the mediastinum.
14. Sarcoma of the mediastinum.
15. Adenoma of the mediastinum, or any combination of these fifteen tumors.

It is evident, therefore, that the **type** of tumor may be inherited from the male parent and its **situation** from the female, and *vice versa.*

As is well known, cancer cells are transplantable into individuals of the same species, although, as the investigations of Fleisher and L. Loeb have shown,[137] the susceptibility to such transplants is also inherited. Hence strains are found into which transplantation of any given type of tumor is frequently successful, and others into which the same type of tumor can but rarely be transplanted.

Similarly the tendency to develop metastases from the transplanted growth varies from one strain to another. Inbreeding very generally lessens the vigor of transplanted tumor growth. This is attributed by Lathrop and Loeb to lessened vigor of growth,[225] but by Whitman[514] to lessened variability of the type.

The almost unanimous conviction of modern investigators is that cancer cells "are in every way similar to those forming normal tissues, but for their increased power of multiplication no longer controlled by the laws which appear to regulate the growth of cells under ordinary conditions."[87] L. Loeb, in concluding an exhaustive comparison of tumor growth and tissue growth observes "that the majority, and ultimately perhaps all, of the phenomena observed in tumor growth find a parallel in the growth of normal tissues under special conditions."[255]

Now we have seen that the law "which regulates the growth of cells under ordinary conditions" is that the accumulated autocatalyst of nuclear synthesis, stored up in the nuclei and prevented from escaping at the moment of nuclear division by the abundance already present in the nutrient medium, finally accelerates the reverse reaction of nuclear synthesis to so great an extent that the velocity of the reverse reaction becomes equal to that of the forward reaction, before the critical nuclear ratio which is requisite for cell-division can be attained. Further cell-multiplication is thus inhibited, and stasis is imposed.

The only means of escape from this stasis, so long as the cell remains confined within the nutrient medium of its community, lies in the reduction of the critical nuclear ratio, with the consequent production of a new physiological type, capable of reproduction in the presence of the prevailing concentration of autocatalyst. Under ordinary circumstances, in the normal development of any of the higher animals or plants, we may suppose that this physiological differentiation occurs in consequence of the inherited variability of the cells, in graded stages, each

successive stage of physiological differentiation arising as the opportunity is created by the inhibition of the multiplication of the preceding cell-types. Pressure relations, nutrient availability, tropistic reactions and the established gradients of autocatalyst-concentration coöperate to guide the new tissues, as they successively arise, into the structural relationships which characterize the particular organism in which they originate. This orderly process of successive unfolding of physiological types at length reaches the limit imposed by the hereditary variability of the cells composing the organism, and senescence, due to the gradual encroachment of types possessing the lower nuclear ratios, is the necessary outcome.

This condition of stasis may at any moment be interrupted by the removal or destruction of some of the cells forming the community. The effect is to upset the balance in that locality and, by the removal of competing cells, to raise the nutrient level of the medium bathing the remainder. Now the stasis imposed by excess of autocatalyst is entirely relative to a definite nutrient level. It must be recollected that stasis of nuclear synthesis occurs when the velocity of the reverse reaction has become equal to the velocity of the forward reaction, and that the velocity of the forward reaction is directly proportional to the nutrient level a which prevails at that moment. An increase of nutrient level, therefore, reinaugurates nuclear synthesis, just as it reinaugurates cell-division in an infusorian which has been isolated from a culture in which multiplication has ceased. Hence the removal of tissue locally reinaugurates nuclear synthesis, through the local rise in nutrient level which is consequent upon decreased consumption. But, it will be observed, and this is a most fundamental consequence of our hypothesis, that the cells which are possessed of lower nuclear ratios will multiply proportionately more rapidly than those which are possessed of higher nuclear ratios. Hence the process of

repair always calls forth the formation of a more differentiated type of tissue than that which is to be replaced.[a]

Normally the degree of differentiation attained in the repair of lost or injured tissue does not exceed that which is still subject to control by the competition of adjacent cells, and the prevailing accumulation of autocatalyst. But this process, if repeated again and again, becomes virtually a process of selective breeding, whereby the cells of the lowest nuclear ratios are favored and enabled to propagate.[b] Should the inherited variability of the cells in that locality enable, at length, the appearance of a type of cell so physiologically differentiated from its competitors, as to be able to multiply freely at the prevailing nutrient level, and in the presence of the prevailing accumulations of autocatalyst, then an outburst of reproductive activity must inevitably ensue, and the successful type of cell will inaugurate, if it is sufficiently differentiated, a malignant tumor.[c]

Obviously this growth will differ in no essential particular from normal tissue, save that it will be "no longer controlled by the laws which appear to regulate the growth

[a] It will be understood that we are referring to **physiological** differentiation, which may or may not result in morphological differentiation in response to the circumstances in which the new cells find themselves.

[b] The idea that differentiation arises out of a struggle for existence between the various cells constituting an organism and consequent "physiological selection" of the "fittest," originated, it appears, with G. H. Lewes, who utilized the conception to interpret the differentiation of the nervous system.[229]

[c] In the ordinary sense in which the term is employed the malignancy of a tumor is usually stated to vary inversely as its differentiation. The differentiation referred to in this generalization is, however, **morphological** differentiation, which is a consequence of preceding physiological differentiation, followed by the assumption of specialized function. It must be remembered that morphological differentiation is relative. In proportion as a tissue is specialized in structure it is considered to be differentiated from the primitive type. The specialization implies the performance of limited functions and this limitation is imposed upon the cell by its inheritance of germ-plasm on the one hand, and the necessities of its environment, on the other. In the case of a tumor the specialized functions (motility, alimentation, etc.) are performed by the tissues of the host, and one group of the exciting causes of histological differentiation is absent precisely in the proportion that the cells of the tumor find it easy to appropriate the nutrients of the host.

of cells under ordinary conditions," and, lacking the hereditary potentialities and predetermined graduated variability of normal embryonic tissue, it will fail, more or less completely, to give rise to organized or morphologically differentiated tissue. The great reduction of the nuclear ratio which is presumably required to permit the neoplasm to gain decisively in the competition for nutrients with the tissues of the host must involve a drastic reduction of hereditary factors. Such tissue retains relatively little potentiality of producing graded variations from the initial type and hence but little power of producing a variety of morphological types.

The tumor, in the light of this hypothesis, owes its origin to an abnormal variability of the nuclear-cytoplasmic ratios in the tissues of the host. This variability may occur in one tissue or organ or in another, but both the type of cell produced (degree of variation) and the tendency of given tissues to display the abnormal degree of variation (situation of the tumor) must evidently be qualities arising out of the genetic constitution of the host. The tendency to develop cancer in response to chronic local destruction of tissue, and consequent repair, must therefore be influenced by hereditary factors precisely in the manner indicated by the experiments of Slye. The new type of cell, subject to the immunity-response of the host,[86,430,496] will find conditions, in respect to nutrient level and autocatalyst concentration, no less suitable for its growth in another animal of the same species than in the original host.

A question may be raised as to the manner in which the tumor cell acquires a nuclear ratio so low as to permit the maintenance of the autokinetic phase while the surrounding cells of the host remain in the autostatic phase. This brings us back again to our ignorance of the precise mechanisms of cell-division (page 219) and consequent ignorance of the way in which physiological differentiation originates during mitosis. We cannot hope to answer

such a question satisfactorily until we possess far more extensive knowledge than we do at present of the physics and chemistry of cell-division. It is not difficult to conceive a mechanism which will permit division of a cell at a lower nuclear ratio than the parent cell, provided that we make certain assumptions regarding the mode of action of the factors which determine the moment of cell-division, but the experimental justification for such assumptions is lacking.

Whatever the mechanism, however, the occurrence of cell-division at a lowered nuclear ratio might happen in either of two ways: the daughter-cells might share a diminished proportion of nuclear materials in equal allotments, or the one cell might retain a full allotment and the other the diminished allotment. The latter phenomenon would involve asymmetrical mitoses and the frequent occurrence of these in pathological neoplasms has repeatedly been pointed out.[170,171,172] Hansemann, indeed, interpreted them in quite another way to that which we have indicated. He supposed that by unequal division the cancer cell gains a disproportionately large allotment of germ-plasm (anaplasia); we have supposed, on the contrary, that it acquires a disproportionately small allotment. But both types of cell may be seen in cancer tissue, together with those resulting from more typical mitoses. Galeotti[156] has shown that similarly unequal mitoses may be artificially induced in the epithelial cells of salamanders by treatment with dilute solutions of narcotic drugs. He has suggested that the unequal mitoses may not be the cause, but rather a consequence of cancer, attributable to the development of poisons by the pathological tissue. But there is no evidence of the production of any such poisons, except indeed we count the nuclear autocatalyst itself as such, in the excessive concentration in which it must be present in cancer tissue.

A very striking analogue to the genesis of cancer from this point of view, and one which gains significance from

the experiments of Galeotti, is supplied by the recently-published observations of Child on head-regeneration in *Planaria* under the influence of narcotic drugs[70] (Fig. 33). In dilute solutions of alcohol the repair of the median region is differentially delayed, so that abnormal head-forms result in which the eyes are fused or absent, or even,

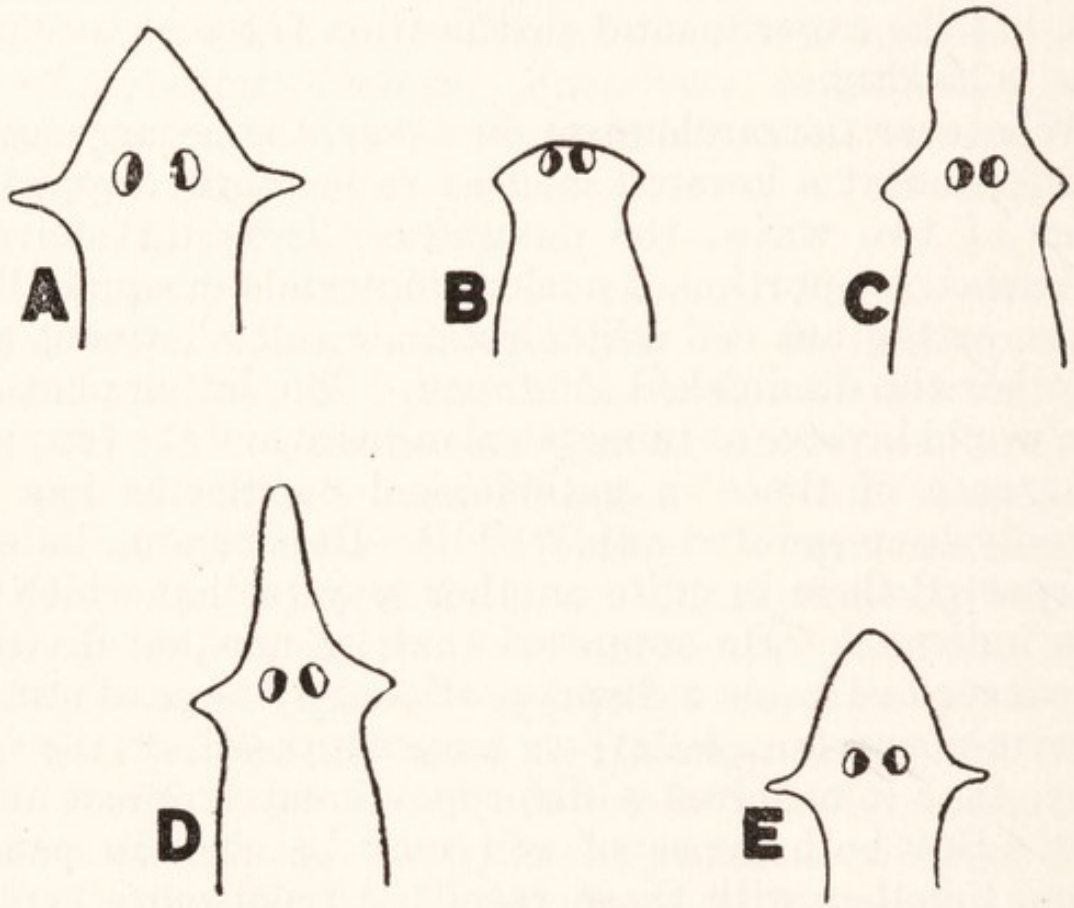


FIG. 33.—A. Planarian with normal head. B. Effect of from sixteen to twenty days' immersion in dilute solutions of alcohol. C and D. Over-development of median region of head during subsequent acclimation. E. Approach to normal head-form after restoration to water. (After Child.)

in more extreme cases, head-formation is altogether inhibited. Child also attributes the cyclopean fishes obtained by Stockard,[478,479] as a result of treatment of embryo with magnesium salts, to similar differential retardation of the growth of the median portion of the head. Under conditions which permit acclimation to occur a differential compensatory acceleration takes the place of differential inhibition in *Planaria,* and excessive over-development of the central portion of the head results. We have inferred

that by selection in reiterated repair, cells are produced which are so adapted to the high autocatalyst-level of the environment that they are enabled to multiply under conditions which totally inhibit the multiplication of the normal cell-types. So also we must suppose that in Child's experiments by the selective effect of prolonged exposure to the narcotic a type is produced which is adapted to its presence and which consequently is able to predominate over the other cells so long as the inhibiting conditions prevail. The observations of Galeotti suggest one means whereby such adaptation might occur.

The synthesis of cytoplasm is a process which is not completed until long after the cessation of cell-division, and in certain tissues, for example muscular tissue,[266] the later growth is almost entirely growth of cytoplasm. Now in any chemical equilibrium it is not absolute mass, but concentration, or mass per unit volume, which determines the station of final equilibrium. Corresponding to this fact we find that cytoplasm in young cells is more dilute than it is in older cells and the embryonic tissues are much richer in water than the corresponding tissues of the adult.[92,128,300,380] Equilibrium between nucleus and cytoplasm is but slowly attained and in embryonic tissues we find it in process of attainment. Cramer and Pringle, however, see in the differing water-content of embryonic and adult tissues a reason rather than a consequence of rapid cell-production.[82,83] They argue that the greater water-content implies less organic content and consequently less consumption of nutrients for the manufacture of embryonic tissue; for this reason, they urge, protoplasmic synthesis is rapid in the embryo, slow in the adult. They have found that the water-content of cancer tissue is similarly high and they consider that cancer is enabled to grow at the expense of the host because, by this peculiarity, it is enabled to produce new cells with more economy.

It would be difficult to assign a reason for the total cessation of the growth of the adult on this basis, for if

rate of growth were inversely proportionate to the nutrients consumed in the manufacture of protoplasm then, in comparison with the rate of growth of cancer tissue, the tissues of an adult not afflicted with cancer should still be increasing at a very perceptible rate. But a more fundamental difficulty may be urged. It has been shown by Aron[20,21] that the calorific value of tissue decreases during starvation, and that this decrease of calorific value is attributable to an increased water-content. After the readmission of food a decided enhancement of growth-rate is observed (Chapter IX) and so far as this goes it appears to be what Cramer's hypothesis would lead us to expect. Actually, however, this is not the case, for the tissue laid down during this period of enhanced growth-rate is not the dilute tissue which is characteristic of starvation, but the more concentrated cytoplasm characteristic of the age and stage of growth of the animal before starvation. Rate of growth is thus clearly perceived to be determined, not by the expenditure requisite to accomplish growth, but by the defect of growth which obtains at the moment. Embryonic tissues, tissues which have been subjected to inanition, and cancer tissue, grow rapidly because they are far from the equilibrium which is attained at the conclusion of growth. Dilution of the cytoplasm enhances growth-rate as dilution of its products accelerates the rate of any chemical reaction, because the restraining effect of the products themselves has been diminished. Physiologically youthful tissues are dilute because the full concentration of the products of their growth has not yet been attained and for the same reason they grow rapidly. It has also been shown that actively proliferating cancer cells are relatively rich in potassium and poor in calcium content, while old and necrotic cancer cells are relatively rich in calcium and poor in potassium,[32] but here, again, we have a difference which obtains between young and old cells of all types, and there is no reason to suppose that it is other than a secondary consequence of rapidity of growth.

Thus the development of cancer may be regarded as a phenomenon of hyper-differentiation of the cells of the host, brought about by repeated selection of types suitable for multiplication under conditions which inhibit the multiplication of cells of the host. The increase of autocatalyst due to the cells of the tumor itself must, however, render the nutrient medium even less suitable than before for nuclear synthesis in the cells of the host, so that senescence of the host should be accelerated by cancer. This effect would be most intense locally and radiate outwards from the cancer tissue, forming an autocatalyst gradient descending from within outwards. The maintenance of normal nuclear ratios by the tissues immediately surrounding the tumor will thus be rendered more and more difficult. It is probably to this factor that the invasive quality of malignant tumors must be ascribed and its magnitude will depend simply upon the difference between the prevailing nuclear ratios in the adjacent tissues and the nuclear ratio which obtains in the neoplasm. As Whitman has pointed out,[514] increase of the rate of multiplication of any tissue by itself would merely lead to hyperplasia and not to invasion and destruction of adjacent tissue. But if the rate of multiplication be sufficiently rapid then this second effect will supervene also and hyperplasia will be succeeded by invasion. For the same reason we can understand why cancer is so preeminently a disease of old age, because the increasing senescence of the normal tissues renders the advancement of their senescence to the point of incompatibility with life a comparatively easy matter, and their invasion by the tumor tissue is facilitated.

In this connection the experiments of Calkins[54] on the effect of cancer tissue extracts upon the vitality of cultures of infusoria (*Didinium*) are particularly suggestive. We have seen in Chapter IV how an increase of autocatalyst-level may inhibit multiplication, or even render impossible the maintenance of normal nuclear ratios in in-

fusorial cultures. We have seen also that the autocatalyst-level in tumor tissues must be exceptionally high. Now Calkins finds that the division-rate of infusoria is much reduced by emulsions of cancer tissue (Flexner-Jobling Carcinoma). With a single dose the average division-rate for 15 days was 1.84 divisions per day per individual, while that for the control series was 2.48 per day. With a double dose the division-rate was 1.38 per day, while that of the control was 2.55. The death rate for the single dose series was the same as that for the control, but in the double dose series it rose to 40 per cent. of all the animals treated, as against 16 per cent. for the controls which were treated with emulsions of normal skin. The effect was not due to poisons elaborated by necrotic tissues for, on the contrary, extract of necrotic tissues actually stimulates, instead of diminishing the division-rate.

The rate of growth of mice which spontaneously develop cancer has been compared throughout the duration of their lives with the rate of growth of animals derived from the same litters which did not develop spontaneous cancer.[424] The differences of growth-rate are not great, but they are of the same type as those displayed by unusually long-lived animals (Chapter VII). That is, the early growth tends to be unusually rapid, while late accretions of tissue do not occur until cancer itself supervenes. This may be interpreted to mean that in animals which do not develop cancer a descent of the nuclear ratio sufficiently small to be safe and merely leading to accretion of a normal type of tissue, anticipates and in a measure forestalls the development of cancer, while in a certain proportion of animals in which this late accretion of normal tissues does not occur, a more drastic reduction of the nuclear-cytoplasmic ratio leads to the production of an invasive neoplasm. The fact that the animals which ultimately develop carcinoma belong to a definable growth-type lends further confirmation, if any were needed, to the view that the tendency to develop neoplasms is governed by hereditary factors.

CHAPTER XI

THE INFLUENCE OF SPECIAL AGENCIES ON GROWTH

1. Lecithin.—The administration of lecithin to animals, by mouth or subcutaneously, leads to a retention of sulphur, phosphorus and nitrogen, and the urinary output of uric acid is diminished.[25] Remarkable effects are elicited in the blood by subcutaneous administration. The erythrocyte count is increased and leucocytosis is observed. The leucocytosis which occurs in response to injection of other substances is also increased by the simultaneous administration of lecithin. The toxic effects upon heart muscle which are exerted by a variety of drugs, for example alcohol, chloral hydrate, ether, chloroform or muscarin, may be partially counteracted by the intravenous administration of an emulsion of lecithin,[227] doubtless owing to solution of the drugs in the emulsion circulating in the blood and consequent prevention of their absorption by the tissues.

The fact that emulsions of lecithin in water are capable of holding in solution or suspension a variety of substances which are otherwise insoluble, as, for example, the higher fatty acids and cholesterol, renders it a difficult matter to ascertain whether the effects which have been enumerated, as well as those about to be described, are due to lecithin itself, or to associated impurities in the preparations employed. In none of these experiments was the lecithin subjected to any very elaborate degree of purification and, furthermore, it was invariably associated with cephalin, since it was prepared by ether extraction from the yolks of eggs. We are therefore uncertain at the present moment whether any or all of the metabolic and growth effects which have been ascribed to lecithin, are in reality attributable to this substance or to cephalin or, on

the other hand, to associated impurities which may separately exert diverse effects. It will be recollected that Devloo found "bios" associated with lecithin,[97] but it appears highly improbable, from what we know of the properties of this substance, that it is identical with lecithin. We should infer, rather, that this substance is readily absorbed or dissolved by lecithin and hence tends to become associated with it during the processes employed in the separation of crude lecithin. The potency of this substance, in comparison with its mass, precludes, indeed, the idea that it can be identical with lecithin, but the probable presence of "bios" in the preparations which were employed in the experiments about to be described must constantly be borne in mind.

It was shown by King that the growth of tadpoles is accelerated by the oral administration of lecithin.[88,89,218] Pigmentation of the skin is inhibited, even when the diet, in the absence of lecithin, would be such as to promote an unusual degree of pigmentation.[210] Recent investigations, however, indicate that this latter effect may have arisen, not through failure of the tadpoles to manufacture pigment, or from inhibition of the growth of any particular tissue, but from retraction of the chromatophores in the skin, either owing to a direct action of lecithin upon the chromatophores, or to an indirect action through an endocrine organ.[202,456]

If lecithin is added to sea-water in which sea-urchin embryos are undergoing development, their further development is inhibited.[395] The experiment cannot be performed until the embryos have escaped from the chorion, because this membrane is dissolved by lecithin and the cleavage cells tend to fall apart, so that development is hindered for purely mechanical reasons. If, however, the embryos are exposed to sea-water containing 0.17 per cent. of lecithin at almost 24 hours after fertilization, when they are free-swimming blastulæ, the inhibition of development is remarkable. In fact after the preliminary stages of

gastrulation (which do not occur until normal larvæ have developed into plutei) an actual reversion of development appears to take place, the archenteron disappears and a somewhat enlarged blastula is reformed, which survives for some time but never develops any further. This observation has been somewhat slightly dismissed by Child as constituting no evidence "of anything more than a toxic effect of the lecithin preparation,"[69] p. 455, and he points out that the establishment of a real reversal of development would necessitate much more extensive investigations. Doubtless this is very true, but it is difficult to avoid gathering the impression that the work of development has been in part undone when one perceives that the archenteron, which was previously very evident, has disappeared and that the organism eight days after fertilization has been reconverted to the form which it exhibited only twenty-four hours after fertilization. If this is a "toxic effect" it is none the less striking, more especially when we consider that the motility of the larvæ, which is very sensitive to poisons, is fully preserved and that these larvæ, in which development was arrested, lived just as long as those in which development had proceeded normally. But in any case, and quite apart from the question whether or not an actual reversal of development has occurred in this instance, it is very evident that so far from being facilitated, as in the tadpole, the development of the sea-urchin at this early stage is decidedly inhibited by lecithin.

Now we have seen that the catalyst in any autocatalytic reaction has the peculiar property of accelerating the reaction at one stage (during the autokinetic phase) and retarding it at another (the autostatic phase). Hence the discovery of a substance capable of exerting totally opposed effects upon growth gave rise to the supposition that lecithin itself might be the nuclear autocatalyst of growth.[395] The considerations which we can now perceive to be opposed to this opinion are the same as those which

militate against the view that the "bios" of Wildiers is identical with lecithin. Its distribution is not identical with or proportionate to that of lecithin. The "bios" is demonstrable in extracts of tissue, and the nuclear autocatalyst is demonstrable in culture fluids which contain exceedingly small quantities of any phospholipin, while the potency of lecithin itself is not at all of the magnitude we would expect if the active material in these extracts and culture fluids consisted of the traces of phospholipin which they presumably contain.

But when we recall the tendency of "bios" to be associated with lecithin and reflect upon the fact which this implies, that the "bios," or nuclear autocatalyst, is soluble in lecithin, then, it appears, we have a clue which may enable us to interpret the peculiar diversity of the effects of lecithin at different stages of growth. If we suppose that lecithin extracts the nuclear autocatalyst from the embryo of the sea-urchin and from the tissues into the circulation of the tadpole, then in each case a reduction of the pericellular concentration of autocatalyst must occur. At the very early stage of development constituted by the sea-urchin blastula or gastrula, the cells are most probably in the autokinetic phase[a] of the growth-curve and reduction of the autocatalyst level in their pericellular fluids must very greatly retard their development or even render it impossible. In the same way we can understand why lecithin retards the multiplication of isolated *infusoria*.[48] In the tadpole, which represents a more advanced stage in the curve of autocatalysis, nuclear synthesis has probably attained the autostatic phase and reduction of the nuclear autocatalyst in the pericellular fluids must accelerate development.[b]

[a] The terms "autostatic" and "autokinetic" were used in a different sense in the article to which reference has been made.

[b] Lecithin, being a colloidal substance, would not readily leave the circulation unless previously modified by chemical changes. Thus it does not penetrate the fertilization membrane of the sea-urchin egg unless it is sufficiently concentrated to dissolve it.

The injection of lecithin emulsions directly into the substance of the tumors decreases the rate of growth of the Flexner-Jobling Carcinoma in rats.[412] This rapidly growing tissue, in which mitoses are frequent, is maintained in the autokinetic phase by the brevity of the intervals at which the nuclei are enabled to discharge their accumulations of autocatalyst, in consequence of the small nuclear ratio necessary to enable cell-division to occur. Withdrawal of the nuclear autocatalyst into an external li-

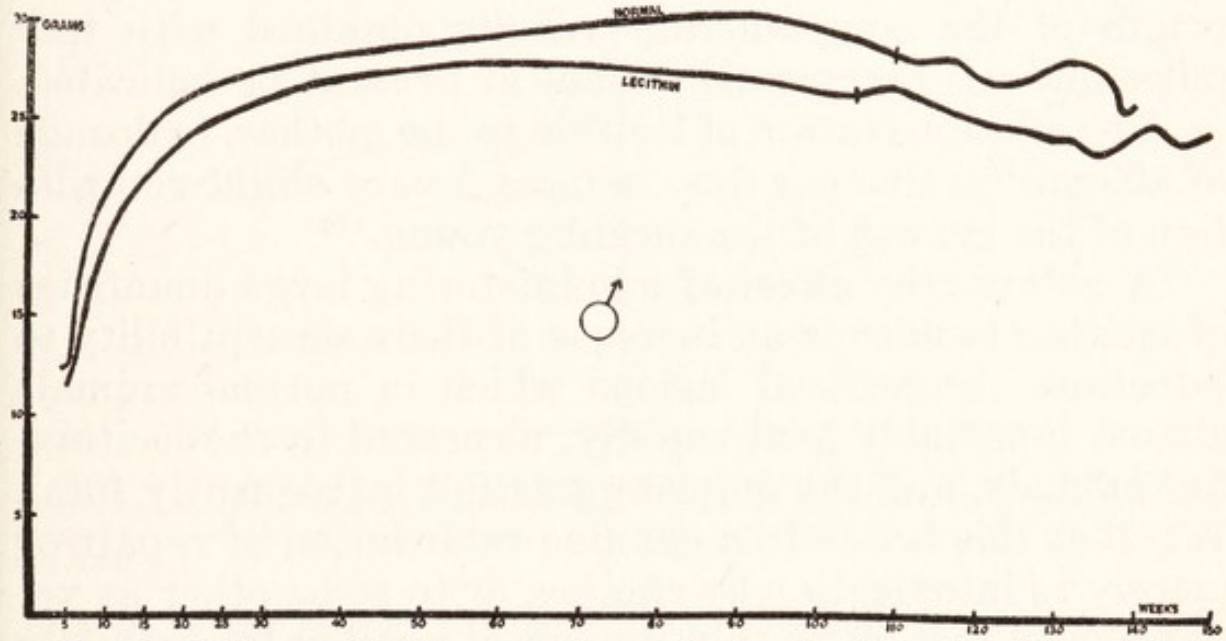


FIG. 34.—Influence of lecithin upon the growth of male white mice. Dosage 40 mg. per day. The vertical cross mark indicates average duration of life. (After Robertson and Ray.)

poidal phase, therefore, retards the rate of multiplication of the cells of the neoplasm. The retardation is not very marked, however, and is valueless therapeutically. Thus the delay in the increase of the diameter of the primary tumor is not comparable with the acceleration which may be induced by cholesterol (Fig. 35) and it is chiefly manifested in the diminution of the number and rate of growth of the metastases.

The oral administration of lecithin to mice in doses of 80 milligrams per day, beginning at 4 weeks after birth, leads to a decided retardation of growth,[404,418,419] amounting, both in males and females, to six times the "probable error" of the differences between the animals which re-

ceived the lecithin and the controls, chosen without selection from the offspring of the same breeding stock, which received the same diet without the addition of lecithin (Fig. 34). It is not easy to interpret this result, the more especially because it is highly uncertain what proportion, if any, of the lecithin administered by mouth is actually absorbed into the circulation as such, since lecithin is very readily split by pancreatic lipase. If a large proportion of the lecithin is split, then the absorption of choline might conceivably exert a toxic effect. In this uncertainty the origin of the contradictory results obtained with tadpoles and with mammals cannot at present be indicated.

The administration of lecithin to the mother, in dosage of 100 milligrams per day, induces a very slight retardation of the growth of the suckling young.[416]

A noteworthy effect of administering large quantities of lecithin to mice is an increase of their susceptibility to infection. Superficial lesions which in normal animals almost invariably heal rapidly, ulcerated in the lecithin-fed animals, and the outcome was not infrequently fatal. Whether this is due to a genuine retardation of repair or merely to intoxication by choline, or to some other as yet unknown effect of lecithin, cannot at present be definitely established, but it will be noted that wound-repair normally involves the production of relatively differentiated tissues.

Against the view that the above effects may actually be attributable to cephalin rather than to lecithin, may be cited the fact that nervous tissue which has been thoroughly exhausted by repeated extractions with acetone is devoid of effect upon the growth of the white mouse even when administered in very large doses.[423] The tissue thus prepared retains the greater part of its lecithin and cephalin, but lecithin is much less abundant in nervous tissues than cephalin. Moreover, lecithin prepared from the anterior lobe of the pituitary body by extraction with alcohol (and therefore comparatively free

from cephalin) was found to exert the same effect upon the growth of mice as the crude lecithin and cephalin mixture which is obtained from eggs by extraction with ether and precipitation with acetone.[404]

2. **Cholesterol.**—Attention was first drawn to the action of cholesterol upon growth by the remarkable effect of administering this substance, in relatively large dosage, to rats which have been inoculated with Flexner-Jobling Carcinoma. The administration of emulsified cholesterol by direct injection into the primary tumor greatly ac-

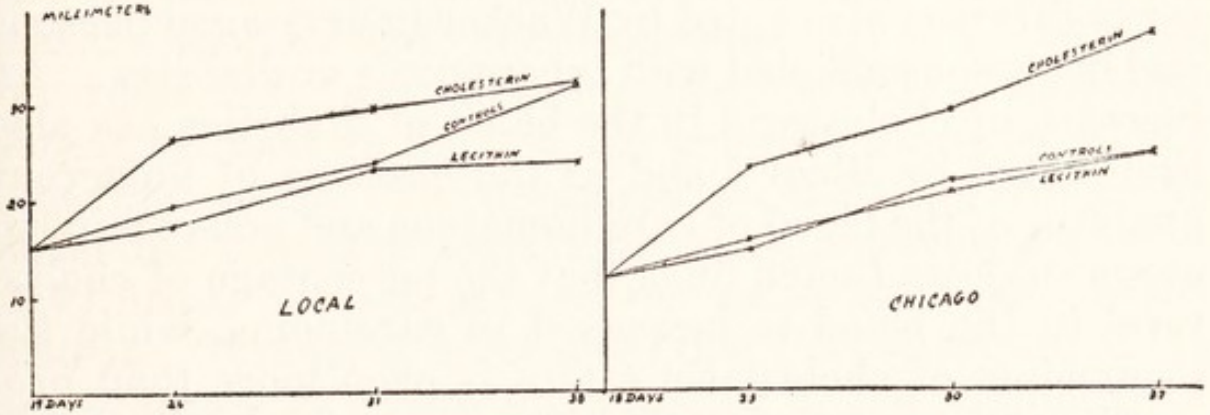


FIG. 35.—Showing the influence of lecithin and cholesterol upon the growth of Flexner-Jobling Carcinoma inoculated into white rats. Dosage 20 mg. of lecithin or 39 mg. of cholesterol every second or third day, beginning with the eighteenth day. (After Robertson and Burnett.)

celerates its growth (Fig. 35). While the incidence of metastases is not increased in these experiments their rate of growth in those cases in which they did occur was much enhanced by the administration of cholesterol.[412]

This observation was subsequently varied in a striking manner by Sweet, Corson-White, and Saxon.[482] They employed for the purpose a strain of Flexner-Jobling Carcinoma which had never been known to yield metastases. The animals, after inoculation, were fed upon a diet to which cholesterol had been added to the extent of 200 milligrams per week. No less than 104 out of 116 animals which were thus treated developed metastases.

An important relationship of cholesterol to the growth and metastatic tendency of carcinoma is thus rendered evident, nor is the significance of the result in any way

diminished by the fact that on a diet deficient in lysine the formation of metastases did not occur even when cholesterol was added. Naturally, cholesterol could not be expected to take the place of this essential factor in the synthesis of protoplasm.

Now it has been shown by Wacker[506] that the cholesterol content of the fatty deposits in the subcutaneous tissues and mesenteries of persons who are afflicted with carcinoma averages no less than 66 per cent. greater than the cholesterol content of the fatty deposits in normal persons. An increase in the cholesterol content of reserve-fats was also noted by Wacker in very aged persons and in persons afflicted with tuberculosis or diabetes. An increase of cholesterol in the blood of diabetics has also been noted by Bloor[35] and as the outcome of numerous analyses of the blood of carcinomatous and non-carcinomatous subjects Luden finds that the percentage of cholesterol in the blood is increased in carcinoma, while the proportion of cholesterol esters is even more than proportionately reduced.[258,259] The cholesterol content of the blood is also increased in syphilis.

The excretion of cholesterol appears to be effected solely by the channel of the bile and a large proportion of the cholesterol thus discharged is reabsorbed.[124] The mechanism for the excretion of unchanged cholesterol is therefore very imperfect. Presumably some other means exists, at present unknown, of reducing the cholesterol content of the body, for otherwise we would rapidly accumulate cholesterol to the very great detriment of the welfare of our tissues.[16,17,24] If, however, advancing senescence of the tissues should reduce the ability of certain organs to destroy cholesterol such accumulation might readily occur, and the increased proportion of cholesterol in the fatty tissues of aged individuals is not improbably attributable to this source. The above-described experiments, however, demonstrate that such a condition would be favorable to the rapid growth of carcinoma and to the

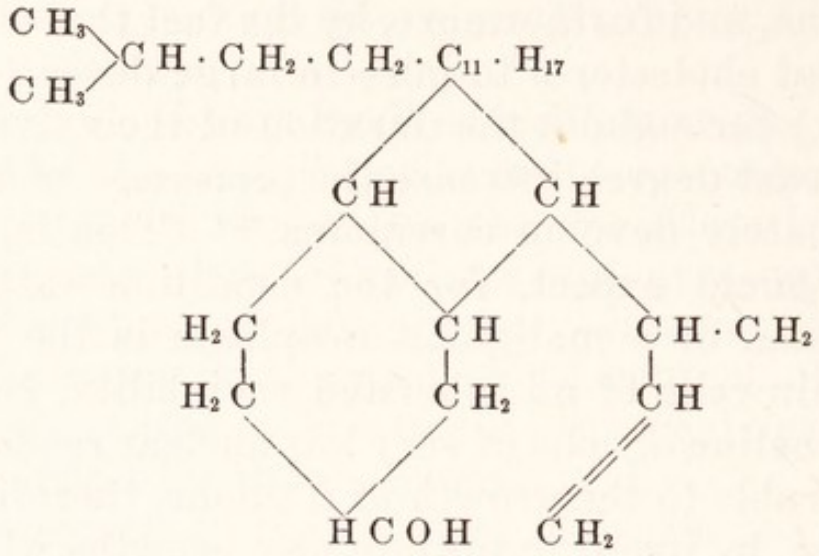
formation of metastases. That this is not the only factor which is concerned, however, is shown by the fact that diabetics and syphilitics do not necessarily develop carcinoma, and furthermore, by the fact that the administration of cholesterol to mice in large doses (40 milligrams daily) throughout the duration of their lives, does not in the least degree increase the percentage of animals which ultimately develop carcinoma.[420] This is, indeed, what we should expect, for the condition essential for the initiation of a malignant neoplasm is the possession by certain cells of an inherited variability, resulting in the production of cells of very low nuclear ratios. A condition favorable to the growth of a tumor, therefore, would not suffice, by itself, to initiate new growths which would not otherwise occur. The accumulation of cholesterol in the blood and tissues of aged individuals, however, may possibly affect our estimate of the age of incidence of carcinoma. It must be recollected that the estimation of the age of incidence of tumors is limited by the possibility of diagnosis. In other words, the necessary conditions precedent to the initiation of tumor-growth may conceivably be present long before the neoplasms attain dimensions sufficient to permit their diagnosis. Any condition, therefore, which favors rapid growth in neoplasms once initiated, may play an important part in determining our estimate of the age of incidence.

The addition of emulsified cholesterol to the culture medium very decidedly accelerates the reproduction of isolated infusoria,[c] so that here, again, as in the case of lecithin, the effect upon the multiplication-rate of isolated infusoria is similar to the effect upon the growth of carcinoma.[48]

The effect of cholesterol upon the growth of carcinoma is dependent upon its ability to form emulsions in water

[c] The experiments of Browder upon *Paramœcium* have recently been repeated and confirmed by the author upon *Enchelys*. At the date of writing these results have not yet been published.

containing soaps or other lipoids.[415] The formula ascribed to cholesterol by Windaus,[525] von Fürth[154] and others, is the following:—



from which it will be perceived that it contains a reduced hydroxy benzene ring. The integrity of this hydroxyl group is essential to the effect. Thus acetyl cholesterol and cholesteryl chloride, in which the hydroxyl group is replaced by acetyl and chlorine respectively, are devoid of any accelerative effect upon the growth of carcinomata. At the same time these compounds fail to form emulsions in hot sodium oleate solutions. Increase of the number of hydroxyl groups in cholesterol enhances its emulsifiability and leaves its effect upon the multiplication of isolated infusoria unaffected.[d] On the other hand metacresol, benzyl alcohol, hexahydrophenol and inositol, substances containing one or more hydroxyl groups associated with an aromatic nucleus, are all devoid of effect upon the growth-rate of inoculated carcinoma in rats.

The accelerative effect of cholesterol is therefore, in all probability, not directly attributable to its hydroxy benzene radicle, but since the presence of the hydroxyl group is essential for the emulsification of the substance and it can only be distributed among the tissues in the form of an emulsion, the integrity of its hydroxyl group

[d] Results not yet published. The preparation employed was the "oxycholesterol" of Lifschütz.[231]

is essential to enable cholesterol to reach the tissues which it affects.

The effect of cholesterol upon the growth of white mice when administered in doses of 40 milligrams daily consists of very great initial retardation (4th to 12th weeks) followed by a slight compensatory acceleration which never enables the animals to attain normal dimensions (Fig. 36). The difference of weight between the cholesterol-fed animals and the normal animals is, during

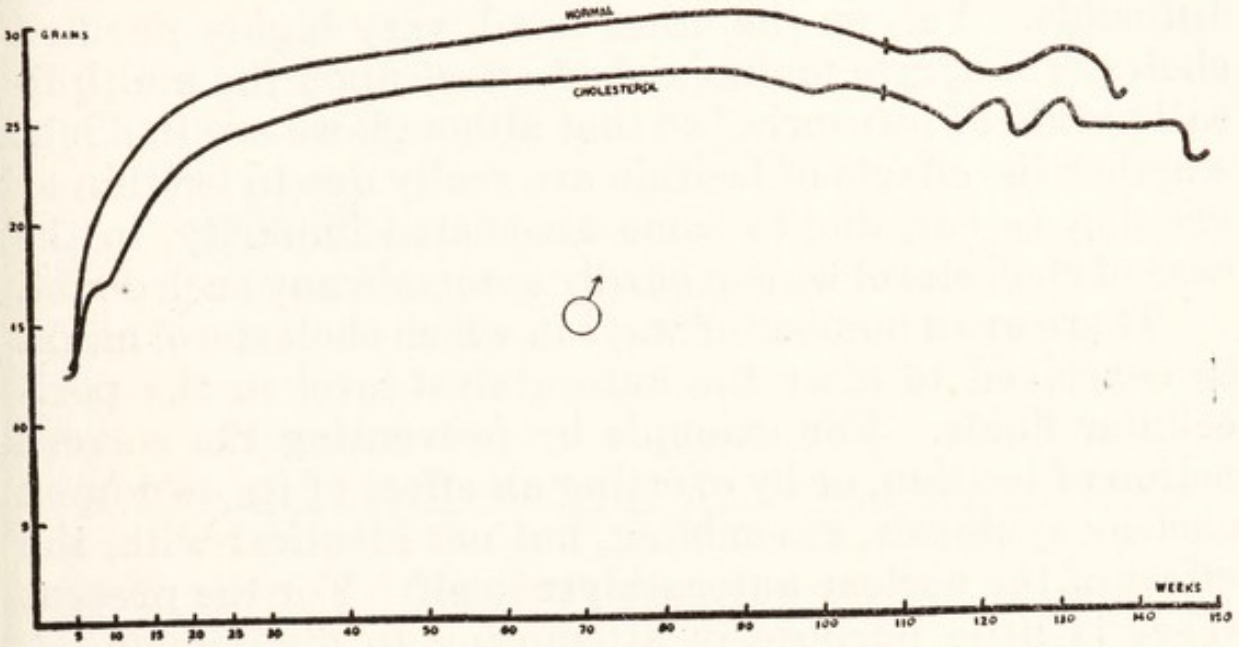


FIG. 36.—Influence of cholesterol upon the growth of male white mice. Dosage 40 mg. per day. The vertical cross mark indicates average duration of life. (After Robertson and Ray.)

the first 26 weeks after initiation of the diet at 4 weeks, no less than from 6 to 6.5 times the probable error of the difference, while subsequently it varies between 2.5 and 4.5 times the probable error.[405,418]

This is, of course, in harmony with the expectation we would form if the accelerative action of cholesterol upon the multiplicative rate of isolated infusoria and carcinoma cells were due to an effect tantamount to an increase of the nuclear autocatalyst in the pericellular fluids. We need not on that account assume that the nuclear autocatalyst is, in fact, cholesterol. The quantitative relations, as in the case of lecithin, display the falsity of any such assumption. The amount of cholesterol required to elicit

its effects is far greater than we have reason to suppose it would be if cholesterol were the nuclear autocatalyst. There is furthermore no evidence that animal cells are able to manufacture cholesterol.[e] Its relationship to the terpenes strongly suggests a vegetable origin. Again, cholesterol is insoluble in water and can only be contained in aqueous media in the form of colloidal indiffusible emulsions, while the occurrence of allelocatalytic effect shows that the nuclear autocatalyst is soluble in water and diffusible. Yet, on the other hand, very highly purified cholesterol exerts undiminished effect upon the multiplicative rate of infusoria,[f] so that although we are in doubt whether the effects of lecithin are really due to lecithin or are not, rather, due to some associated impurity, in the case of cholesterol we can hardly entertain any such doubt.

There are a number of ways in which cholesterol might be conceived to alter the autocatalyst-level in the pericellular fluids. For example by preventing the solvent action of lecithin, or by exerting an effect of its own upon nuclear synthesis, resembling, but not identical with, the effect of the nuclear autocatalyst itself. For the present there is little purpose in attempting to distinguish between these and other possible alternatives, for we lack the information necessary to make a choice among them. We may merely note the analogy of its effects to those of the nuclear autocatalyst and proceed to ascertain how far this analogy extends.

One effect of increasing the autocatalyst-level in the pericellular fluids would be, we should imagine, to encourage the production of relatively differentiated tissues. This, however, would be limited by the hereditary tendencies to vary among the cells of the organism. In the absence of such variation, no such favoring effect could be observed, but in any individual in which a wide range of

[e] The increase of blood cholesterol after the ingestion of cholesterol-free fat, noted by Chauffard, Laroche and Grigaut,[64] does not necessarily imply that the cholesterol was not previously present in the tissues.

[f] Results not yet published.

variation of the nuclear-cytoplasmic ratio is possible, cholesterol should encourage late accretions of tissue of which the development of cancer would constitute an extreme manifestation. Individuals endowed with variable cells would thus come to depart widely from those composed of less variable cells and in any group of animals the spontaneous differences between individuals would tend to become exaggerated in the later stages of develop-

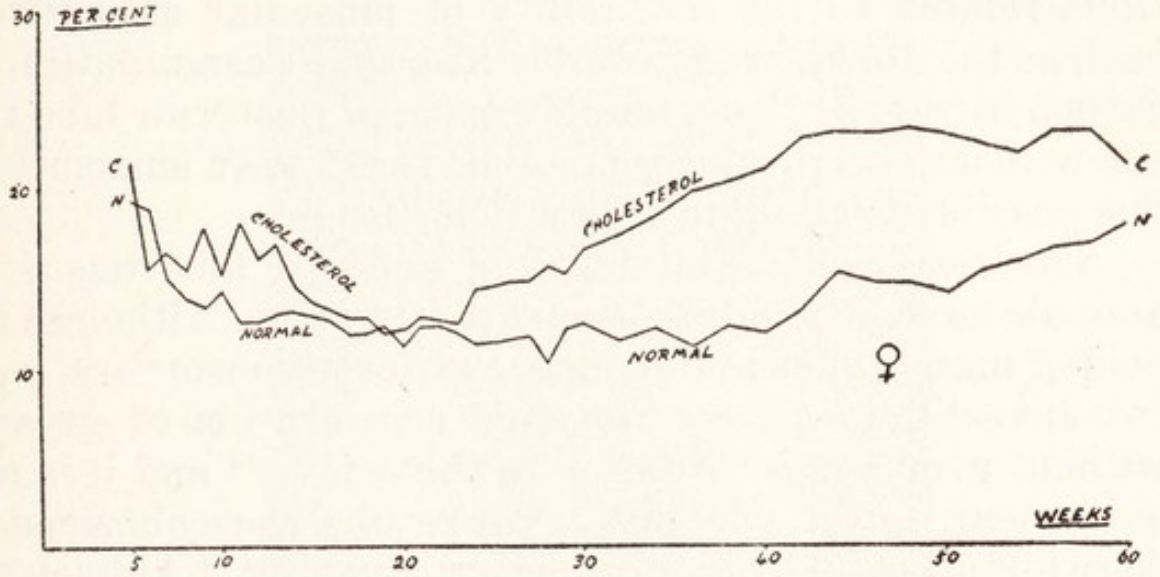


FIG. 37.—Comparison of the variability (ratio of standard deviation to mean weight) of female white mice receiving cholesterol (40 mg. per day by mouth) with that of normal female white mice.

ment.[g] Now cholesterol-fed animals, especially the females, become abnormally variable in weight after the first six or seven months. This is illustrated by the accompanying curve of comparative variabilities (Fig. 37), the magnitude of the variability having been computed in the usual statistical manner.[h]

3. **The Anterior Lobe of the Pituitary Body.**—The discovery by Marie[272] of the abnormalities of the pituitary body which accompany gigantism and acromegaly in

[g] This, of course, would be true only so long as the autocatalyst-level did not rise so high as to suppress the multiplication of all of the cell-types possible to the species.

[h] As the ratio of the square root of the mean square of the deviations from the average, to the magnitude of the average itself. Deviations from the average, equal to or less than the magnitude of the variability thus determined, will be displayed by two-thirds of the individuals comprising the group,[94] p. 16.

human beings has led many investigators to endeavor to reproduce these effects by the administration of anterior lobe tissue to growing animals. The clinical observations of Cushing and others, which are summarized in Cushing's Monograph,[85] unite in showing that the excessive development of bony and epithelial tissues which occurs in these cases is attributable to hyperfunctioning of the anterior lobe. The posterior lobe has quite other functions related to the irritability of muscular structures, such as the uterus, and possibly also to the assimilation of carbohydrates. The administration of posterior lobe tissue to mammals produces no other result than emaciation, due possibly to the diarrhœa which ensues.

The results of administering anterior lobe tissue to animals have, in general, been disappointing. Although repeated many times and by many different observers, only two investigators have reported acceleration of growth without previous retardation in the rat[163,274] and it is not quite clear, as yet, why divergent results were obtained in these instances. All other observers, Cushing,[84] Aldrich,[9,10] Wulzen,[531] Schafer,[440] Pearl,[369] Maxwell,[279] and Robertson and Ray[418] are agreed that the early effects of administration by mouth to growing rats (Aldrich, Schafer), fowls (Wulzen, Pearl, Maxwell), or mice (Robertson and Ray) are confined to a slight retardation of growth in weight. Subsequently to this, however, Schafer, Wulzen and Robertson all noted an acceleration which partially, but not wholly, compensates for the initial retardation. The whole effect of administering to mice 125 milligrams of fresh anterior lobe of the ox-pituitary (one-twelfth part of a gland) *per diem* is merely to retard their growth during the first seven months to an extent equal to twice the "probable error" of the difference of weight of the experimental and the control animals in the males, and four times the "probable error" in the females[418] (Fig. 38). The effect can hardly be other than a real one since it is attested by so many observers employing such diverse

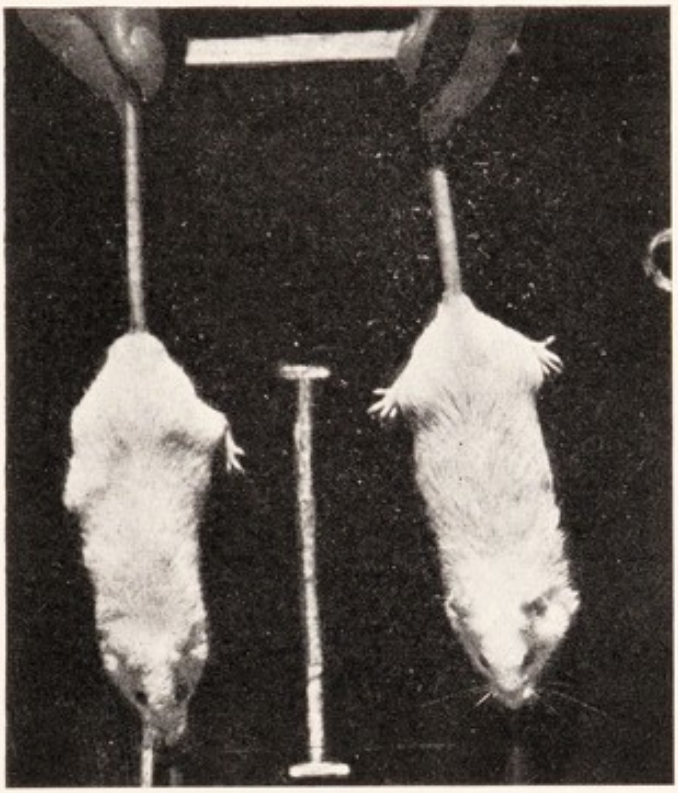


FIG. 38.—Approximation to acromegaly in the white mouse. On the left, a normal male, 409 days old and 30 grams in weight. On the right a pituitary-fed male, 396 days old and 37 grams in weight. It will be noted that the pituitary-fed mouse is of approximately the same linear dimensions as the normal, yet it is nearly 25 per cent. heavier, and of a distinctly sturdier and more compact build. Dosage: 0.125 grams of fresh anterior lobe tissue (ox) per mouse per day.

subjects of experimentation, and in the case of female mice, at all events, the retardation of growth exceeds the "probable error" by a sufficient amount to establish its reality. Still this effect is much less than we should anticipate from the clinical accounts, although an approximation towards acromegaly is indicated by the relatively compact build of the animals which received pituitary tissue throughout the duration of their lives.

There are several reasons why such a result is not altogether surprising. In the first place the anterior lobe of the pituitary body is one of the tissues most richly supplied with blood vessels. There is evidently a very rapid interchange of materials between the blood and the tissues of the gland, so that the amount of active material contained in the gland at the moment of death may represent only a very small proportion of that which is produced in twenty-four hours. Again, there is no necessary reason why administration of any endocrine organ tissue by mouth should exert the effects of the gland *in situ*. The fact that thyroid tissue does so must not mislead us into supposing that such a result is always to be expected, for the active material in the tissue may be destroyed by the digestive fluids, or it may not be capable of passing through the intestinal epithelium. The results obtained with pituitary (anterior lobe) tissue which have been cited tend to indicate that the active material in this instance is not totally destroyed by digestive juices, nor totally unassimilable from the digestive tract, but either of these factors nevertheless may have contributed to reduce the effect of the administration.

Very different results have been obtained in consequence of the administration of pituitary (anterior lobe) tissue to amphibians. Smith[455,456] and Allen[11] working with tadpoles, Uhlenhuth[497] with *Amblystoma* larvæ, and Huxley and Hogben[202] with Axolotls, have all obtained increase of growth in larval amphibians fed with anterior lobe tissue. The dosages employed in these experiments,

however, far exceeded those employed in the mammalian experiments above cited, for the amphibian larvæ were fed upon a diet consisting exclusively of the anterior lobe tissue, a procedure which is, naturally, impossible in the case of rats or mice. This mode of administration, while it has the advantage of ensuring maximal effects, is associated with the disadvantage that the experimental and control diets are no longer comparable in other respects. However, the control diets (earthworms in Uhlenhuth's experiments, meat in the experiments of Huxley and Hogben) differ in the various experiments so widely that the uniform result obtained probably points to a specific effect of the pituitary tissue. The experiments of Uhlenhuth will be further considered in connection with the effects of tethelin, which are about to be described.

The lack of correspondence between the results obtained with amphibian larvæ and those obtained with mammals may arise from several sources. In the first place there is the immense disparity of the dosages employed. Still more important, however, is the difference in the stages of development attained by the two types at the time of administration. It is impossible to administer dietary additions to mammals during the periods in which cellular multiplication is most rapid unless, indeed, it chances to prove possible to influence the growth of the young through the maternal circulation. In the case of the pituitary no effect of tethelin upon the young can be elicited through administration to the mother, although from the moment that the young obtain access to the mother's food, even although they may still derive the greater part of their nutrition from the mother's milk, the characteristic retardation of growth occurs.[417] By this time, however, the majority of the cells comprising the animal are already in the autostatic phase. Inspection of the normal curve of growth shows that the second growth-cycle is nearly completed and the third is far advanced.[402] In amphibian larvæ, on the contrary, the opportunity

is presented of investigating the effect of pituitary tissue upon earlier and possibly autokinetic phases of growth. We shall see that the effect of the active constituent of the pituitary (tethelin) is, in such types of growth, invariably accelerative.

The effect of hypodermic administration of emulsified pituitary (anterior lobe) tissue to rats inoculated with carcinoma affords a striking illustration of this fact. The

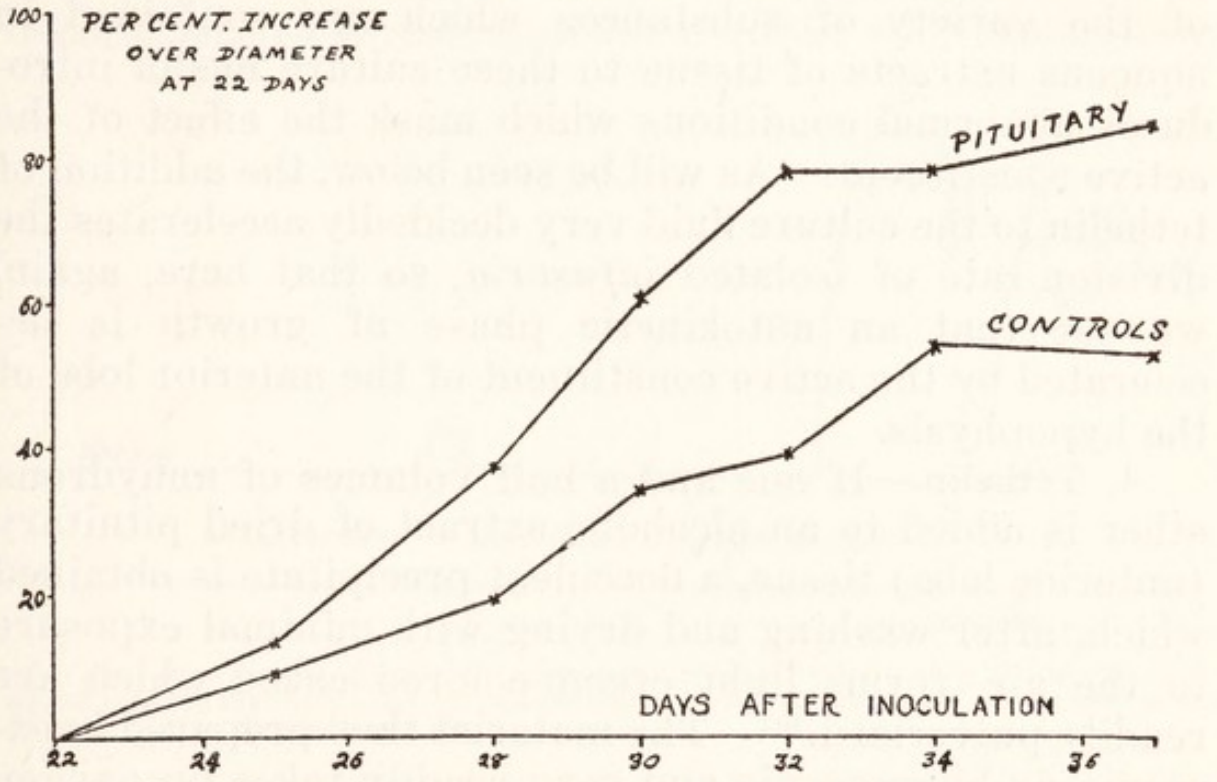


FIG. 39.—Influence of fresh pituitary tissue (anterior lobe of the ox-pituitary) upon the growth of Flexner-Jobling Carcinoma inoculated into rats. Dosage: 0.5 grams of emulsified tissue per rat every second or third day, beginning with the twenty-second day. (After Robertson and Burnett.)

neoplasm, by the very fact that it is so actively proliferating, is evidently in the autokinetic phase. The administration of 0.5 grams of emulsified tissue every other day to the animals 22 days after inoculation produces a most remarkable acceleration of the growth of the primary tumor.[413] This is illustrated in Figure 39. Fifteen days after the commencement of administration the diameter of the tumors in the control animals had increased 52 per cent., in the treated animals 84 per cent. Recollecting that the volume of tumor tissue varies as the cube of the mean diameter, these increases of diameter correspond to

volume increases of 250 per cent. and 523 per cent., respectively, in other words, the tumors in the animals which received pituitary emulsion grew twice as rapidly as the tumors in the animals which did not receive pituitary emulsion.

It is stated by Shumway[328,450] that the division-rate of protozoa is not increased by the addition of anterior lobe extract to culture medium. It is possible that the addition of the variety of substances which are contained in aqueous extracts of tissue to these culture media introduces abnormal conditions which mask the effect of the active constituent. As will be seen below, the addition of tethelin to the culture fluid very decidedly accelerates the division-rate of isolated *infusoria,* so that here, again, we see that an autokinetic phase of growth is accelerated by the active constituent of the anterior lobe of the hypophysis.

4. **Tethelin.**—If one and a half volumes of anhydrous ether is added to an alcoholic extract of dried pituitary (anterior lobe) tissue, a flocculent precipitate is obtained which, after washing and drying with minimal exposure to the air, forms light cream-colored cakes which are readily pulverized.[403] The material thus prepared is exceedingly hygroscopic and very readily takes up oxygen and darkens. The iodine-number is simultaneously diminished. This oxidation is accelerated by traces of water vapor, a combination of qualities which renders the preparation of the material a matter of exceptional difficulty and necessitates the most scrupulous care in avoiding contamination by water vapor or exposure to air while drying. It is not known, however, whether the physiological effects of the substance, which are about to be described, are adversely affected by oxidation or not. Material of the highest obtainable purity having always been employed for experimental purposes, the effect of oxygen absorption upon its activity, if any, has been avoided, and not sought.

There is nothing to show that this substance is a chemical individual. It may be a mixture of substances, and, in fact, the phosphorus which was originally supposed to be an essential constituent has now been found to arise in part from an admixture of phosphates, which may be removed by adequate purification[i] without any loss of physiological activity. Although the method of preparation suggests probable contamination by inositol, the undecomposed substance does not yield the color reactions of

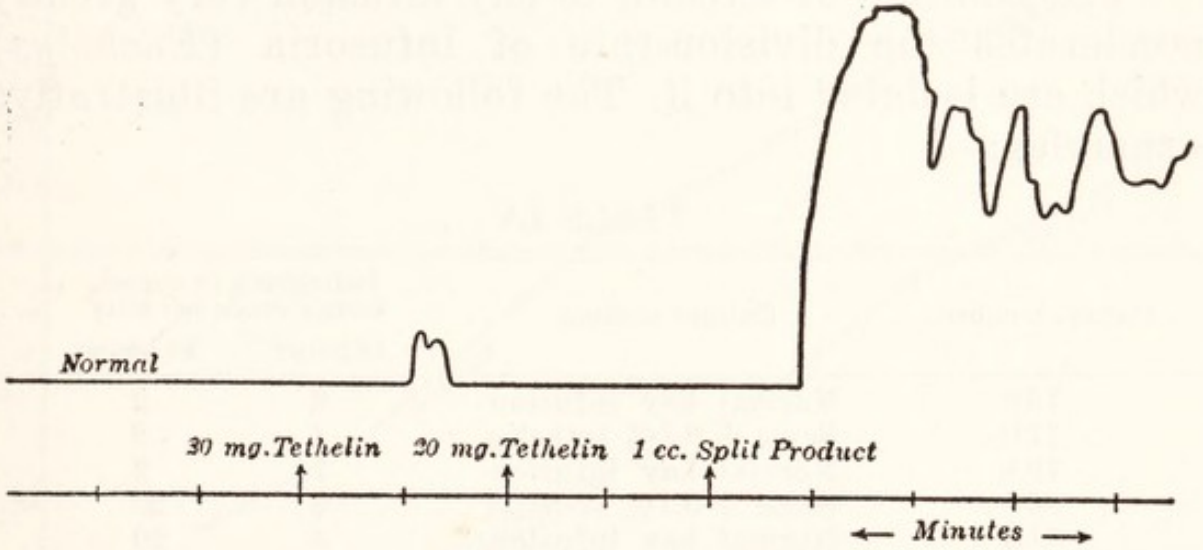


FIG. 40.—Tracing showing the absence of effect of tethelin upon the uterus of the guinea pig, and the effect of split products obtained by gentle heating of the tethelin in acid solution. (After Schmidt and May.)

inositol. Nevertheless, after hydrolysis, not only may Scherer's reaction be obtained in the mixture of resultant products, but crystalline inositol has been separated from them and identified by its melting-point. The hydrolyzed substance also causes contraction of the virgin guinea-pig uterus[441,442] while the unaltered material has no effect upon the uterus (Fig. 40). This fact is of peculiar significance in view of the anatomical relations of the anterior lobe, which suggest that it supplies material to the posterior lobe, possibly for the elaboration of its characteristic hormone.[180] The substance itself exerts no immediately obvious physiological effects when injected intravenously, beyond a slight fall of blood pressure which

[i] Unpublished observations.

is possibly attributable to contamination by the depressor substances which are found in a wide variety of tissue-extracts[26] and a slight diminution of diuresis previously induced by diuretic agents.[320] From its effect upon the growth of animals this substance has been designated *tethelin* ($\tau\varepsilon\theta\eta\lambda\omega\varsigma$ = growing).[j]

The content of tethelin in the pituitary gland of the ox is, in the average, ten milligrams. The substance is completely soluble in water.

The addition of tethelin to hay infusion very greatly accelerates the division-rate of infusoria (*Enchelys*) which are isolated into it. The following are illustrative examples:—[k]

TABLE LV

Culture number	Culture medium	Individuals produced from a single cell after	
		18 hours	24 hours
76B	Normal hay infusion	2	2
77B	Same + 0.1% tethelin	4	8
79A	Normal hay infusion	1	2
80A	Same + 0.1% tethelin	8	28
81A	Normal hay infusion	4	20
82A	Same + 0.1% tethelin	10	96
81B	Normal hay infusion	4	16
82B	Same + 0.1% tethelin	15	78
85A	Normal hay infusion	2	—
86A	Same + 0.1% tethelin	16	—
85B	Normal hay infusion	2	—
86B	Same + 0.1% tethelin	30	—
93A	Normal hay infusion	—	1
95A	Same + 0.1% tethelin	—	4
93B	Normal hay infusion	—	2
95B	Same + 0.1% tethelin	—	4

The subcutaneous administration of tethelin to rats which are inoculated with Flexner-Jobling Carcinoma

[j] The process employed in the manufacture of tethelin has been patented in the United States and Great Britain and the patents are held in trust by the Regents of the University of California. The proceeds arising from a small royalty on sales, after deduction of the expenses involved in procuring the patents, are devoted by the terms of the trust wholly to the prosecution of medical research under the direction of the Regents of the University of California.[408] The substance is manufactured by the H. K. Mulford Company, of Philadelphia.

[k] Not previously published.

leads to an extraordinary acceleration of the growth of the neoplasm.[414] The dose employed was 30 milligrams, administered every second day, starting upon the 21st day after inoculation. The results are depicted in Figure 41. Fourteen days after the commencement of administration the average volume of the tumors in the control animals had increased 272 per cent., while in the treated animals it had increased 445 per cent., or nearly twice

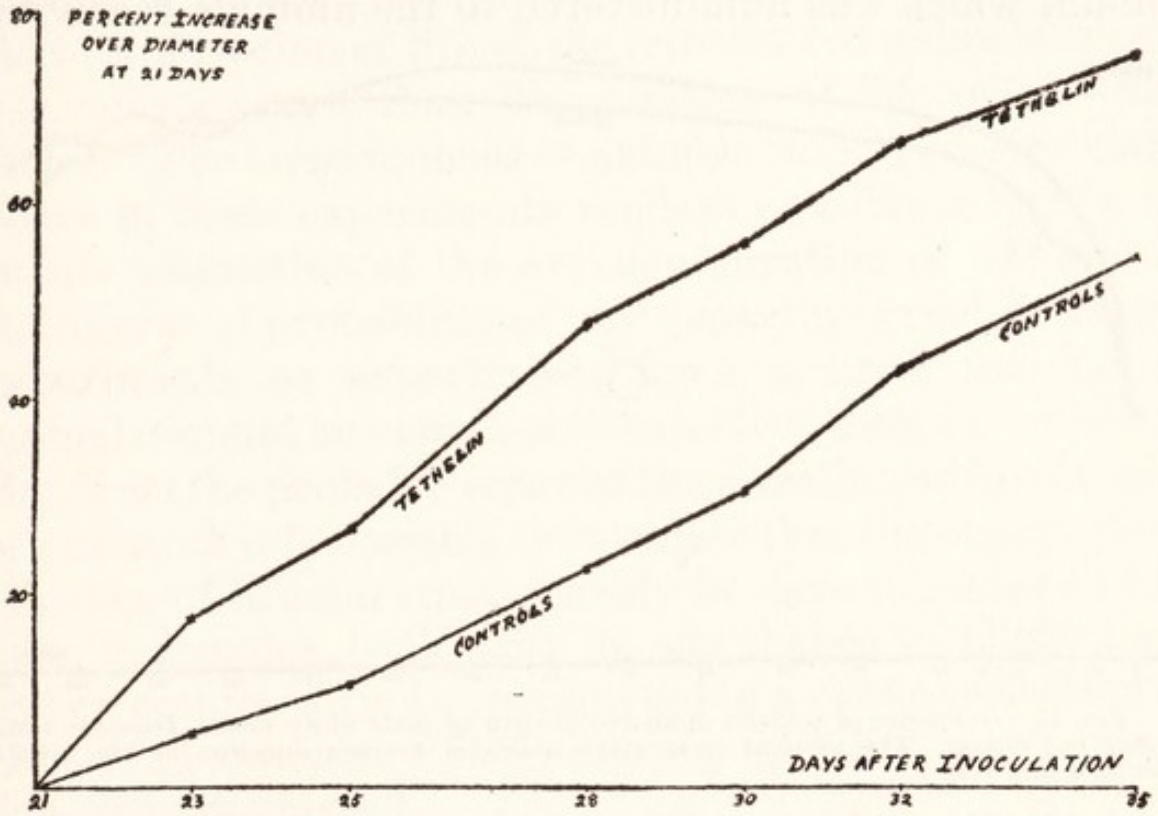


FIG. 41.—Influence of tethelin upon the growth of Flexner-Jobling Carcinoma inoculated into rats. Dosage: 30 mg. (three bovine anterior lobes) per rat every second or third day, beginning with the twenty-first day. Injection on the side remote from the tumor. (After Robertson and Burnett.)

as rapidly. The proportion of metastases was increased from 9 per cent. in the controls to 35 per cent. in the treated animals. Other fractions derived from extracts of the pituitary body (anterior lobe) were devoid of effect. The effect of tethelin, therefore, and that of no other fraction investigated, reproduces exactly the effect obtained with anterior lobe tissue emulsion. From this and from the effect upon the multiplication-rate of isolated infusoria, we may infer that the autokinetic phase of growth is accelerated by tethelin.

The effect of tethelin upon the growth of animals is complex and varies greatly with the period and duration of the administration. The small proportion which is contained in the gland permits the administration of quantities equivalent to an amount of tissue which would be impracticable to administer to such animals as mice. A dose of 4 milligrams per day corresponds to two-fifths of an ox pituitary, or 0.6 grams of tissue, or five times the amount which was administered to the animals receiving

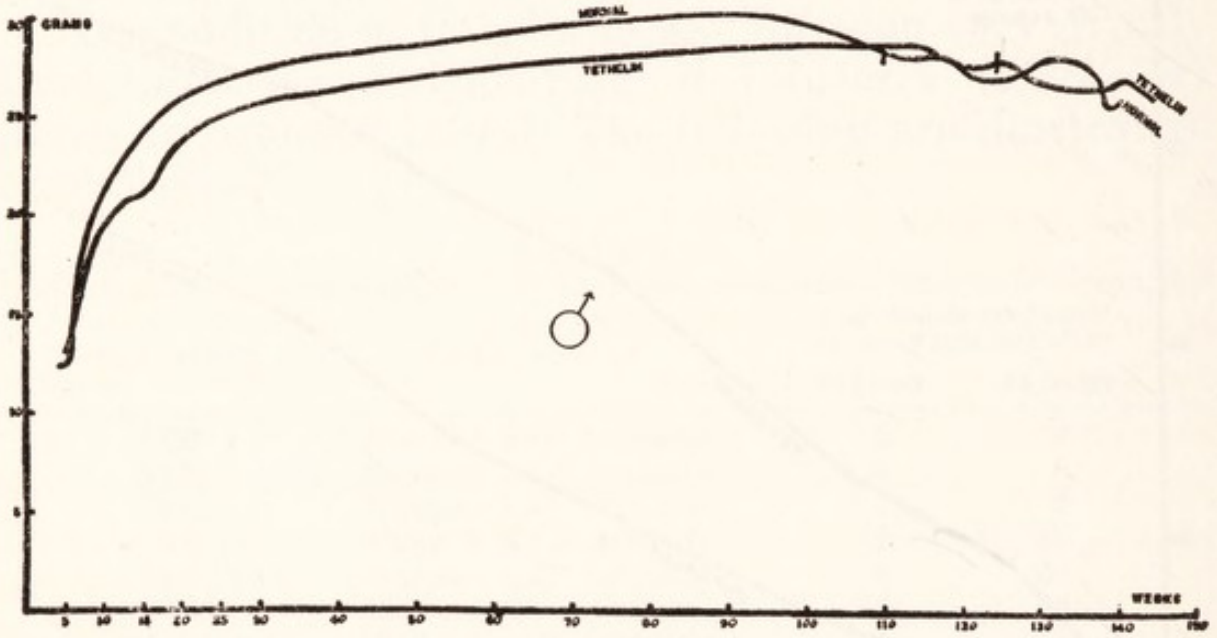


FIG. 42.—Influence of tethelin upon the growth of male white mice. Dosage: 4 mg. per day per mouse. The vertical cross mark indicates average duration of life. (After Robertson and Ray.)

fresh pituitary tissue. The effects are similar in kind, but much greater in extent than those which are obtained by the administration of the fresh tissue.[418,421] They are illustrated in the accompanying chart (Fig. 42) from which it will be seen that the first effect (4th to 15th weeks) is a noteworthy retardation of growth, followed by acceleration which partially, but never wholly, compensates for the initial retardation. The result is indeed much what one might have expected to obtain had the result with anterior lobe tissue been multiplied fivefold, in proportion to the dosage.

The late effects are, however, of even greater interest. During the period lasting from the 40th to the 110th weeks

of life in the males, and from the 30th to the 100th weeks of life in the females, the growth-curve of the tethelin-fed animals is a straight line which is very slightly inclined upwards. That is, a very slight accretion is occurring at an absolutely uniform rate. In normal animals the line is more curved and its slope is greater, in other words, more accretion of tissue is occurring late in life. Corresponding to this difference (*vide* Chapter VII) we find that the duration of life of the tethelin-fed animals is considerably greater than the duration of life of the controls.[419] The large number of animals employed over many years in these experiments renders possible a highly accurate estimation of the average duration of life and of the degree of probability of any departure from it. Single experiments or experiments upon a small number of animals, would be valueless to establish such a conclusion. But from the probable error of the duration of life of mice of our stock it is possible to calculate that the observed extensions of life-duration, namely 99 days in males and 81 days in females, could only by one chance in 11,000 have arisen accidentally, *i.e.*, originated in a chance selection of long-lived animals. This remote possibility may without danger be dismissed.

We have, then, a genuine prolongation of life accompanied, as we should expect it to be, by a lack of tissue production or multiplication of highly differentiated cell-types in late life. It is of interest here to observe that Smith found that administration of anterior lobe tissue delays metamorphosis (resulting from differentiation) in the tadpole, so that the larvæ grow to unusual dimensions without metamorphosing.[455]

The effect upon the incidence of spontaneous carcinoma is at first sight very surprising, in view of the acceleration which tethelin causes in the growth of inoculated carcinomata in rats. In our stock of mice car-

cinoma occurred spontaneously in 27 per cent. of all the males and in 39 per cent. of all the females. This figure corresponds closely with the average incidence found by Lathrop and Loeb in a variety of different strains of mice.[224] The incidence in the tethelin-fed males was 35 per cent., and in the females 25 per cent., figures which do not differ sufficiently from the average to establish any definite effect upon the numerical frequency of carcinoma in these animals, but the **age of incidence, duration of life of the carcinomatous animals,** and **the mode of incidence** were all profoundly affected.[420] Thus the prolongation of life of the tethelin-fed animals which exhibited carcinoma at death was even more marked than the prolongation of life of the whole group, amounting to 132 days in the males and 249 days, or 33 per cent. of the whole normal duration of life in the females. Since no other dietary classes in these experiments have ever exhibited the slightest tendency to prolongation of life of the carcinomatous individuals, we must regard this effect as distinctively attributable to the administration of tethelin.[420]

Since carcinoma is essentially a disease which accompanies a measure of senescence, we may infer that the delay of the death of the carcinomatous animals was due to the fact that the onset of senescence in the tethelin-fed animals is deferred, and the spontaneous origin of carcinoma suffered even more than proportionate delay. Lathrop and Loeb, in their investigations upon tumor incidence and tumor age in mice,[224] do not report the death of any animals having tumors at ages exceeding 2 years, and our observations upon mice of all dietary classes, other than the tethelin-fed groups, support the conclusion that the oldest animals do not develop carcinoma. In the tethelin-fed groups, however, the reverse was true, and the carcinomatous growths were encountered most abundantly in animals which survived longest.

Even more marked than the delay of the incidence of carcinoma, however, was the delay of its growth in the tethelin-fed animals. A majority of the animals exhibiting carcinoma in other dietary classes died as a result of the growth of the tumors, and consequent interference with the functions of the organs in their vicinity. In the tethelin-fed animals, on the contrary, death in nearly all cases occurred from other and undefined causes, possibly diffuse senescent atrophy of one or more organs, not evident to the naked eye, and the carcinoma was confined to one or more minute spots of from 1 to 6 mm. diameter in the lungs. In normal animals, on the contrary, the primary growth usually originates in the axilla or the groin and occasionally in the ovary.

The clinical investigations of Marie[272] and Cushing[85] have shown us that hyperfunction of the pituitary (anterior lobe) may lead to either of two conditions. If the period of hyperfunction is post-adolescent, when the bones have ceased to grow in length because of the hardening of the epiphyses, the result is the development of the condition known as acromegaly. The bones have become thickened, especially at the ends, although they do not increase in length. The skin becomes thick and coarse, and there is usually an excessive growth of hair. The bodily proportions are modified, so that the ratio of weight to height greatly exceeds the normal; in other words the bodily density or "ponderal index" is increased. If the period of hyperfunction is pre-adolescent, while the bones are still increasing in length, their growth is accelerated and gigantism ensues. The bodily density in such cases is not necessarily increased, or in fact may even be subnormal because the linear dimensions are enormously increased. Individuals of over seven or even eight feet in height are produced. If the period of hyperfunction extends into post-adolescent ages then enlargement of the extremities, as in acromegaly, may also be observed. In

any case the hyperfunction is usually transient and is succeeded by hypofunction.[1]

Both of these conditions have been experimentally induced in mice by the administration of tethelin. When this substance is administered in doses of 4 milligrams daily from the fourth week of age, the animals, although in the average smaller than normal animals, become remarkably compact in build. Weight for weight they are smaller, but size for size they are much heavier than the normals. The "ponderal index" has obviously been increased. The contours of their surface are more rounded and youthful and, most remarkable of all, their coats retain, even at 14 months of age, the glossy, silky appearance of the coats of young animals (stimulation of the growth of hair), while 6 months or more prior to this age the coats of normal males are already shaggy, staring and discolored.

These differences are clearly displayed in the accompanying photograph (Fig. 43) in which a normal and a tethelin-fed male of the same age (1 year) and of the same weight (28.0 grams) are compared. The normal animal on the left has a shaggy, staring and discolored coat, while the tethelin-fed animal has a smooth, glossy, and pure white coat. The normal animal is irregular in outline and loosely built, while the contour of the tethelin-fed animal is rounded, and its build is compact and stocky. It is obviously shorter than the normal animal of equal weight.

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[1] The "Ponderal Index," or ratio

$$\frac{100\sqrt[3]{\text{weight in grams}}}{\text{Height in centimetres}}$$

has, in the normal male, a value lying between 23 and 24,[276] p. 246. In a case of extreme acromegaly instanced by Cushing, in which the height was 6 feet 1 inch, and the weight 247 pounds, the ponderal index was 25.8. In a case of gigantism which is described by the same author, the weight was 275 pounds and the stature 8 feet 3 inches. The ponderal index was, therefore, 19.8. The "index of body density" or

$$\frac{100 \times \text{weight in grams}}{(\text{Height in centimetres})^3}$$

is 1.3 to 1.4 in the normal male. In the acromegalic individual it was 1.74, and in the giant 0.78.

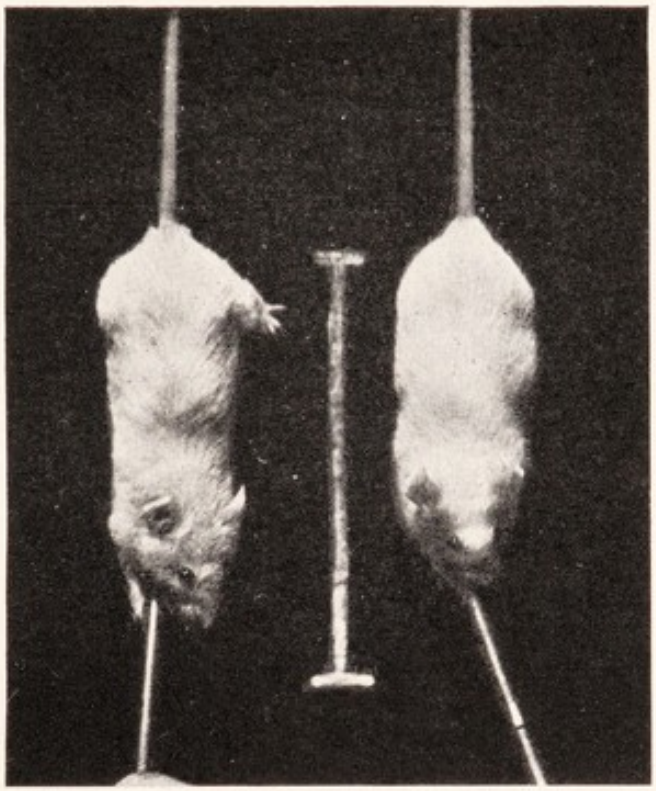


FIG. 43.—Approximation to acromegaly in the white mouse. Comparison of a normal (left) and a tethelin-fed male (right), both one year old and 28 grams in weight. Note the smooth coat and compact form of the tethelin-fed mouse, as contrasted with the loose form and rough coat of the normal animal.

The continuous administration of tethelin appears to prevent gigantism through acceleration of the hardening of the epiphyses, so that the body becomes confined to a small frame and all that subsequent growth can accomplish is to enhance the bodily density. Or, to express the effect in more general terms, the favoring of (relatively) undifferentiated tissues prevents the development of physiologically differentiated tissues which would normally complete the growth of the animal. The re-

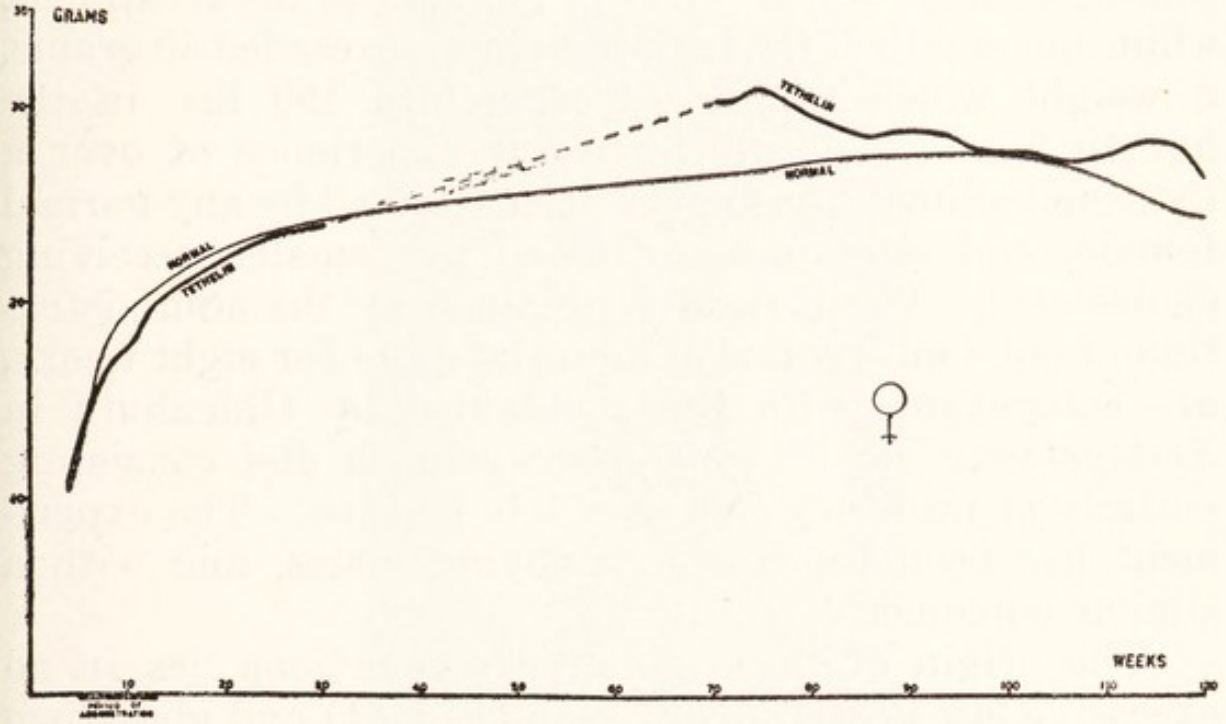


FIG. 44.—The influence of a brief administration of tethelin upon the subsequent growth of female white mice. Dosage: 4 mg. per day per mouse from the fifth to the thirteenth week after birth. Subsequent diet normal. No weighings from the thirtieth to the seventieth week. Thick line represents growth of tethelin-fed animals. Thin line that of controls.

sultant condition is as near to human acromegaly as we could expect it to be in an animal which differs so widely from man in its external aspect as the mouse.

If, however, the administration of tethelin is confined to the pre-adolescent period, results of a very different character are obtained. These effects may be elicited by administering tethelin (4 milligrams per day) from the fourth to the twelfth weeks of age and then discontinuing the administration. The curve of growth of these animals in comparison with that of normals is shown in the accompanying chart (Fig. 44), and their external appear-

ance at 500 days old is compared in the accompanying photograph. The increase of bodily dimensions is extraordinary (Fig. 45). The average weight of the treated females at 500 days was 31 grams, while that of the normal females was only 25 grams. An increase of 25 per cent. in average weight was thus obtained corresponding in human females to an increase from the average normal weight of about 120 lbs. to an average weight of 150 lbs. But the average was considerably diminished by a small proportion of animals which failed to respond to the treatment, while one-eighth of the treated animals exceeded 40 grams, a weight which would correspond to 190 lbs. in the human female and which, in our experience of over a thousand animals, has never been attained by any normal female, and only once or twice by females receiving cholesterol. These results, obtained by the administration of but 4 milligrams of material daily for eight weeks, are comparable with those obtained by Uhlenhuth in *Amblystoma*[497,498,499] by administering a diet consisting entirely of pituitary (anterior lobe) tissue. The experiment has been repeated, employing males, and with a similar outcome.[421]

The origin of these gigantic proportions lies in an excessive late accretion of tissue (Fig. 44) and gigantism is not displayed in its full degree until the animals attain the age of about 500 days, or over a year after the discontinuation of the administrations.[m] Corresponding to this we find, in accordance with the general rule that late accretion of tissue is unfavorable to longevity (Chapter VII), that the duration of life of these animals was abbreviated rather than enhanced.[422]

The full interpretation of these results cannot be attempted in the present state of our knowledge, but it appears evident that the effect of the pituitary autocoid is in some way related to a change in the average con-

[m] Acceleration of growth, however, is manifested very soon after the discontinuation of the administrations.[421]

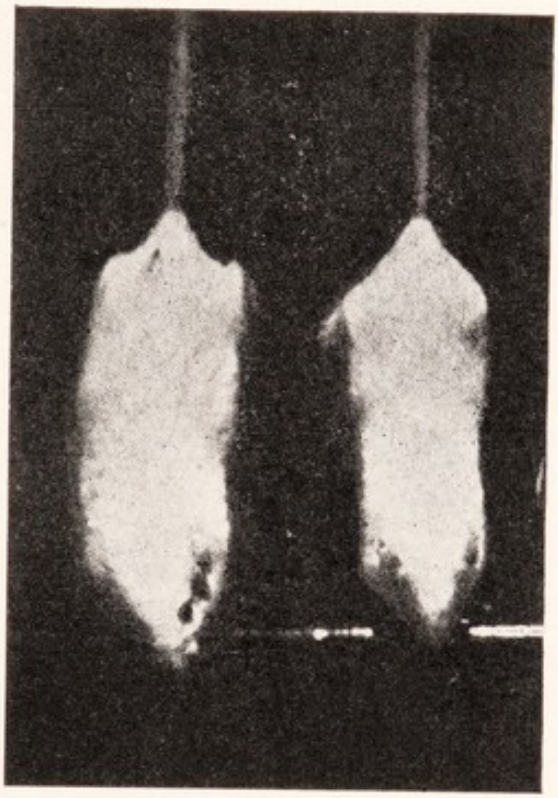


FIG. 45.—Approximation to gigantism in the white mouse. The animal on the right (25 grams) is an average normal animal, 500 days old. The animal on the left (31 grams) is an average tethelin-treated animal of the same age. Dosage: 4 mg. per mouse per day for eight weeks, from the fifth to the thirteenth week after birth. Subsequent diet, normal.

Normal
TBIL
Normal
Talk

centration or distribution of the nuclear autocatalyst. The striking specificity of the effect, in stimulating the growth of bony and epithelial tissue, lends a measure of encouragement to the latter view (alteration of the distribution of autocatalyst). In general, however, the effect of tethelin may be summarized as consisting in a delay of the development of (physiologically) differentiated tissues, so that senescence of the animal is deferred and its bodily dimensions are decreased. Other tissues, less physiologically differentiated, are comparatively favored, and if administration is discontinued while active growth is still proceeding, the restoration of the normal proportions of the several tissues necessitates the attainment of excessive bodily dimensions, whereby the underlying stimulation of growth is rendered manifest and senescence now occurs at the usual age or even earlier than usual.

Clinically, the employment of tethelin has been proposed for the stimulation of repair in slow-healing superficial wounds and in ununiting fractures. It is applied to superficial wounds after asepsis has been established, in admixture with lanoline,[n] with which it is readily incorporated after solution in water. In the treatment of ununiting fractures it is injected in the form of aqueous solution into the adjacent soft parts. Striking instances of success in inducing repair of very long standing and extensive lesions have been reported.[28,58,407] The effect upon superficial lesions is peculiar. A part of the newly-formed epithelium, instead of growing out from the uninjured edge of the lesion, as it usually does, appears in islands on the surface of the granulation tissue. Whether these represent dormant epithelial cells in which multiplication has been induced by the tethelin, or whether they represent cells which have wandered off from the un-

[n] Application to infected wounds is useless [78] and in any case stimulation of repair in wounds which are still infected is undesirable.

injured edge into the granulation tissue, has not yet been ascertained.

Smith[456] has failed to restore normal growth in hypophysectomized tadpoles by the administration of tethelin, while he was able to do so by administering the residue after boiling aqueous or alcoholic extraction of the dried glandular tissue. He therefore infers that tethelin is not the growth exciting constituent of the pituitary, but that this is contained in the insoluble residues.° The tethelin was, however, added to the water in which the animals were swimming and since it is rapidly dissolved by water there is no evidence that they actually ingested any of it. In fact the only proof advanced by Smith of consumption of the material by the tadpoles was their enhanced motility after its addition to the water. This is not very convincing. On the other hand, tethelin is extracted from the dried glandular tissue only very slowly by boiling alcohol, and there is no proof that its complete removal is secured by 48 hours of extraction,[403] either by alcohol or by water. But whatever residue of the substance was contained in the extracted tissue must perforce have been eaten by the tadpoles for this constituted their only food. The lack of effect of tethelin and aqueous and alcoholic extracts which was noted by Smith, therefore, may have arisen solely from a deficiency of dosage in comparison with that received by the animals fed upon the extracted residues.

5. **Thyroxin (Thyroid Autocoid).**—The discovery by Gudernatsch[166,167] that frog larvæ may be induced to

° The statement of Smith that the acceleration of growth which occurs after discontinuance of the administration of tethelin to mice shows that it is not a growth-accelerating agent is manifestly incorrect. A parallel inference would be that since post-adolescent hyperpituitrism does not cause gigantism, therefore, hyperfunction of the pituitary has no effect upon the growth in length of bone. Growth of an animal is not a unit phenomenon like the growth of a crystal, but the development of a series of tissue-types in a certain sequence and proportion. If one of these types is favored at the expense of another that is none the less an effect of stimulation of growth and the subsequent restoration of normal proportions, rendered possible by discontinuance of the administration, permits the actual stimulation which has occurred to manifest itself in increased dimensions of the animal.

metamorphose prematurely by the administration of thyroid tissue has been repeatedly confirmed.[483,484,485] A most ingenious application of this discovery has been made by Laufberger,[226] Jensen,[209] Huxley,[201] and Huxley and Hogben.[202] In the group of tailed amphibians, the newts and salamanders, development is arrested at various stages corresponding to larval stages of development in the frogs and toads. The retention of the tail is one feature of affinity to the frog larvæ, but in addition the Axolotl (*Amblystoma*), *Proteus* and *Necturus* and the *Sirenidæ* retain gills throughout the duration of their lives. It had been shown that metamorphosis, with absorption of the gills, may be induced in the Axolotl by prolonged enforcement of air breathing,[43,65] but in order to induce metamorphosis by this means from three to four months is required. By administering thyroid tissue Jensen induced metamorphosis in from 17 to 50 days, Huxley and Hogben[202] in from 24 to 32 days; moreover the initial size of the animals makes little difference to the time required for metamorphosis, although growth in weight and length, both in frog and axolotl larvæ, is arrested by the administration of the thyroid tissue. Hence the metamorphosed animals are smaller than they would have become in the absence of the thyroid administration.

In *Proteus* and *Necturus* no similar effect could be induced, although a slight reduction of the gills occurs in *Necturus*.

Recalling the fact that the thyroid autocoid probably determines the "nutrient level" of amino-acids by determining the rate of their deaminization, it is evident that the effect of administering excess of thyroid must be to reduce the nutrient level of amino-acids, and in higher animals this leads to progressive loss of weight. We have seen that inanition tends to favor the cells which are possessed of high nuclear ratios—the physiologically undifferentiated, or ontogenetically primitive cells—because the cytoplasm of all the cells in the body is called

upon to supply both protein and the deficiency of calories, while the non-protein constituents of the nucleus are called upon only to restore the nutrient level of their own substrates. The cytoplasm, in a word, bears a double burden in inanition; the nucleus but one.

But at the same time any cells which are still able to undergo division at the lowered nutrient level will continue to multiply, while all those with higher nuclear ratios for division must necessarily cease to multiply. The cell which is able to undergo mitosis is always in a favored position, for by the discharge of its accumulations of autocatalyst it is rejuvenated and starts nuclear synthesis afresh. It must be recollected that the nutrient level, except, perhaps, at the verge of death from inanition, is always in excess of that necessary to permit nuclear synthesis if the velocity of the reverse reaction, due to accumulated autocatalyst, did not prevent the synthesis from occurring. Cancer continues to grow in the starving animal and the fetus, with its relatively small accumulations of autocatalyst, is able to rob the mother of nutrition and grow at her expense if necessary. Hence if, in the animal to which thyroid is being fed, cells exist with a sufficiently small nuclear ratio to permit its attainment at the lowered nutrient level, while all other cells will cease to multiply and even suffer loss of substance, these (physiologically) differentiated cells will increase and in consequence morphological differentiations will arise. The absence of such cells in *Necturus* and *Proteus* would explain the failure of these forms to metamorphose even when thyroid tissue is administered to them.[p] The absence of these cells would be due to the lack of the necessary genetic factors which are responsible for the tendency of nuclei to divide at lower nuclear ratios.

[p] This possibility has been suggested by Huxley and Hogben.[302] They incline, however, to the view that in these forms the necessary genetic factors to reproduce differentiation are present, but that the cells fail to respond to thyroid autocoid.

According to Swingle[483,484,485] the administration of iodides or of iodine to frog larvæ is as effective as thyroid tissue or thyroxin to induce metamorphosis. Jensen, Huxley and Hogben failed, however, to secure metamorphosis in axolotls by the administration of iodine, while in *Salamandra* and *Triton* in which metamorphosis normally occurs, it was decidedly accelerated by iodine. In the opinion of Swingle all tissues, but especially the thyroid, are able to synthesize the metamorphosis-accelerating agent from iodine, and its amount, presumably, depends to a large extent upon the amount of iodine available.

6. **Other Special Agencies.**—A complete review of the special agencies which affect growth would involve a discussion of the entire field of endocrine research. We must here be content merely to allude with extreme brevity to a few of the most clearly established instances. A serious difficulty, which attaches to all endocrine investigations, is that which arises out of the physiological interrelationships of the endocrine organs, so that a given result may not be directly attributable to the endocrine organ under investigation, but indirectly to its effect upon some other endocrine organ. Thus the adrenals may conceivably influence growth through their effect upon the thyroid.[55]

A striking instance of an apparently specific growth-hormone has been discovered by L. Loeb.[250,251,252,253,254] He finds that in the virgin guinea-pig, for a certain period succeeding ovulation, while the *corpus luteum* is still developing, any irritation or incision of the uterus results in the outgrowth of a placenta. The injection of extracts of *corpora lutea* brought about a similar sensitization of the uterus, and he concludes that the *corpora lutea* furnish a substance to the circulation which specifically stimulates the formation of decidua. The suggestion has been made, however, that the *corpora lutea* control the circulation in the uterus,[270] so that the possibility exists that the remarkable sensitization of the uterus, observed by Loeb, may

be a secondary effect arising from alteration of the blood supply.

The secondary sexual characters of the male, such as the growth of the beard and deepening of the voice in man, the development of horns in the ram, and of the comb and tail-feathers in the cock, have long been known to be attributable to the development of the testes. Castration has long been practiced both in man and in animals for the purpose of preventing the development of secondary sexual characters, and of bringing about the psychic and metabolic modifications which also accompany the excision of these organs. The removal of the testes in man before the onset of puberty prevents the appearance of the beard and the deepening of the voice which characterizes that period of development. In sheep the castration of the male suppresses the development of the horns, and similarly in cocks, castration prevents the development of the comb and tail-feathers. If, however, the excised testicle be implanted in another part of the body, as, for example, in the peritoneal cavity, then the secondary sexual characters develop normally, the penis grows to its normal dimensions, the seminal vesicles and the prostate develop as if the testes were actually functioning as generative organs, and yet, not only are the testes prevented by lack of communication with the vas deferens from discharging spermatozoa, but, as a matter of fact, the spermatogenic tissues of the testes dwindle away and the production of spermatozoa ceases. The effect of this organ upon the development of the secondary sexual characters is therefore not attributable to its spermatogenic tissues, and appears to be due to the interstitial cells, which are normally present between the seminal tubules and become increased in number in the transplanted organ.[468,469,470,471]

In the female, the excision of the ovaries leads to a more or less pronounced tendency towards the acquirement of masculine characteristics. If ovarian tissue is transplanted into the body of a castrated male of the same species, not only do the secondary sexual characters

of the male fail to develop, but those of the female take their place, even to the development of the mammary glands. Here again the effect appears to be attributable to the interstitial elements of the ovary rather than to the reproductive elements.

A remarkable instance of the converse effect, namely, suppression of female characteristics by secretions from the male organs of generation is supplied by the sterility which is almost the invariable rule in the females of heterosexual twins in cattle. A female of this type is known to cattle breeders as a Free-martin. It has been ascertained by F. R. Lillie[233] that in cattle a twin pregnancy is almost always a result of the fertilization of an ovum from each ovary and development begins separately in each horn of the uterus. The ova, in the course of development, however, meet and fuse and the blood vessels from each side anastomose in the connecting part of the chorion, so that each embryo receives part of its blood supply from the other. Both the arterial and venous circulations overlap, so that a constant interchange of blood takes place. If both are males, or both are females, no harm results, but if the one is a male and the other female, the reproductive system of the female is largely suppressed in its development, and certain male organs even develop in the female. The effect of this is to render the female incapable of reproduction.

We have in these facts evidence of specific hormones which stimulate the growth of certain tissues, those, namely, which give rise to the secondary sexual characters. This is not hard to understand, but if we regard this effect as arising from direct action of the hormones, the simultaneous inhibition of the opposite type of character is exceedingly difficult to interpret. We have seen in Chapter VIII, however, how the development of one type of tissue may suppress the development of another by prior appropriation of nutrients and the establishment of gradients of autocatalyst concentration, and we can understand in the light of these facts how a stimulator of one

type of tissue growth may, by the very fact that the growth occurs, lead indirectly to the suppression of an alternative type of tissue growth. Let but the one type of tissue atrophy and the other, to the extent that atrophy has occurred, will tend to take its place. Thus in human beings, and especially in the Australian aboriginal, the atrophy of the female generative organs at the climacteric is succeeded by a measure of development of male characteristics. Both possibilities are present. Which is to develop is determined by the inherited interstitial tissue. The development of the one type of tissue, and not the interstitial tissue itself, inhibits the development of the characters of the opposite sex.

That this is the true explanation of the facts is shown by a remarkable experiment of Morgan's. There are certain fowls, known as Sebrights, in which the males are always hen-feathered. Now, when these hen-feathered cocks are castrated they develop the long highly-colored feathers of the ordinary male,[313] p. 244. If the ovary of a hen of an ordinary breed is removed she also develops the plumage of the cock.[164] Hence, the interstitial tissue of the Sebright cock resembles, so far as feathering is concerned, the interstitial tissue of an ovary. This stimulates the growth of the hen type of feather and thus indirectly inhibits the development of the male type of feather. But the full possibility of developing male feathers is latent, and if the influence which favors hen feathers is removed the male type of feather is enabled to develop.

According to McCord[294] the pineal gland has a function in relation to the development of sexual characters, the administration of this tissue to fowls accelerating the attainment of sexual maturity and the development of secondary sexual characters. The small amount of clinical evidence which is available also supports this view. The action is possibly an indirect one, however, through an effect of pineal autocoid upon the development of interstitial tissue in the testes and ovaries.

CHAPTER XII

GROWTH AND EVOLUTION

Our analysis of the phenomena of growth has led us to the conclusion that the diversity of cells originates in a diversity of relationships between nucleus and cytoplasm. The communal life of the metazoa, and to a lesser extent that of the multicellular plants, led to the acquirement of a certain degree of independence of short period fluctuations in the nutrient environment, and a relative constancy of the nutrient level in the pericellular fluids. In this way the first step was taken towards the evolution of the communally-controlled environment which is exemplified in its most elaborate form in the homoiothermal animals. To say that relative independence of the environment was acquired because it was advantageous to the forms acquiring it is not to invoke a teleological explanation of the facts. The forms of organization which arose in consequence of each successive liberation from environmental caprice could not by any means have arisen had not the corresponding measure of environmental control been previously or simultaneously acquired. The organism may in fact, in the majority of cases, have been the spontaneous outcome of the changed conditions of life, consequent upon the acquirement of an additional measure of environmental invariance, and the operations of selection may have been directed rather to the internal environmental modification than to its somatic consequences.

But the acquirement of environmental invariance carried with it the penalty of mortality imposed by the pericellular accumulation of the nuclear autocatalyst. Mortality, however "disadvantageous" in a personal sense to the individual, was of no disadvantage to the race, for gametic reproduction ensured its continuance. The

mortality of higher forms may thus truly have arisen by selection, as Weismann supposed, but not in the manner or for the reason that he supposed. It was impossible for Weismann to explain how mortality could arise through the operation of selection, seeing that it occurs after reproduction has ensured the continuity of the race. Mortality, regarded as an independent quality of the race, could not facilitate its perpetuation because perpetuation has been accomplished before death supervenes. The object which it was supposed to facilitate has been attained before it occurs. But viewing mortality, as we do, as the inevitable outcome of a positive advantage, namely invariance of the cellular environment, then its perpetuation by selection becomes an obvious necessity.

The stasis of growth which is imposed upon multicellular communities by the accumulation of autocatalyst in the pericellular fluids is deferred for a longer or shorter period by the step-by-step reduction of the nuclear ratio at division, whereby nuclear synthesis is enabled to proceed because it is periodically reinaugurated by the discharge of autocatalyst into the medium. For this too a penalty has to be paid in the loss of genetic characters consequent upon diminution of the nuclear content at each successive differentiation. The differentiated cell bears a mutilated germ-plasm. When the utmost degree of differentiation has been attained then stasis can no longer be deferred and the ultimate consequence is senescence and the termination of the existence of the individual.

This hypothesis to which our analysis has led throws an unanticipated light upon certain evolutionary problems.

In the first place a fundamental difficulty in the interpretation of organic evolution has always been constituted by the fact that the less organized forms, even to the most lowly, continue to coexist with the higher. Darwin, indeed, argued that they survive because, "under very simple conditions of life a high organization would be of

no service—possibly would be of actual disservice, as being of a more delicate nature, and more liable to be put out of order and injured,"[91] p. 93. But this, unfortunately, is an argument which cuts both ways and its reverse is fatal to the origin of species by natural selection. The point need not be labored, because modern genetic research has shown beyond question that the origin of species lies elsewhere than in the cumulative selection of fortuitous variations and that natural selection merely determines which of the immense number of variations presented by nature shall for the time being survive. The continued existence of the less organized forms becomes thus more intelligible; out of the infinite multitude of forms created by the varying interrelations of germ-plasm and cytoplasm many are "fit" and consequently survive, and mere complexity of organization constitutes in itself no criterion of "fitness." But the invariability of many of these forms still constitutes a stumbling block to our comprehension. Many organisms have survived without change of type from the remotest geological epochs while, during the same period, others have originated the most astounding diversity of types. Indeed when one considers the comparatively slight variation which man has displayed in the four or five hundred thousand years during which he has inhabited the earth[332] and compares these trivial changes of skull and skeleton with the vast evolutionary interval which separates *Pithecanthropus* from *Amœba*, one can scarcely avoid the conjecture that in our own species a slowing of variation has occurred, and that evolution has not always taken place at this snail's pace. Would the most generous estimate of our earth's age suffice for an evolutionary process so prolonged?[a] On the other hand witness the swift rise and rich variety of the reptiles of the Cretaceous period. We see in evolution, now swift, now slow, checked

[a] The most recent estimate of the geological age of the earth is 1500 million years (E. Blackwelder, *Science*, LV, 1922, p. 83).

here and blossoming forth there, an irregular arborescence rather than a concatenated progress.

Every new character in a multicellular form originates in a fresh differentiation which we have supposed to arise from a diminution of the nuclear-cytoplasmic ratio and consequent impoverishment of the germ-plasm remainder. We cannot suppose that all these step-by-step diminutions are of equal size—it is far more likely that they are of variable dimensions. All the differentiation that occurs in the ontogeny of *Planaria* does not deprive its tissues of the power to restore almost the whole animal, but the differentiation attained in the 32- or 64-cell stage of the *Echinoderm* embryo suffices to destroy the totipotentiality of its cells for reproduction. But if in any type the individual steps in physiological differentiation are large, that type will the sooner reach the limit of differentiation and the number of cell-types and consequent degree of organization will be small. It has failed to economize its germ-plasm resources, and has consequently attained all its realizable variational potentialities at an early stage of organization. On the contrary, in a type in which the step-by-step diminutions are small an immense variety of tissues arises, and moreover, by their existence other types of tissue are enabled to survive, so that differentiations impossible to the more primitive type become accessible to the type which is already highly organized. The differentiation that produced nervous tissue rendered possible a host of differentiations which must otherwise have been checked by their uselessness or positive harmfulness to the organism, or by the lack of mechanism to support their peculiar varieties of metabolism. But even here a limit is set beyond which differentiation may not proceed. Reduction to the single determinant pictured by Weismann as the formative element of the individual cell manifestly could proceed no further.

The continued existence of unvarying primitive types is therefore a consequence of the extravagant mode of

evading developmental stasis which they have adopted. We need not, indeed we cannot, suppose that in any form the ultimate indivisible unit of germ-plasm has actually been attained by all its tissues. That would involve destruction of all the earlier work of development and of tissues upon which the existence of the later-developed types has come to depend. But when the relative proportion of most highly differentiated tissues becomes too great, variation must necessarily be rendered increasingly difficult. It is for this reason, no doubt, that in the creation of new forms evolution has so repeatedly harked back to earlier types, or to relatively primitive tissues in the more advanced types. The branch is continued to a certain limit and then ceases to proliferate and a fresh outgrowth from a more primitive stem takes its place and overshadows it. Viewing the phylogenetic history of man in this light, we may perhaps harbor the speculation that if a superhuman type is ever to appear upon our earth it will not arise from a human stock. Since man is now engaged in rapidly exterminating all animal competitors, this eventuality may perhaps be rightly deemed impossibly remote.

Undoubtedly it may be argued that enormous changes have taken place in the brain of man since he first made his appearance upon the earth, and that these changes have anticipated and rendered unnecessary the changes of form and skeleton which might otherwise have occurred. We have little ground upon which either to affirm or to deny this proposition; but if cerebral volume be regarded as affording any criterion of intellectual capacity, and there is much evidence to show that within limitations it may be so regarded,[34] then there has been little or no change in the intellectual capacity of man for thirty thousand years.[333] In seeking to account for the enormous development of the tools and powers of man during this period, it must be remembered that man, by his ability to transmit the products of his thought to others, has acquired an

external heritage, the environment, namely, that his intellectual labor has created. In this environmental form of inheritance, unlike his physical heritage, acquired characters are transmitted. All that it has been necessary for nature to contribute to this form of development has been a brain which is capable of far more, and more various potentialities than the individual can ever cultivate, for a large proportion of our neurones are never used.[38] In this way, although no individual will ever display all the powers of the race, the totality of individuals, in the process of adaptation to their varying environments, may actually do so. It may still be urged that the realization of these potentialities hangs upon the presence of structures in which certain special qualities of the mind arise—for example, persistence, industry, or adaptability; and that failing the presence of these, even infinite potentialities may be useless. The question whether such distinctions are psychologically sound need not be raised. We might suppose that variation and development have occurred most conspicuously in the physical substrata of these qualities. But, should we admit this to be the case, then if thirty thousand years have been requisite to develop the moderate measure of these qualities which man now possesses, could 1500 million years have sufficed to traverse the interval between *Pithecanthropus* and *Amœba?* It seems impossible to escape the conclusion that variation in man has been much slower than it was in prehuman types. Man, like the primitive invariable forms which coexist with him, appears to be approaching the conclusion of the period of differentiation during which his distinctive species has arisen.

In the case of man it is manifestly difficult to reach unassailable conclusions on account of the intangibility of his most conspicuous qualities, but in many other types the slackening of variation or its total absence for whole geological epochs is readily demonstrable. It has always been implicitly or explicitly assumed that the coexistence

of variable and invariable types depends upon the nature of the processes which determine variation. In the mechanism which has been described in the preceding pages the origin of these rhythms of variation may readily be perceived. Variation in any tissue can only take place by differentiation and this involves the loss of germ-plasm capital by the differentiated tissues. When the expenditure of this capital has reached the point at which further loss of germ-plasm becomes incompatible with the performance of those functions which the rest of the cell community can support in that situation then variation must of necessity cease, or else arise in some other tissue.

These considerations have led us back to the fact that variation arises by differentiation, and differentiation, in our conception, involves a loss of genetic elements in the differentiated cell. We have in this a curious parallel with the conclusions of modern genetic research, and an unexpected light is thrown upon their physiological origin.

It will be recollected that the researches of Mendel, which have now been tested and proven in a multitude of special instances,[30] showed that the characters of organisms are transmitted to the offspring in indivisible units. The few instances in which characters are transmitted in diluted form, as in the descent of the color of the skin in crosses between the white man and colored races, are exceptional and may represent the occasional fractionation of the unit-elements[31] but in the vast majority of instances an individual "is not a sort of pool into which each tributary ancestral stream has poured something, but rather a conglomerate of ingredient characters taken from his progenitors in such a way that some ingredients are represented and others omitted,"[31] p. 23.

These ingredient-characters may be grouped in pairs, technically known as allelomorphs, of which one, the dominant character, is incompatible with and suppresses the development of the other, the recessive character. When a pair of allelomorphs is crossed, each of the germ-cells of

the offspring contains both the characters, but only the dominant character may be expressed, and so the offspring in the first generation have the uniform appearance of the dominant allelomorph. In their germ-cells, however, the allelomorphs become separated. Each cell contains either the dominant or the recessive allelomorph and, in the long run, as many germ-cells are produced of the one kind as of the other. If two such hybrid parents are now crossed, the consequences of this segregation become manifest. If either spermatozoön or ovum carries the dominant allelomorph, then the offspring will display the dominant character, but if two recessive allelomorphs are paired, that is on the average in one instance out of four, then the offspring displays the recessive character and, moreover, the offspring of this type never again display the dominant character unless they are once more crossed with hybrid or pure dominants.

For some time the inevitable meaning of these facts was not perceived, but now, thanks to the work of many investigators, we have attained to the conception of dominance as representing the presence of some determining factor, recessiveness as representing its absence.[31] If the factor is transmitted through either parent then the offspring must inevitably display the corresponding character; if it is not so transmitted, as in the pure recessive type, then it can by no possibility display the character. Just in what this presence or absence consists may be readily inferred from the fundamental discovery of Morgan[313] that the characters which are transmitted from parent to offspring are latent in particulate fragments, indivisible units, of the chromatin elements of the nucleus. Presence of the requisite fragment involves dominance; its absence involves recessiveness.

But what constituted the great difficulty in perceiving the identity of dominance and recessiveness with "presence and absence" of factorial units was the fact that the recessive character so frequently appears as an

addition to the structure or diversity of tissues in the organism, while just as frequently dominance appears as the absence or lack of development of some tissue or quality. The white leghorn fowl is not white because of the absence of factors which determine color, but because of the presence of factors which arrest the development of color. The assumption of the existence of these inhibitory factors in this instance and in many others of similar character has been deemed by many to be artificial, owing to the difficulty of conceiving their mode of inheritance or action, and to some investigators this has appeared to constitute a very serious weakness of Mendelian genetics.

But from our analysis of the mechanisms of growth we are now in a position to understand how these difficulties arose. The development of any new tissue and of the characters arising out of this development necessitates the dropping of certain genetic elements, a loss of nuclear, and presumably of chromatin material, from the new type of cell. This is the expression of a deficiency of transmitted germ-plasm. It cannot arise unless the defect is represented in the germ-plasm parcel from which by reproduction of its like, or of its like with parts left out, the whole organism has originated. At least, if it does so arise it can only do so as a forced adaptation to circumstances, an "acquired character" which cannot be inherited. We conclude that **positive additions to multicellular structures must be recessive.**

Now the investigators of genetics have in latter years encountered and recorded very numerous instances of spontaneous production of new varieties, but in every well-established instance these spontaneous variations have proved to be recessive. The testimony of Bateson is as follows:—"Of the origin of new forms **by loss** there seems to me to be fairly clear evidence, but of the **contemporary acquisition** of any new factor I see no satisfactory proof, though I admit that there are rare examples which may be so interpreted,"[31] p. 12. This fact has led

him to a curious, and at first sight, almost incredible hypothesis which he has expressed in the following words (*loc. cit.*, p. 17) :—"If then we have to dispense, as seems likely, with any addition from without we must begin seriously to consider whether the course of evolution can at all reasonably be represented as an unpacking of an original complex which contained within itself the whole range of diversity which living things present.....as we have got to recognize that there has been an evolution, that somehow or other the forms of life have arisen from fewer forms, we may as well see whether we are limited to the old view that evolutionary progress is from the simple to the complex, and whether after all it is conceivable that the process was the other way about."

If, indeed, we were compelled in consequence to look upon the original *Amœba* as a veritable homunculus containing in miniature but preformed, all the immense variety of living things that were ultimately to spring from it, this hypothesis would indeed be legitimately distasteful, for it would be patently absurd. But this is not at all what it involves. Its author clearly perceived, what we may now illustrate in terms of the specific mechanism of differentiation, that the immense variety of cell-types may all arise by modifications through loss as easily as by modifications through gain. All that is determined by germ-plasm is the physiological type of cell which may be produced. Environment, competition and the coöperation of other cells combine to force upon this physiological type a morphological aspect which it must necessarily assume or else fail to survive. Given the diversity of cell-types in multicellular organisms, then diversity of structure and function follows by force of circumstances. The structures did not lie concealed in the earliest prototype of life, but merely the ability to produce a variety of physiological types. The existence of this ability, constituting the very foundation of organic evolution, has recently been the subject of most exhaustive demonstra-

tion by Jennings.[206] Viewed in this manner the hypothesis presented by Bateson is the most reasonable which we can conceive; it is the converse view in fact which meets with truly insuperable difficulties; "..... is it easier to imagine that these powers could have been conveyed by extrinsic additions? Of what nature could these additions be? Additions of material surely cannot be in question. We are told that salts of iron in the soil may turn a pink hydrangea blue. The iron cannot be passed on to the next generation. How can the iron multiply itself? The power to assimilate iron is all that can be transmitted..... These illustrations may seem too gross; but what refinement will meet the requirements of the problem, that the thing introduced must be, as the living organism itself is, capable of multiplication and subordinating itself in a definite system of segregation?" [31] p. 18.

We have seen that the accumulation of the nuclear autocatalyst in the pericellular fluids automatically compels the members of a cell-community to cease multiplying or else alternatively to produce a type of cell in which a lesser extent of nuclear synthesis is prerequisite for division. This may be accomplished by the elimination from the nucleus of certain of its elements. Thereby a new physiological type is created which by division may relatively rejuvenate itself, and which reacts to its environment in a manner characteristically different to that in which the parent type reacted. A new morphological type thus arises and it too passes through the cycle of growth which the parent type of cell experienced. In unicellular communities new types similarly arise, as Peters has shown, in consequence of prolonged inhabitation of a limited volume of culture medium,[372] and Jennings has shown that the morphological and physiological characteristics of such types may be inherited.[206] The multicellular community differs from the unicellular community only in this respect—that the circumstances compel a more elaborate degree of morphological differen-

tiation, and this is rendered possible by the coöperation of the constituent members of the community whereby they supplement each other's physiological and metabolic deficiencies.[b]

In whatever way we attempt to interpret the mode of action of the germ-plasm in moulding the morphological characters of an organism, we are compelled to start from its effect upon the cell. We may suppose that the morphogenetic units separately convey to the cell as a whole certain qualities which may be dropped, step by step, if the nutritional and physiological circumstances permit and compel it. The multicellular animal has devised means of procuring such circumstances mainly by delegation of function, so that the functions no longer possible to one type may yet be possible to another, and the totality of function satisfies the complete requirement for existence of each of the constituent units of the community. The single-celled animal or plant, on the contrary, cannot delegate function and its germ-plasm must be supposed to be adequate, or more than adequate to support all the activities essential for the existence of the cell. The one-celled animal or plant is not therefore of necessity a simple type of life and in harmony with this view, which we have deduced by retracing the various steps in the mechanism of differentiation, modern investigations in the field of unicellular cytology reveal complexities of structure in the one-celled animals which were formerly unrealized.[98] The *Protista,* in fact, do not represent a morphologically simpler group than the multicellular phylœ, they merely represent a group which has utilized its potentialities for complexity in a different way, an individual rather than a communal way, and the less differentiated types of multicellular organism may actually represent a lower stage of comparative evolution than that which has been attained by the more complex types of one-

[b] A most elaborate manifestation of such mutually supplemental activity is afforded by the manufacture of hormones by the endocrine organs.

celled organism. We must not be misled by confusing physiological with morphological differentiation. Physiologically the *Protista* are undifferentiated because, as a necessity arising out of the many parts they are called upon to play, they contain a large or at least a varied parcel of nuclear components. Morphologically they are highly differentiated because the very fullness of their genetic equipment renders great morphological complexity possible. In general we may say, both in ontogeny and in phylogeny that **the potential morphological complexity of a cell stands in inverse relation to its degree of physiological differentiation.**

APPENDIX

In the second column of the accompanying table (Table LX) are found the various values of $K\ (t - t_1)$ which correspond to the values of $\frac{x}{A}$ enumerated in the first column, employing logarithms to the base 10. The tabulated values of $\frac{x}{A}$ range from 0.000 to 0.500 inclusive, increasing successively by 0.001. For values of $\frac{x}{A}$ less than 0.500 the corresponding values of $K\ (t - t_1)$ are *negative*. For values of $\frac{x}{A}$ greater than 0.500 the corresponding values of $K\ (t - t_1)$ may be obtained from the table by finding the value of $K\ (t - t_1)$ in the second column which corresponds to $1 - \frac{x}{A}$ in the first column and prefixing a *positive* sign. Thus, for the value $\frac{x}{A} = 0.200$, the corresponding magnitude of $K\ (t - t_1)$ is -0.602; while for the value of $\frac{x}{A} = 0.800$ the corresponding magnitude of $K\ (t - t_1)$ is $+0.602$.

Employing logarithms to the base 10, the equation to the curve of autocatalysis may be written

$$\phi = x - \frac{Ae^{2.3026\,K(t-t_1)}}{1 + e^{2.3026\,K(t-t_1)}} = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (69)$$

Let us suppose that approximate but arbitrarily chosen values of the constants A, K and t_1 have been found, and that the *most probable* values of these constants are, respectively,

$$A + \alpha, \qquad K + \beta, \qquad t_1 + \gamma,$$

then we can proceed from a number of experimental determinations of x and t to compute the values of α, β and γ in the following way:[a]

Inserting the above values of the constants and expanding ϕ in equation (69) by Taylor's Theorem we obtain

$$\phi = x - \frac{Ae^{2.3026\,K(t-t_1)}}{1 + e^{2.3026\,K(t-t_1)}} + \frac{d\phi}{d\,(A + \alpha)}\,\alpha + \frac{d\phi}{d\,(K + \beta)}\,\beta + \frac{d\phi}{d\,(t_1 + \gamma)}\,\gamma = 0.$$

Since α, β and γ are assumed to be *small* corrections of the magnitude of

[a] Cf. M. Merriman, A Text-book on the Method of Least Squares (Ed. 8, New York, 1910), p. 200.

the constants, this equation may be written, with a very close approximation to exactitude,

$$\phi = x - \frac{Ae^{2.3026K(t-t_1)}}{1+e^{2.3026K(t-t_1)}} + \frac{d\phi}{dA}\alpha + \frac{d\phi}{dK}\beta + \frac{d\phi}{dt_1}\gamma = 0. \quad . \quad . \quad (70)$$

Inserting the experimentally ascertained values of x and t, we obtain a series of values of

$$\frac{Ae^{2.3026K(t-t_1)}}{1+e^{2.3026K(t-t_1)}} - x,$$

which we may designate by the symbol θ. Hence

$$\frac{d\phi}{dA}\alpha + \frac{d\phi}{dK}\beta + \frac{d\phi}{dt_1}\gamma = \theta \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (71)$$

From the form of the equation (69) it is evident that

$$\frac{d\phi}{dA} = \frac{e^{2.3026K(t-t_1)}}{1+e^{2.3026K(t-t_1)}}$$

$$\frac{d\phi}{dK} = tA \times \frac{2.3026\, e^{2.3026K(t-t_1)}}{\left(1+e^{2.3026K(t-t_1)}\right)^2}$$

$$\frac{d\phi}{dt_1} = -KA \times \frac{2.3026\, e^{2.3026K(t-t_1)}}{\left(1+e^{2.3026K(t-t_1)}\right)^2}$$

Writing

$$\frac{2.3026\, e^{2.3026K(t-t_1)}}{\left(1+e^{2.3026K(t-t_1)}\right)^2} = v$$

then we have

$$\frac{d\phi}{dA} = \frac{x}{A} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (72)$$

$$\frac{d\phi}{dk} = tAv \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (73)$$

$$\frac{d\phi}{dt_1} = -KAv \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (74)$$

$$\frac{dx}{dt} = KAv \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (75)$$

The values of v corresponding to the values of $\frac{x}{A}$ from 0.000 to 0.500 are enumerated in the *third* column of the table. The values of v corresponding to the values of $\frac{x}{A}$ greater than 0.500 are those which correspond to the value of $1 - \frac{x}{A}$. Thus for $\frac{x}{A} = 0.200$ we have $v = 0.368$, which is also the value of v for $\frac{x}{A} = 0.800$.

Inserting the experimentally ascertained values of x and t and the values of v which correspond to the experimental values of x in equations (72) to (75), we obtain a series of *observation equations* of the form

$$X\alpha + Y\beta + Z\gamma = \theta$$

corresponding to which we obtain the normal equations:

$$(\Sigma X^2)\,\alpha + (\Sigma XY)\,\beta + (\Sigma ZX)\,\gamma = \Sigma X\theta \quad . \quad . \quad . \quad . \quad . \quad \text{(I)}$$
$$(\Sigma XY)\,\alpha + (\Sigma Y^2)\,\beta + (\Sigma YZ)\,\gamma = \Sigma Y\theta \quad . \quad . \quad . \quad . \quad . \quad \text{(II)}$$
$$(\Sigma ZX)\,\alpha + (\Sigma YZ)\,\beta + (\Sigma Z^2)\,\gamma = \Sigma Z\theta \quad . \quad . \quad . \quad . \quad . \quad \text{(III)}$$

the solution of which equations yields the most probable values of α, β and γ.

We will now proceed, with the assistance of the table, to apply the equation of autocatalysis to the observations (enumerated in Table XV on p. 35).

We first determine *approximate* values of A, K and t_1 in the following manner:

The conclusion of the first year of post-natal growth in man coincides, approximately, with the conclusion of a "cycle" of growth, that is to say, with the completion of one of the series of autocatalyzed reactions which underlie the total growth of man. The value of A for this "cycle" is therefore, probably, greater, but not very much greater than the value of x (= weight in ounces) at twelve months. The average weight of British male infants at twelve months of age is 314.3 ounces. Hence we may take A as being, approximately, 320 ounces.

Now t_1 is the value of t when $x = \frac{1}{2}A = 160$ ounces. We note that at one month British male infants weigh 147.0 ounces, and at two months 169.0 ounces. Hence, approximately,

$$t_1 = 1 + \frac{160 - 147}{169 - 147} = 1.59 \text{ months.}$$

From the observed values of t and x we now compute the corresponding values of K at each age, as follows:

TABLE LVI

Age in months	x	$\frac{x}{A}$	$K(t-t_1)$ (from table)	$t-t_1$ (observed)	K
1	147.0	0.459	— 0.071	— 0.59	0.120
2	169.0	0.528	+ 0.049	+ 0.41	0.120
3	193.8	0.606	+ 0.187	+ 1.41	0.133
4	218.6	0.683	+ 0.333	+ 2.41	0.138
5	233.7	0.730	+ 0.432	+ 3.41	0.127
6	251.8	0.787	+ 0.568	+ 4.41	0.129
7	268.6	0.839	+ 0.717	+ 5.41	0.133
8	276.2	0.863	+ 0.799	+ 6.41	0.125
9	282.8	0.884	+ 0.882	+ 7.41	0.119

The values of K $(t - t_1)$ are obtained from the second column in the table and are the values which correspond to the observed values of $\frac{x}{A}$. The average value of K determined in this way is 0.127.

Multiplying the observed values of $t - t_1$ by this value of K, we obtain a series of values of K $(t - t_1)$, and from these, with the assistance of the table, a series of "calculated" values of x, which are the amounts which the infants should weigh at the various ages considered, provided the equation

$$\log_{10} \frac{x}{320 - x} = 0.127\,(t - 1.59) \quad . \quad . \quad . \quad . \quad . \quad . \quad (76)$$

correctly represented the course of growth of these infants during the first nine months. As a matter of fact, of course, we find greater or less divergencies $(= \theta)$ between the calculated and the observed weights. In this way we obtain the following series of values:

TABLE LVII

Age in Months	$K(t - t_1)$ Calculated	$\frac{x}{A}$ Calculated	x Calculated	Divergence $= \theta$
1	— 0.075	0.457	146.2	— 0.8
2	+ 0.052	0.530	169.6	+ 0.6
3	+ 0.179	0.602	192.6	+ 1.2
4	+ 0.306	0.669	214.1	+ 4.5
5	+ 0.433	0.730	233.6	+ 0.1
6	+ 0.560	0.784	250.9	+ 0.9
7	+ 0.687	0.829	265.3	+ 3.3
8	+ 0.814	0.867	277.4	— 1.2
9	+ 0.941	0.897	287.0	— 4.2
				$\Sigma\theta = + 4.4$

If only an *approximate* comparison of theory with observation is required, the above may suffice, but to obtain a more accurate comparison we proceed to correct the approximate values of the constants A, K, and t_1 as follows, obtaining the values of v corresponding to the observed values of $\frac{x}{A}$ from the third column of the table:

TABLE LVIII

Age in Months	θ	$\frac{d\varphi}{dA} = \frac{x}{A}$ Observed	v	$\frac{d\varphi}{dK} = tAv$	$\frac{d\varphi}{dt_1} = - KAv$
1	— 0.8	0.459	0.572	183.0	— 23.24
2	+ 0.6	0.528	0.574	367.4	— 23.33
3	+ 1.2	0.606	0.550	528.0	— 22.35
4	+ 4.5	0.683	0.499	738.7	— 20.28
5	+ 0.1	0.730	0.454	726.4	— 18.45
6	+ 0.9	0.787	0.386	741.1	— 15.69
7	+ 3.3	0.839	0.311	696.6	— 12.64
8	— 1.2	0.863	0.272	696.3	— 11.05
9	— 4.2	0.884	0.236	679.7	— 9.59

From these figures we obtain the following series of observation equations:

Age in months	X		Y		Z		θ	"Weight"
1	0.459α	+	183β	—	23.2γ	=	-0.8	86
2	0.528α	+	367β	—	23.3γ	=	$+0.6$	117
3	0.606α	+	528β	—	22.4γ	=	$+1.2$	134
4	0.683α	+	739β	—	20.3γ	=	$+4.5$	114
5	0.730α	+	726β	—	18.5γ	=	$+0.1$	124
6	0.787α	+	741β	—	15.7γ	=	$+0.9$	105
7	0.839α	+	697β	—	12.6γ	=	$+3.3$	90
8	0.863α	+	696β	—	11.1γ	=	-1.2	94
9	0.884α	+	680β	—	9.6γ	=	-4.2	85

All of these observation equations are not of equal value, since the first, for example, was derived from the determination of the mean weight of 86 infants, while the third was derived from measurements made upon 134 infants. Hence, in order to ascribe to each observation its just value, we must *"weight"* the equations in proportion to the number of infants observed. This we do by multiplying every term in each equation by the number of infants weighed.[b] In this way we obtain the following *weighted observation equations:*

Age in months	X		Y		Z		θ
1	39.47α	+	15740β	—	1995γ	=	-68.8
2	61.78α	+	42940β	—	2726γ	=	$+70.2$
3	81.20α	+	70750β	—	3001γ	=	$+160.8$
4	77.86α	+	84250β	—	2314γ	=	$+513.0$
5	90.52α	+	90020β	—	2294γ	=	$+12.4$
6	82.64α	+	77810β	—	1689γ	=	$+90.5$
7	75.51α	+	62730β	—	1134γ	=	$+297.0$
8	81.12α	+	65420β	—	1043γ	=	-112.8
9	55.14α	+	57800β	—	816γ	=	-357.0

From these we obtain the following normal equations:

$$45.12\,\alpha + 399.1\,\beta - 1080.0\,\gamma = 54.30 \quad \text{I}$$
$$0.51\,\alpha + 451.2\,\beta - 12.5\,\gamma = 0.49 \quad \text{II}$$
$$1.25\,\alpha + 1080.0\,\beta - 36.9\,\gamma = 1.83 \quad \text{III}$$

Whence

$$\alpha = -1.98;\ \beta = -0.0004;\ \gamma = -0.133.$$

Hence the most probable values of the constants in equation (69) are

$$A = 320 - 1.98 = 318$$
$$K = 0.127 - 0.0004 = 0.127$$
$$t_1 = 1.59 - 0.13 = 1.46$$

From which we infer that the equation which affords the best fit to the observations enumerated above is

$$\log_{10}\frac{x}{318 - x} = 0.127\,(t - 1.46) \quad (77)$$

[b] Cf. M. Merriman, *loc. cit.*, p. 36.

Inserting the observed values of t and computing therefrom the corresponding values of 0.127 (t — 1.46), we can find by referring to the table the corresponding values of $\frac{x}{318}$ and, consequently, compute the corresponding "calculated" values of x. The observed and calculated values are compared below:

TABLE LIX

Age in Months	Weight in ounces		Divergence $= \theta$
	Observed	Calculated	
1	147.0	148.2	+ 1.2
2	169.0	171.4	+ 2.4
3	193.8	194.3	+ 0.5
4	218.6	215.6	— 3.0
5	233.7	234.7	+ 1.0
6	251.8	251.5	— 0.3
7	268.6	265.5	— 3.1
8	276.2	277.0	+ 0.8
9	282.8	286.5	+ 3.7
			$\Sigma\theta = + 3.2$

The correspondence between "theory" and observation is, it will be observed, somewhat closer when the corrected values of the constants are employed than it is when the uncorrected constants are employed.

It will be observed that the *rate of growth* corresponding to any given value of $\frac{x}{A}$ may be ascertained from the tables by multiplying the corresponding value of v in the third column by KA, the values of K and of A having been determined in the manner described above. The rate of growth which corresponds to any given value of t may be directly determined, without previously estimating $\frac{x}{A}$ by computing K $(t - t_1)$ from the ascertained values of K and t_1 and multiplying by KA the value of v corresponding to this computed magnitude of K $(t - t_1)$ in the second column of the tables.

The table was constructed with the aid of the following formulæ:

Reciprocal of the Modulus of Common Logarithms = log 10 = 2.3026.

Logarithm to base 10 of the reciprocal = 0.3622.

$$q = \frac{\frac{x}{A}}{1 - \frac{x}{A}}$$

Where

$$q = e^{2.3026\,K(t-t_1)}$$

$$\log_{10} q = K\,(t - t_1)$$

$$v = 2.3026\,\frac{x}{A}\left(1 - \frac{x}{A}\right)$$

In the computations four-place logarithms were employed for values of $\frac{x}{A}$ from 0.000 to 0.300 inclusive; from 0.301 to 0.500 inclusive five-place logarithms were employed.

The values given in the table are correct to within one half-unit in the last significant figure. They have all been checked by the method of differences.

TABLE LX

VALUES OF $K(t-t_1)$ AND OF $\frac{1}{KA}\frac{dx}{dt}$ CORRESPONDING TO VALUES OF $\frac{x}{A}$ IN THE CURVE OF AUTOCATALYSIS

$\frac{x}{A}$	$K(t-t_1)$	$v=\frac{1}{KA}\frac{dx}{dt}$	$\frac{x}{A}$	$K(t-t_1)$	$v=\frac{1}{KA}\frac{dx}{dt}$
0.000	$\pm\infty$	0.000	0.036	± 1.428	0.080
0.001	± 3.000	0.002	0.037	± 1.415	0.082
0.002	± 2.698	0.005	0.038	± 1.403	0.084
0.003	± 2.522	0.007	0.039	± 1.392	0.086
0.004	± 2.396	0.009	0.040	± 1.380	0.088
0.005	± 2.299	0.011	0.041	± 1.369	0.091
0.006	± 2.219	0.014	0.042	± 1.358	0.093
0.007	± 2.152	0.016	0.043	± 1.347	0.095
0.008	± 2.093	0.018	0.044	± 1.337	0.097
0.009	± 2.042	0.021	0.045	± 1.327	0.099
0.010	± 1.996	0.023	0.046	± 1.317	0.101
0.011	± 1.954	0.025	0.047	± 1.307	0.103
0.012	± 1.916	0.027	0.048	± 1.297	0.105
0.013	± 1.880	0.030	0.049	± 1.288	0.107
0.014	± 1.848	0.032	0.050	± 1.279	0.109
0.015	± 1.817	0.034	0.051	± 1.270	0.111
0.016	± 1.789	0.036	0.052	± 1.261	0.114
0.017	± 1.762	0.038	0.053	± 1.252	0.116
0.018	± 1.737	0.041	0.054	± 1.244	0.118
0.019	± 1.713	0.043	0.055	± 1.235	0.120
0.020	± 1.690	0.045	0.056	± 1.227	0.122
0.021	± 1.669	0.047	0.057	± 1.219	0.124
0.022	± 1.648	0.050	0.058	± 1.211	0.126
0.023	± 1.628	0.052	0.059	± 1.203	0.128
0.024	± 1.609	0.054	0.060	± 1.195	0.130
0.025	± 1.591	0.056	0.061	± 1.187	0.132
0.026	± 1.574	0.058	0.062	± 1.180	0.134
0.027	± 1.557	0.060	0.063	± 1.172	0.136
0.028	± 1.541	0.063	0.064	± 1.165	0.138
0.029	± 1.525	0.065	0.065	± 1.158	0.140
0.030	± 1.510	0.067	0.066	± 1.151	0.142
0.031	± 1.495	0.069	0.067	± 1.144	0.144
0.032	± 1.481	0.071	0.068	± 1.137	0.146
0.033	± 1.467	0.073	0.069	± 1.130	0.148
0.034	± 1.454	0.076	0.070	± 1.123	0.150
0.035	± 1.440	0.078	0.071	± 1.117	0.152

TABLE LX—*Continued*

$\frac{x}{A}$	$K(t-t_1)$	$v = \frac{1}{KA}\frac{dx}{dt}$	$\frac{x}{A}$	$K(t-t_1)$	$v = \frac{1}{KA}\frac{dx}{dt}$
0.072	± 1.110	0.154	0.122	± 0.857	0.247
0.073	± 1.104	0.156	0.123	± 0.853	0.248
0.074	± 1.097	0.158	0.124	± 0.849	0.250
0.075	± 1.091	0.160	0.125	± 0.845	0.252
0.076	± 1.085	0.162	0.126	± 0.841	0.254
0.077	± 1.079	0.164	0.127	± 0.837	0.255
0.078	± 1.073	0.166	0.128	± 0.833	0.257
0.079	± 1.067	0.168	0.129	± 0.829	0.259
0.080	± 1.061	0.169	0.130	± 0.826	0.260
0.081	± 1.055	0.171	0.131	± 0.822	0.262
0.082	± 1.049	0.173	0.132	± 0.818	0.264
0.083	± 1.043	0.175	0.133	± 0.814	0.266
0.084	± 1.038	0.177	0.134	± 0.810	0.267
0.085	± 1.032	0.179	0.135	± 0.807	0.269
0.086	± 1.026	0.181	0.136	± 0.803	0.271
0.087	± 1.021	0.183	0.137	± 0.799	0.272
0.088	± 1.016	0.185	0.138	± 0.796	0.274
0.089	± 1.010	0.187	0.139	± 0.792	0.276
0.090	± 1.005	0.189	0.140	± 0.788	0.277
0.091	± 1.000	0.190	0.141	± 0.785	0.279
0.092	± 0.994	0.192	0.142	± 0.781	0.281
0.093	± 0.989	0.194	0.143	± 0.778	0.282
0.094	± 0.984	0.196	0.144	± 0.774	0.284
0.095	± 0.979	0.198	0.145	± 0.771	0.285
0.096	± 0.974	0.200	0.146	± 0.767	0.287
0.097	± 0.969	0.202	0.147	± 0.764	0.289
0.098	± 0.964	0.204	0.148	± 0.760	0.290
0.099	± 0.959	0.205	0.149	± 0.757	0.292
0.100	± 0.954	0.207	0.150	± 0.753	0.294
0.101	± 0.950	0.209	0.151	± 0.750	0.295
0.102	± 0.945	0.211	0.152	± 0.747	0.297
0.103	± 0.940	0.213	0.153	± 0.743	0.298
0.104	± 0.935	0.215	0.154	± 0.740	0.300
0.105	± 0.931	0.216	0.155	± 0.737	0.302
0.106	± 0.926	0.218	0.156	± 0.733	0.303
0.107	± 0.922	0.220	0.157	± 0.730	0.305
0.108	± 0.917	0.222	0.158	± 0.727	0.306
0.109	± 0.913	0.224	0.159	± 0.723	0.308
0.110	± 0.908	0.225	0.160	± 0.720	0.309
0.111	± 0.904	0.227	0.161	± 0.717	0.311
0.112	± 0.899	0.229	0.162	± 0.714	0.313
0.113	± 0.895	0.231	0.163	± 0.711	0.314
0.114	± 0.891	0.233	0.164	± 0.707	0.316
0.115	± 0.886	0.234	0.165	± 0.704	0.317
0.116	± 0.882	0.236	0.166	± 0.701	0.319
0.117	± 0.878	0.238	0.167	± 0.698	0.320
0.118	± 0.874	0.240	0.168	± 0.695	0.322
0.119	± 0.870	0.241	0.169	± 0.692	0.323
0.120	± 0.865	0.243	0.170	± 0.689	0.325
0.121	± 0.861	0.245	0.171	± 0.686	0.326

TABLE LX—*Continued*

$\frac{x}{A}$	$K(t-t_1)\ v = \frac{1}{KA}\frac{dx}{dt}$		$\frac{x}{A}$	$K(t-t_1)\ v = \frac{1}{KA}\frac{dx}{dt}$	
0.172	± 0.683	0.328	0.222	± 0.545	0.398
0.173	± 0.680	0.329	0.223	± 0.542	0.399
0.174	± 0.677	0.331	0.224	± 0.540	0.400
0.175	± 0.674	0.332	0.225	± 0.537	0.402
0.176	± 0.670	0.334	0.226	± 0.535	0.403
0.177	± 0.667	0.335	0.227	± 0.532	0.404
0.178	± 0.664	0.337	0.228	± 0.530	0.405
0.179	± 0.661	0.338	0.229	± 0.527	0.407
0.180	± 0.659	0.340	0.230	± 0.525	0.408
0.181	± 0.656	0.341	0.231	± 0.522	0.409
0.182	± 0.653	0.343	0.232	± 0.520	0.410
0.183	± 0.650	0.344	0.233	± 0.517	0.412
0.184	± 0.647	0.346	0.234	± 0.515	0.413
0.185	± 0.644	0.347	0.235	± 0.513	0.414
0.186	± 0.641	0.349	0.236	± 0.510	0.415
0.187	± 0.638	0.350	0.237	± 0.508	0.416
0.188	± 0.635	0.352	0.238	± 0.505	0.418
0.189	± 0.633	0.353	0.239	± 0.503	0.419
0.190	± 0.630	0.354	0.240	± 0.501	0.420
0.191	± 0.627	0.356	0.241	± 0.498	0.421
0.192	± 0.624	0.357	0.242	± 0.496	0.422
0.193	± 0.621	0.359	0.243	± 0.493	0.424
0.194	± 0.619	0.360	0.244	± 0.491	0.425
0.195	± 0.616	0.361	0.245	± 0.489	0.426
0.196	± 0.613	0.363	0.246	± 0.486	0.427
0.197	± 0.610	0.364	0.247	± 0.484	0.428
0.198	± 0.608	0.366	0.248	± 0.482	0.429
0.199	± 0.605	0.367	0.249	± 0.479	0.431
0.200	± 0.602	0.368	0.250	± 0.477	0.432
0.201	± 0.599	0.370	0.251	± 0.475	0.433
0.202	± 0.597	0.371	0.252	± 0.473	0.434
0.203	± 0.594	0.373	0.253	± 0.470	0.435
0.204	± 0.591	0.374	0.254	± 0.468	0.436
0.205	± 0.589	0.375	0.255	± 0.466	0.437
0.206	± 0.586	0.377	0.256	± 0.463	0.439
0.207	± 0.583	0.378	0.257	± 0.461	0.440
0.208	± 0.581	0.379	0.258	± 0.459	0.441
0.209	± 0.578	0.381	0.259	± 0.457	0.442
0.210	± 0.575	0.382	0.260	± 0.454	0.443
0.211	± 0.573	0.383	0.261	± 0.452	0.444
0.212	± 0.570	0.385	0.262	± 0.450	0.445
0.213	± 0.568	0.386	0.263	± 0.448	0.446
0.214	± 0.565	0.387	0.264	± 0.445	0.447
0.215	± 0.562	0.389	0.265	± 0.443	0.448
0.216	± 0.560	0.390	0.266	± 0.441	0.450
0.217	± 0.557	0.391	0.267	± 0.439	0.451
0.218	± 0.555	0.393	0.268	± .0436	0.452
0.219	± 0.552	0.394	0.269	± 0.434	0.453
0.220	± 0.550	0.395	0.270	± 0.432	0.454
0.221	± 0.547	0.396	0.271	± 0.430	0.455

TABLE LX—*Continued*

$\frac{x}{A}$	$K(t-t_1)\,v = \frac{1}{KA}\frac{dx}{dt}$		$\frac{x}{A}$	$K(t-t_1)\,v = \frac{1}{KA}\frac{dx}{dt}$	
0.272	± 0.428	0.456	0.322	± 0.323	0.503
0.273	± 0.425	0.457	0.323	± 0.321	0.503
0.274	± 0.423	0.458	0.324	± 0.319	0.504
0.275	± 0.421	0.459	0.325	± 0.317	0.505
0.276	± 0.419	0.460	0.326	± 0.315	0.506
0.277	± 0.417	0.461	0.327	± 0.313	0.507
0.278	± 0.414	0.462	0.328	± 0.311	0.508
0.279	± 0.412	0.463	0.329	± 0.310	0.508
0.280	± 0.410	0.464	0.330	± 0.308	0.509
0.281	± 0.408	0.465	0.331	± 0.306	0.510
0.282	± 0.406	0.466	0.332	± 0.304	0.511
0.283	± 0.404	0.467	0.333	± 0.302	0.511
0.284	± 0.402	0.468	0.334	± 0.300	0.512
0.285	± 0.399	0.469	0.335	± 0.298	0.513
0.286	± 0.397	0.470	0.336	± 0.296	0.514
0.287	± 0.395	0.471	0.337	± 0.294	0.514
0.288	± 0.393	0.472	0.338	± 0.292	0.515
0.289	± 0.391	0.473	0.339	± 0.290	0.516
0.290	± 0.389	0.474	0.340	± 0.288	0.517
0.291	± 0.387	0.475	0.341	± 0.286	0.517
0.292	± 0.385	0.476	0.342	± 0.284	0.518
0.293	± 0.383	0.477	0.343	± 0.282	0.519
0.294	± 0.380	0.478	0.344	± 0.280	0.520
0.295	± 0.378	0.479	0.345	± 0.278	0.520
0.296	± 0.376	0.480	0.346	± 0.277	0.521
0.297	± 0.374	0.481	0.347	± 0.275	0.522
0.298	± 0.372	0.482	0.348	± 0.273	0.522
0.299	± 0.370	0.483	0.349	± 0.271	0.523
0.300	± 0.368	0.484	0.350	± 0.269	0.524
0.301	± 0.366	0.485	0.351	± 0.267	0.524
0.302	± 0.364	0.485	0.352	± 0.265	0.525
0.303	± 0.362	0.486	0.353	± 0.263	0.526
0.304	± 0.360	0.487	0.354	± 0.261	0.527
0.305	± 0.358	0.488	0.355	± 0.259	0.527
0.306	± 0.356	0.489	0.356	± 0.257	0.528
0.307	± 0.354	0.490	0.357	± 0.256	0.529
0.308	± 0.352	0.491	0.358	± 0.254	0.529
0.309	± 0.350	0.492	0.359	± 0.252	0.530
0.310	± 0.347	0.492	0.360	± 0.250	0.531
0.311	± 0.345	0.493	0.361	± 0.248	0.531
0.312	± 0.343	0.494	0.362	± 0.246	0.532
0.313	± 0.341	0.495	0.363	± 0.244	0.532
0.314	± 0.339	0.496	0.364	± 0.242	0.533
0.315	± 0.337	0.497	0.365	± 0.240	0.534
0.316	± 0.335	0.498	0.366	± 0.239	0.534
0.317	± 0.333	0.499	0.367	± 0.237	0.535
0.318	± 0.331	0.499	0.368	± 0.235	0.536
0.319	± 0.329	0.500	0.369	± 0.233	0.536
0.320	± 0.327	0.501	0.370	± 0.231	0.537
0.321	± 0.325	0.502	0.371	± 0.229	0.537

TABLE LX—*Continued*

$\frac{x}{A}$	$K\,(t-t_1)\;v = \frac{1}{KA}\frac{dx}{dt}$		$\frac{x}{A}$	$K\,(t-t_1)\;v = \frac{1}{KA}\frac{dx}{dt}$	
0.372	± 0.227	0.538	0.422	± 0.137	0.562
0.373	± 0.226	0.538	0.423	± 0.135	0.562
0.374	± 0.224	0.539	0.424	± 0.133	0.562
0.375	± 0.222	0.540	0.425	± 0.131	0.563
0.376	± 0.220	0.540	0.426	± 0.130	0.563
0.377	± 0.218	0.541	0.427	± 0.128	0.563
0.378	± 0.216	0.541	0.428	± 0.126	0.564
0.379	± 0.214	0.542	0.429	± 0.124	0.564
0.380	± 0.213	0.542	0.430	± 0.122	0.564
0.381	± 0.211	0.543	0.431	± 0.121	0.565
0.382	± 0.209	0.544	0.432	± 0.119	0.565
0.383	± 0.207	0.544	0.433	± 0.117	0.565
0.384	± 0.205	0.545	0.434	± 0.115	0.566
0.385	± 0.203	0.545	0.435	± 0.114	0.566
0.386	± 0.202	0.546	0.436	± 0.112	0.566
0.387	± 0.200	0.546	0.437	± 0.110	0.567
0.388	± 0.198	0.547	0.438	± 0.108	0.567
0.389	± 0.196	0.547	0.439	± 0.106	0.567
0.390	± 0.194	0.548	0.440	± 0.105	0.567
0.391	± 0.192	0.548	0.441	± 0.103	0.568
0.392	± 0.191	0.549	0.442	± 0.101	0.568
0.393	± 0.189	0.549	0.443	± 0.099	0.568
0.394	± 0.187	0.550	0.444	± 0.098	0.568
0.395	± 0.185	0.550	0.445	± 0.096	0.569
0.396	± 0.183	0.551	0.446	± 0.094	0.569
0.397	± 0.182	0.551	0.447	± 0.092	0.569
0.398	± 0.180	0.552	0.448	± 0.091	0.569
0.399	± 0.178	0.552	0.449	± 0.089	0.570
0.400	± 0.176	0.553	0.450	± 0.087	0.570
0.401	± 0.174	0.553	0.451	± 0.085	0.570
0.402	± 0.172	0.554	0.452	± 0.084	0.570
0.403	± 0.171	0.554	0.453	± 0.082	0.571
0.404	± 0.169	0.554	0.454	± 0.080	0.571
0.405	± 0.167	0.555	0.455	± 0.078	0.571
0.406	± 0.165	0.555	0.456	± 0.077	0.571
0.407	± 0.163	0.556	0.457	± 0.075	0.571
0.408	± 0.162	0.556	0.458	± 0.073	0.572
0.409	± 0.160	0.557	0.459	± 0.071	0.572
0.410	± 0.158	0.557	0.460	± 0.070	0.572
0.411	± 0.156	0.557	0.461	± 0.068	0.572
0.412	± 0.154	0.558	0.462	± 0.066	0.572
0.413	± 0.153	0.558	0.463	± 0.064	0.572
0.414	± 0.151	0.559	0.464	± 0.063	0.573
0.415	± 0.149	0.559	0.465	± 0.061	0.573
0.416	± 0.147	0.559	0.466	± 0.059	0.573
0.417	± 0.146	0.560	0.467	± 0.057	0.573
0.418	± 0.144	0.560	0.468	± 0.056	0.573
0.419	± 0.142	0.561	0.469	± 0.054	0.573
0.420	± 0.140	0.561	0.470	± 0.052	0.574
0.421	± 0.138	0.561	0.471	± 0.050	0.574

TABLE LX—*Continued*

$\frac{x}{A}$	$K(t-t_1)\ v = \frac{1}{KA}\frac{dx}{dt}$		$\frac{x}{A}$	$K(t-t_1)\ v = \frac{1}{KA}\frac{dx}{dt}$	
0.472	± 0.049	0.574	0.487	± 0.023	0.575
0.473	± 0.047	0.574	0.488	± 0.021	0.575
0.474	± 0.045	0.574	0.489	± 0.019	0.575
0.475	± 0.043	0.574	0.490	± 0.017	0.575
0.476	± 0.042	0.574	0.491	± 0.016	0.575
0.477	± 0.040	0.574	0.492	± 0.014	0.575
0.478	± 0.038	0.575	0.493	± 0.012	0.576
0.479	± 0.037	0.575	0.494	± 0.010	0.576
0.480	± 0.035	0.575	0.495	± 0.009	0.576
0.481	± 0.033	0.575	0.496	± 0.007	0.576
0.482	± 0.031	0.575	0.497	± 0.005	0.576
0.483	± 0.030	0.575	0.498	± 0.003	0.576
0.484	± 0.028	0.575	0.499	± 0.002	0.576
0.485	± 0.026	0.575	0.500	± 0.000	0.576
0.486	± 0.024	0.575			

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