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STUDIES IN THE BIOLOGY OF TUMOR CELLS.*

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PLATES I TO VI.

SYNOPSIS.

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I. INTRODUCTION.

Tumors have been studied from many standpoints and yet there is little exact knowledge concerning the essential or basic conditions that determine their incidence, progress and proliferative ca-

pacities, or of the factors that govern the morphology, growth habits and capacity, and the arrangement and differentiation of their structural elements.

It is our purpose to discuss in the following pages the results of a study of certain aspects of the biology of tumor cells and to correlate them with certain recently established facts concerning the biology of cells in general, and especially those whose life histories are best known, namely, certain protozoa, sex cells, thymus cells, and certain functionally active metazoan cells, such as gland, ganglion and muscle cells.

These various kinds of cells possess many attributes in common. Protozoa, sex cells and tumor cells are capable under certain circumstances of unlimited growth, and both tumor cells and ova may survive the host from whom they spring and in whom they temporarily reside, and, further, each may live and grow within certain limits after transplantation.

R. Hertwig (1904) has compared tumor cells in their growth habits to a colony of protozoa, and another zoölogist, Moroff (1908), has pointed out certain close resemblances between protozoa and sex cells. The growth, multiplication and fate of protozoa and of sex cells may be modified experimentally in various ways, and, under these conditions, may exhibit certain variations in their physiology and morphology, as well as certain regulatory phenomena not always so evident in the normal state. As we shall show, tumor cells may and often do give evidence of the same attributes, and are governed in general by the same laws.

In order that the facts which we have established and the conclusions to be drawn therefrom may be clearly understood and correlated, it is necessary briefly to review the structure of protoplasm and nucleus, the relation of cell structure to function and certain other evidences of differentiation, depression, physiological degeneration and death of cells, the morphological and other changes in nucleus and cytoplasm in physiological degeneration and in recovery therefrom, the nucleus-plasma relation and its importance in cell life; and, finally the regulatory processes of protozoa, of sex cells, and of certain tissue cells.

II. GENERAL CELLULAR BIOLOGY.

THE STRUCTURE OF PROTOPLASM AND THE MODIFICATIONS PRODUCED BY FUNCTION.

The divergent views as to the structure of protoplasm which have from time to time predominated have been due, in large part, to the kind of cell used for study. The correctness of Bütschli's conception of the alveolar structure of protoplasm can no longer be doubted. The foam-like nature of cytoplasm is best seen in protozoan cells, but even here there are all gradations in the size of the alveoli, from the extremely minute ones met with in *Paramœcium,* for instance, alveoli so small as to give the protoplasm a finely granular appearance, to the coarsely meshed protoplasm of *Actinosphærium,* for example, in which the cytoplasm is sponge-like. In egg cells and in tumor cells the correctness of Bütschli's views can also be readily enough ascertained. In the case of metazoan cells it is in those with little or no functional differentiation that the alveolar structure is best marked. In protozoa it is the undifferentiated endoplasm which most clearly exhibits a typical "Schaumplasma."

As soon as an individual cell becomes functionally specialized and begins to take on activities peculiar to cells of its kind or species there occurs a more or less wide departure from the simple alveolar structure. Diversity of function leads to structural modifications and these give rise to the morphological differences which characterize the process known as differentiation. Depending upon the type of cell chosen for study, much evidence can be found to uphold the older ideas of the fibrillar structure of protoplasm of Remak, Max Schultze, Flemming, and others, and those of the net-like structure of Fromman and of Heitzmann. Even if one is unwilling to accept Altmann's bioblast theory in its entirety, one must admit that protoplasm may appear granular and that the granules present are, in some cases at least, living functional material, as has been amply shown in the case of enzyme secreting cells.

The fibrillar nature of the protoplasm of tumor cells has of recent years received strong confirmation by the work of Mallory, Thompson, Wolbach and others. The net-like structure is apparent in the

intestinal cells of amphibia and other lower animals and in large ganglion cells.

Whenever, however, the simple alveolar structure of cytoplasm is departed from, the cell, whether metazoan or protozoan, is one of such unusual size as to require supporting fibrils or net-works, or of such highly specialized function that the departure from the alveolar structure must be considered the morphological evidence of the specialized physiological activity of the cell.

NUCLEUS AND CYTOPLASM AND THE PART OF EACH IN CELLULAR ACTIVITY.

Just as the diversity of opinion as to the structure of the cell resulted from the type of cell studied and gave way finally to unanimity when the proper material was used by a mind able properly to value individual minutiæ and give them their place in a generalized whole, so also there have been various theories as to the chief seat of cell function. Again the diversity of opinion was dependent upon the type of cell selected for study. And, to carry the analogy between the two lines of cell study still further, again controversy is giving way to unanimity because of the study of the proper material.

In the earliest cell studies originated by Schleiden, Schwann, and their immediate followers the distinguishing morphological characteristic which made possible the identification of the individual cell as such was the delimiting membrane. To this was ascribed great functional importance.

With the development of knowledge and the recognition that protoplasm could undergo modifications, the cytoplasm became the all important portion of the cell, and the variations in the size, shape and structure of cells resulting from such modifications were looked upon as the morphological expression of cytoplasmic activity.

Investigations into the phenomena of maturation, division, and fertilization of sex cells brought to light the important rôle taken by the nucleus in a number of highly complex processes. The pendulum swung in the opposite direction—the nucleus became the essential element of the cell. Up to within very recent years the controversy has been maintained and much experimental work

has been done to show, on the one hand, that denucleated portions of protoplasm might manifest the evidences of living matter and that non-nucleated cells might, theoretically at least, exist in nature; and, on the other hand, that nuclei might live after they had been freed of cytoplasm and that the latter was only of secondary importance in the life of the cell.

The earlier work of Maupas (1888, 1889) and of R. Hertwig (1889) upon the conjugation of the Infusoria, involving as it did at the same time the consideration of processes much like those which had led to the enthronement of the nucleus, and the study of cells able to manifest a great variety of activities other than those concerned in the so-called sexual process, was the beginning of a transitional period. Further work upon protozoa by R. Hertwig and his pupils led to the postulation by him (R. Hertwig, 1903 *a* and *b*, 1904) of a mutual interdependence of nucleus and cytoplasm. This doctrine of the nucleus-plasma relation has been the stimulus for a large amount of confirmative work, which enables one to state briefly that nucleus and cytoplasm are dependent upon each other for their existence and for the expression of their particular functions; that cellular differentiation, whether the cell be a free living or colony forming protozoön, or a metazoan sex or tissue cell, is the expression of this interdependence; that, of the various structures which help in producing morphological differentiation, certain ones are directly, others more indirectly, nuclear derivatives, and still others cytoplasmic derivatives formed probably under the influence of the nucleus; and, that, finally, differentiation is the means by which the single cell, whether it be free living or a member of a colony or tissue aggregate, exercises the highly specialized functions demanded of it as an individual.

RICHARD HERTWIG'S NUCLEUS-PLASMA RELATION THEORY.

Based on Gerassimow's (1902) experiments upon the alga *Spirogyra*, and his own on protozoa and egg cells, R. Hertwig (1903*a*, 1903*b*, 1904) pointed out that for each cell there is an optimum size relation between nuclear mass and protoplasm mass which may not permanently be departed from in either direction without serious consequences, and in this connection he propounded

his nucleus-plasma relation theory. According to this theory the nucleus-plasma relation of a cell is represented by the quotient obtained by dividing the nuclear mass by the protoplasm mass. This figure is constant for each kind of cell and, as long as it remains within certain physiological limits, the cell functions normally. If this is upset to the advantage of either nucleus or protoplasm the cell is abnormal, and before it can function normally it must restore its normal nucleus-plasma relation. Hence, there is a struggle between nucleus and plasma which tends to preserve a normal nucleus-plasma balance or equilibrium. R. Hertwig further explains cell division upon the basis of his nucleus-plasma relation theory as follows: In a recently divided cell the functional growth of the nucleus is relatively slow in comparison with that of the protoplasm, until, shortly before the next division, there occurs an upset in the nucleus-plasma relation to the advantage of the protoplasm, producing a nucleus-plasma tension which is the determining factor in division. At this critical point, there sets in a rapid growth of the nucleus—divisional growth—changing the nucleus-plasma relation in favor of the nucleus and ending in cell division, which results in a normal nucleus-plasma relation for the daughter cells. These observations and conclusions of Hertwig have been confirmed by Popoff's (1908) recent exact measurements of the volume relation between nucleus and protoplasm in *Frontonia*.

CELL GROWTH.

Popoff's (1908) *Frontonia* work is of unusual importance because the conditions under which the cultures were kept could be held uniform from division to division. Since all cellular activities were, therefore, uniform from generation to generation one can leave out of consideration all functions except those concerned in cell growth. The variations within physiological limits of the relationship of nucleus and plasma could be accurately determined for every period in the life of the individual. This normal physiological variation is determined by the normal rate of growth of protoplasm for this particular cell.

Differences in the Rates of Cytoplasmic and Nuclear Growth in Frontonia at 25° C.

When examples of *Frontonia* were kept at a uniform temperature of 25° C. and were daily given regular amounts of fresh water and food, division occurred every seventeen hours. The results of exact measurements made at regular intervals gave the growth curves given in Popoff's Figure 9 (Chart 1).

During the entire period, from one division to another, the bulk of the cytoplasm increases gradually with uniform regularity. The nucleus, however, shows, at first, slight decrease in size (which may be disregarded) and then a slower rate of growth than does the cytoplasm. This continues until the difference in the amount of growth reaches its greatest point during the fifteenth hour.

The Production of Nucleus-Plasma Tension and the Inauguration of Division.

This point of maximum disproportion determines the moment of nucleus-plasma tension which inaugurates the divisional growth of the nucleus. During the sixteenth and seventeenth hours the nucleus grows rapidly, the increase in bulk being considerably greater during these two hours than during the entire previous fifteen hours. The disproportion in the relationship between plasma volume and nucleus volume, a disproportion which has been greatly in favor of the former, is overcome, and at the end of the seventeenth hour plasma and nucleus again bear the same relation to each other that they did at the beginning of the first hour. The volume of each, however, has been doubled and division occurs with the formation of two cells each with the volume of plasma and of nucleus, and with the relationship between the two, that are characteristic for *Frontonia* kept at 25° C.

By dividing the cytoplasm volume by the nucleus volume, obtained by measurements taken at regular periods, the nucleus-plasma relationship is mathematically expressed and plotted by Popoff in the curve given in Chart 2.

Leaving out of consideration the irregularity of the curve during the first four hours, due to the initial actual decrease in the size of the nucleus, the disproportion in the rate of cytoplasm growth

as compared with that of nucleus growth is seen to be a gradually increasing one up to the end of the fifteenth hour, the moment of nucleus-plasma tension which inaugurates the rapid divisional

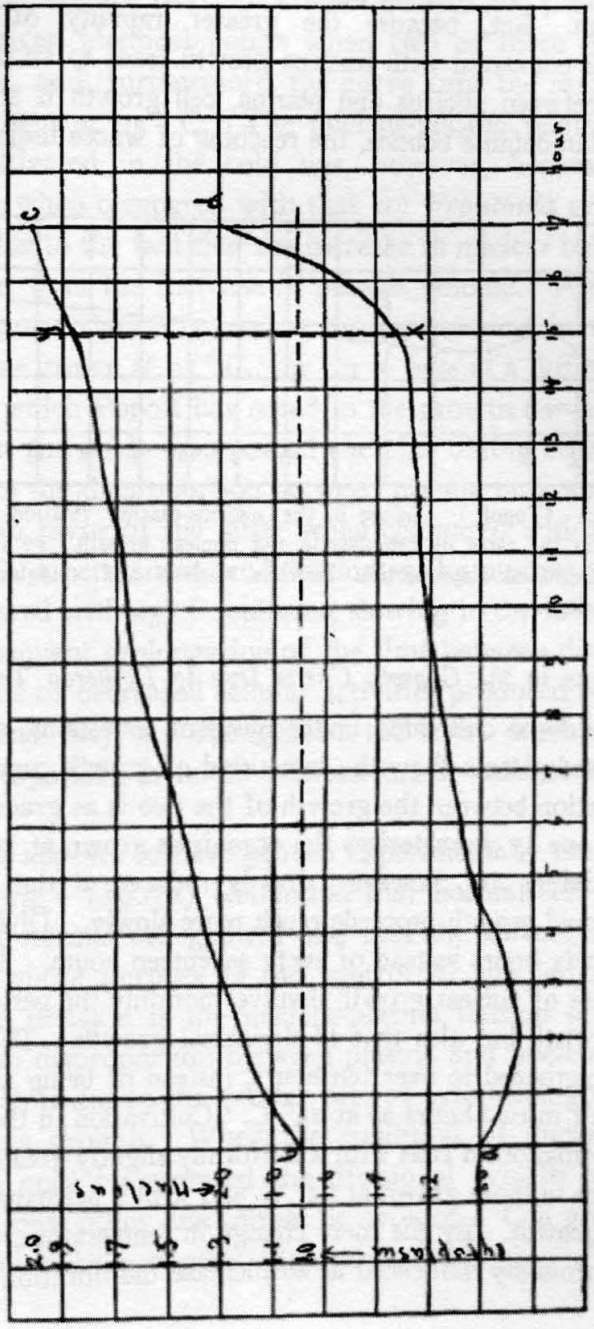


CHART 1. (Popoff.) Growth curves of *Frontonia* at 25° C. *ad* represents curve of nuclear growth; *bc*, curve of cytoplasmic growth; *xy*, the point of maximum disproportion between nucleus and cytoplasm (nucleus-plasma tension); *xd*, divisional growth of nucleus; *ax*, functional growth of nucleus.

growth of the nucleus. At the end of the seventeenth hour, when division occurs, the relation between nucleus and plasma is again the same as at the beginning.

A uniform rate of cell growth is a property inherent in living protoplasm. But, because the greater rapidity of cytoplasm growth as compared with nucleus growth leads to an upset in the relation between nucleus and plasma, cell growth is sharply confined within definite bounds, the reaching of whose limits results in division.

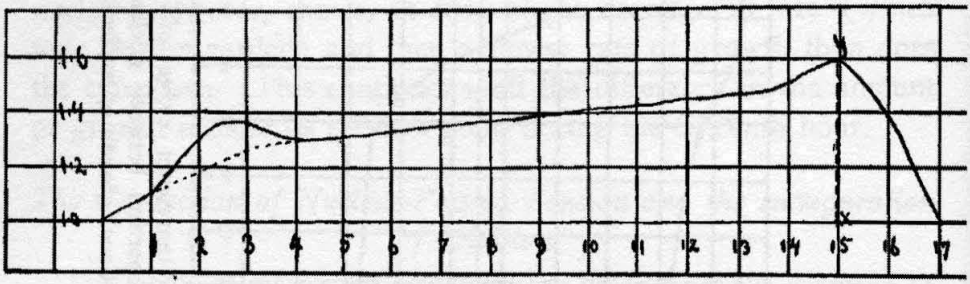


CHART 2. (Popoff.) Change in the nucleus-plasma relation, due to the variations in the rates of cytoplasmic and nuclear growth. *xy* represents the point of maximum disproportion between nucleus and cytoplasm (nucleus-plasma tension).

Alterations in the Growth Curve Due to Lowered Temperature.

In *Frontonia* cultivated under uniform conditions at 14° C., nucleus and plasma show the same kind of growth curves, and the disproportion between the growth of the two is as gradually an increasing one as characterizes the organisms grown at 25° C. Cellular activities are, however, greatly reduced at the lower temperature and growth proceeds much more slowly. Division occurs every ninety hours instead of every seventeen hours. The slowing of the rate of nuclear growth involves not only the period of functional growth but also that of divisional growth. The latter becomes lengthened to over ten hours, instead of being able to complete itself in two hours as at 25° C. Cultivation in the cold leads to the formation of cells with a uniformly slightly greater size than is the case in those grown at 25° C. and with a permanently slower rate of growth. By the mere change in temperature, cell growth is so profoundly influenced as to increase the duration of the life

of the individual over five hundred per cent. The important point, however, is that, although the size of the individual is permanently increased and the rate of nucleus and plasma growth permanently slowed, the volume relation between nucleus and plasma is the same at any given identical points when two or more generations are compared, and, furthermore, the curve runs the same course as in animals kept at 25° C. The nucleus-plasma relation of the animals cultivated in the cold has, however, become permanently changed, when compared with that for *Frontonia* grown at 25° C. This is due to the fact that the increase in nuclear bulk, due to cold, is greater than the increase in plasma volume. Although the nucleus-plasma relation curve has the same course in 14° C. animals as in those grown at 25° C., the curve runs at a different level. The changes which Popoff has noted in the growth curves of *Frontonia* grown in the cold—increase in the size of the cells, a permanent change in the disproportion between plasma bulk and nucleus bulk slightly in favor of the latter when compared with animals grown at 25° C., a permanent modification in the nucleus-plasma relation as compared with 25° C. cultures, slowing in the rate of cell growth and consequent prolongation of the time between divisions—all are the results of decreased cellular activities produced by the action of cold. And they are changes of the most profound importance when comparison is made between tumor cells and normal tissue cells.

Popoff appears to have proven experimentally the correctness of R. Hertwig's (1903*b*) contention that normal cell division is the result of normal cell growth and that the former is a regulatory process whose purpose is the control of the latter. The prime factor in division is the nucleus-plasma tension produced by the maximum disproportion between plasma and nucleus, a disproportion which is due to the greater rate of cytoplasm growth over that of nucleus growth. When the condition of nucleus-plasma tension has once inaugurated the divisional growth of the nucleus, division must occur and cannot be avoided. Cell growth and division automatically control each other.

Prolongation of the Period of Growth by the Prevention of Nucleus-Plasma Tension.

That the factor of chief importance in division in its relation to normal cell growth is the condition of nucleus-plasma tension, which occurs at the time of greatest disproportion between nucleus growth and plasma growth, has been shown experimentally by Popoff in *Frontonia*. By removing a portion of the cytoplasm at a time before the divisional growth of the nucleus had begun, that is, at a time when the disproportion between nucleus and plasma had not yet reached its height, division of the cell could be delayed for varying periods. In one case, by repeating the operation from day to day as the removed cytoplasm was renewed by growth, but always at a period before the growth had reached such a maximum as to produce nucleus-plasma tension, division was delayed for a period of twelve and one-half days. The life of the individual was increased from ninety hours to three hundred and ninety hours —an increase of four hundred and thirty-three per cent., and this in a 14° C. animal, in which low temperature had already prolonged the growth period five hundred per cent. as compared with a normal 25° C. animal. Cell growth is, therefore, a property of normal protoplasm which can be almost indefinitely prolonged if conditions are so altered by slowing the rate of cytoplasm growth that the moment at which the preponderance of cytoplasm volume over nucleus starts the divisional growth of the nucleus is postponed.

Indefinite Prolongation of the Growth Period not Possible because of the Exhaustion of Growth Energy when Division is Prevented.

Growth is not indefinite because the initial growth energy with which a cell is endowed is sooner or later used up even if nuclear division can be prevented. In Popoff's *Frontoniæ*, whose division was prevented by repeated operative removal of portions of cytoplasm, the cells finally went into physiological degeneration and died without division. The animals died with all the evidences of senility—sluggishness of motility, inability to take food and condensation of protoplasm. For the renewal of the energy used

up in growth there become necessary certain phenomena other than the usual asexual division.

UPSET OF THE NUCLEUS-PLASMA RELATION.

In Protozoa.

It has been established by the work of Maupas, Calkins, R. Hertwig, and the latter's pupils, that certain protozoa, when cultivated for a considerable period of time, cease to multiply, go into depression and, finally, undergo physiological degeneration and die. Before complete degeneration and death take place, there occur alternate periods of depression and recovery with active multiplication. R. Hertwig (1900) discovered that during the depression periods the nuclear material is not only relatively but actually increased in relation to the protoplasm; that is, that the normal nucleus-plasma balance is upset in favor of the nucleus. In *Paramœcium, Frontonia* and *Stylonychia* in this condition larger and smaller portions of the macronucleus are broken off and destroyed. In *Actinosphærium eichhorni,* a multinucleated heliozoön, R. Hertwig (1904) has shown that in overfed cultures, there occur periods of rapid multiplication alternating with periods of deep depression. During the latter, there occur marked evidences of increase of nuclear material, as is shown by nuclear hypertrophy (and at certain stages producing giant nucleus formation), hyperplasia and hyperchromatism, associated with marked degenerative changes in the protoplasm, such as retraction, thickening and loss of pseudopodia, plasmogam, condensation and thickening of the protoplasm, and even areas of necrosis of the latter. The degenerative changes of the nuclei are pronounced and varied, and embrace dissolution and resorption of nuclei (the protoplasm of this animal has developed to a high degree the property of breaking down nuclear material), nuclear fusion, and, most remarkable of all, the reduction of nuclear material by the passage of chromidia from the nuclei into the protoplasm, where they are either broken down, often with pigment formation, or extruded. Thus by destruction of superabundant nuclear material accumulated during excessive function and rapidly repeated divisions, this animal may reorganize or regulate

itself, and, so, recovering from depression, it may again feed and multiply. After depression and recovery have repeated themselves several times, the animals fall into fatal depression with hypernucleosis and markedly degenerated protoplasm—physiological degeneration—and the culture dies out. One of us (Howard, 1908) has confirmed and amplified Hertwig's findings in depressed *Actinosphærium.*

In Sex Cells.

R. Hertwig (1903 *a*), Goldschmidt and Popoff (1907), Popoff (1907 *a* and *b*) and Moroff (1909) have called attention to the occurrence of chromodiosis previous to division in sex cells. Popoff (1907*a*) has shown that after the rapidly repeated divisions of the spermatogonia and the ovogonia stages, the cells are in depression, make abortive attempts at division and finally regulate themselves by chromodiosis before division can start again. A large proportion fail to regulate themselves and die at this stage. It seems probable, indeed, that the maturation divisions of egg cells are in part, at least, regulatory phenomena. Sex cells after the maturation process are still in depression, for, unless conjugation occurs, they perish.

In Tissue Cells.

It is a well-established fact that the cells of the blood have a relatively short life. The older leucocytes present various evidences of depression, including condensation and lobulation of their nuclei. Ganglion cells of old animals present striking evidences of an upset in the nucleus-plasma relation. Of the various somatic cells of which we have accurate studies, the thymus cells bear the most striking relation to our theme.

The development of the thymus and the life history of its cells have been studied recently in *Hypogeophis rostratus* by Marcus (1908). His findings may be epitomized as follows:

The cells of the thymus anlage are at first exactly like those of the neighboring intestinal epithelium from which it is derived—cylindrical epithelial cells with oval nuclei and a small nucleus-plasma relation. During a period of relatively slow growth the

nuclei of the cells become larger, the cytoplasm decreases in amount, and the epithelium becomes lower, resulting in a nucleus-plasma relation somewhat in favor of the nucleus. The nuclei are now from 7.5 to 9.0 micra in diameter and the cell bodies are of considerable size. With the separation of the thymus vesicle from the intestine, there occurs an intensive growth, in which the cell divisions take place so rapidly that the daughter cells do not have the chance to reach the size of the mother cells. Thus the vesicle becomes a compact mass of small cells, the nuclei of which at the end of this stage have about half the surface of those at the outset. The cytoplasm is diminished even more in proportion, some cells possessing very little recognizable cytoplasm. In this manner arise the so-called "lymphoid" cells of the thymus, which are depressed cells with a nucleus-plasma relation markedly in favor of the nucleus. The nuclei have not only gained in size relatively, but are highly hyperchromatic.

While the chromosomes of the dividing nuclei in the previous stage are slender, delicate rods, now they are deformed, clumped in large, square or round ball-like masses, unfitted for exact division. This leads to pathological mitoses, and thus there occur all transitions from normal to degenerative nuclear and division structures—heteropolar mitoses, clumped chromosomes and single chromatic balls. At this time the organ is composed of a single type of cell, the "lymphoid" thymus cell, whose characteristics are indicated above. It can no longer divide and its functional and assimilation capacities must be at a low ebb: it must die unless reparation in some form occurs.

The next stage in the evolution of the organ is marked by the appearance of cells of the "epithelioid" type, originating from the "lymphoid" cells, which increase in size by growth of the cytoplasm. The nuclei of the "epithelioid" cells are hyperchromatic, increased to twice the normal size, and are diplocaryotic nuclei in Boveri's sense. When these cells attempt to divide, their chromosomes are ill-shaped and abnormally large, often to such a degree that division is arrested. From this abnormal state various reparative and degenerative changes may be traced. By reduction of

the nuclei by chromidiosis and by growth of the cytoplasm the may be produced very large cells with pale nuclei. The most i portant degenerative changes are the production of achromat nuclei, vacuolization and hyaline degeneration of the cytoplas and the degeneration of chromidia with the formation of larg and smaller masses of nucleolar substance, giving rise to eosin philous granules in the cytoplasm. Further, in some nuclei the occur appearances strikingly suggestive of the synapsis stage sex cells. Many of these large "epithelioid" cells fuse by pro esses (plasmogamy), as often observed in depressed protozoa, a form large protoplasmic masses with two or more nuclei. Tl central portions of such masses show various stages of degener tion and thus the Hassall bodies are formed.

Marcus sets forth the direct bearing of these various stages the development of the thymus cell upon Hertwig's nucleus-plasm relation theory, and indicates the striking analogies between tl depression states of the thymus cell and phenomena observed b Hertwig, Popoff, himself and others in protozoa and in sex cell Marcus also indicates the relation of the changes in the thymus ce with metaplasias occurring in this organ—the development of mu cous cells, striated muscle cells and ganglion cells—and with tl various histological differentiations found in dermoid cysts.

THE NUCLEOLAR SUBSTANCE.

In what has been said above, the ubiquitous relationship of th nucleus to the biological processes occurring in normal and abno mal cell life has been pointed out. In the diverse functions of th nucleus and in its varying relationships to the cytoplasm the aci staining material known variously as oxychromatin, nucleolar sul stance, plastin, pyrenin, etc., plays an important rôle. In what to follow, the term karyosome will be used in preference to nucle lus and plasmosome. Whether the plastin present in the intran clear body is combined or not with chromatin is a matter of seco dary importance. The chief point is the presence in the karyoson of plastin.

The Relation of the Nucleolar Substance to Vegetative Function and to Reproduction.

Moroff (1908), in his exhaustive study of several species of *Aggregata,* concludes that the karyosome has a purely vegetative function. Applying the doctrine of nuclear duality to nuclei which are morphologically single, he concludes that the karyosome is the somatic nucleus of the cell and is directly comparable to the macronucleus of the *Ciliata.* Up to a certain point one must agree with this author. The participation of plastin in the secretory activities of plant and animal cells is known. In these cells it may occur diffused throughout the nucleus, either combined or uncombined with chromatin, or it may be collected into a single larger intranuclear mass either free of chromatin as a true nucleolus or plasmosome or united with chromatin as an amphinucleolus or compound karyosome. But it is also a well-established fact that plastin takes part in the formation of chromosomes. Furthermore, the material of the karyosome plays an active part in the formation of those intranuclear, amitosis-like figures which are formed during the division of a number of protozoan nuclei, nuclei belonging to the type termed centronucleus by Boveri. The karyosome and its constituent nucleolar substance may have, therefore, not only vegetative or somatic functions, but also animal or reproductive properties.

The Rôle of the Nucleolar Substance in the Organization of Chromatin.

The chemical differences between plastin and chromatin rest upon too insecure a basis to permit an attempt at the correlation of function and chemical composition. The recently expressed view of Růžička (1908), that plastin is a complex albuminoid or albuminoid-like substance built up from more labile protoplasmic compounds and that it may be broken down into the relatively less complex chromatin, is not at variance with known facts but adds nothing essentially fundamental for the explanation of those facts.

However varied or important may be the part taken by the nucleolar substance in nuclear and cellular phenomena, the chief virtue of the material lies in the relationship it bears to the chro-

matin. The most acceptable expression of this relationship we owe to R. Hertwig (1902*a*). Ignoring, in the present state of our knowledge of cytochemistry, the question of the nature of the nucleolar substance, Hertwig demonstrates the intimate relationship between this substance and chromatin, and explains the functions of the former by means of this relationship. According to him, the material from which chromatin is derived is formed in the cytoplasm and exists there in a form not to be recognized, because it has, at this stage, no characteristic staining reactions. The union, within the nucleus, of this prochromatin, if one may coin the word, with the nucleolar substance results in the formation of the basic staining material which we call chromatin, and in this organization the chromatin becomes visualized.

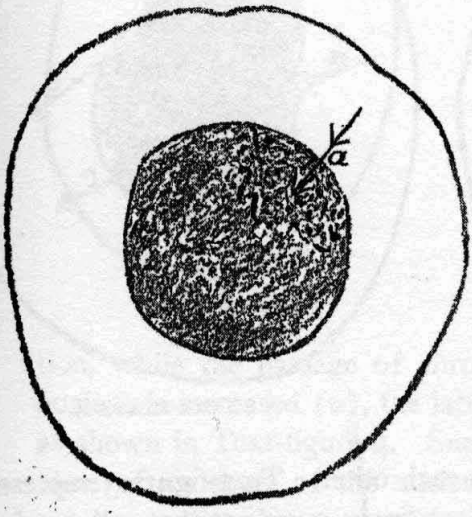
The Utilization and Fate of Chromatin in Cellular Function.

Excessive nucleolar substance may be stored in the karyosome, to be used in the further production of chromatin as may be required by the activities of the cell. From the nucleus, chromatin and its derivatives return to the cytoplasm to be used in the vegetative functions of the cell and in the formation of many of the morphological differentiations associated with these functions. When the chromatin leaves the nucleus in the form of granules or masses, the nuclear origin of these bodies is apparent from their property of being stained with the basic dyes. In the further changes which these chromidia may undergo, the basic staining property gradually becomes lost and the granule or mass takes the characteristic oxychromatin stain. An identical series of changes can be seen in the karyolysis and karyorrhexis which occur in a number of degenerative processes. As the basic staining material disappears from the nuclei the plastin again comes into view. Finally, this also loses its identity. It has been recently pointed out by one of us (Schultz, 1909, in association with Corlett) that in the production of keratohyalin in the epidermis there occurs a similar succession of changes.

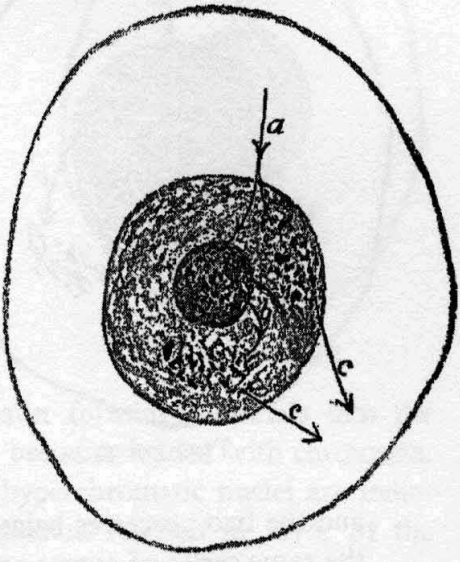
Chromatin would seem to be, then, an unstainable material derived from the cytoplasm plus nucleolar substance. The latter is the material by which, on the one hand, the nucleus derives from

the cytoplasm the substances necessary to the former and by means of which, on the other hand, the nucleus controls the varying activities of the cell. The nucleolar substance, therefore, is of prime importance in the interdependence which exists between nucleus and cytoplasm and is necessary for the maintenance of a proper nucleus-plasma relation.

Hertwig's idea concerning the nucleolar substance may be expressed diagramatically, and the same means may be used for amplifying this idea and for making clear certain of the events which occur in the regulatory phenomena necessary for maintaining the proper balance between the nucleus and plasma. In the diagrams the chromatin is represented by black, and nucleolar substance by shading.



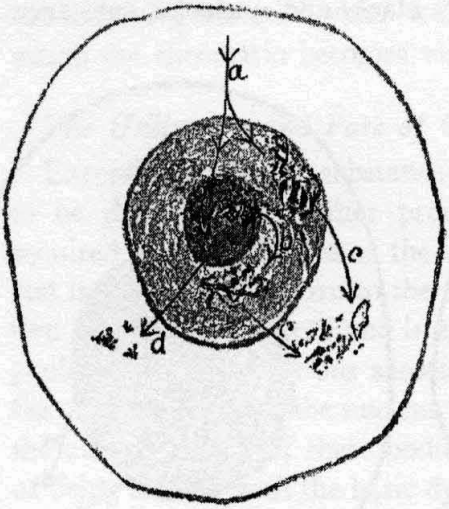
TEXT-FIGURE 1.



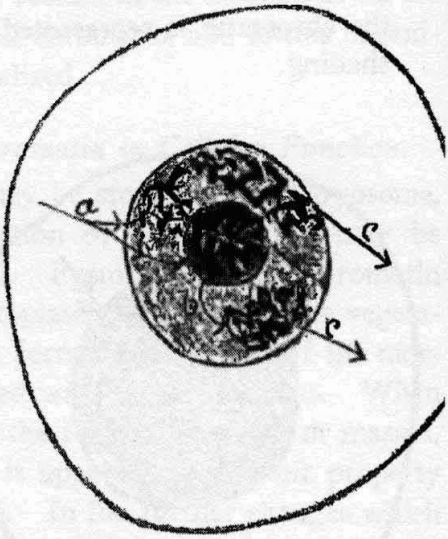
TEXT-FIGURE 2.

The participitation of the nucleolar substance in the formation of chromatin is illustrated by Text-figure 1, the passage of materials into the nucleus, where they become visualized as chromatin, being indicated in this and in Text-figures 2, 3, 4, 5, and 6 by the arrow (*a*). If there is no excess of nucleolar substance stored in a morphologically differentiated intranuclear body, this process results in the formation of a nucleus with chromatin more or less diffused. This chromatin visualization is only one side of the nucleus-plasma

equation. In Text-figure 2 is shown a further stage, the passa of chromatin derivatives into the cytoplasm, there to be used in t activities of the cell. This step is designated by the arrow ((If the nucleus contains a nucleolus, chromatin may be deposited he and then passed on in order to become diffused throughout the r cleus, as represented by the arrow (*b*). Text-figure 2 illustrates, diagrammatic fashion, the course of events in the great majori of tissue cells, those cells with a not very active function. Fro the nucleus, materials are constantly given off to the cytoplasm unstainable material, fresh chromatin is being constantly forme



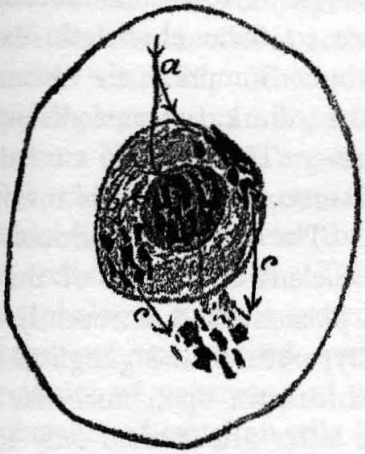
TEXT-FIGURE 3.



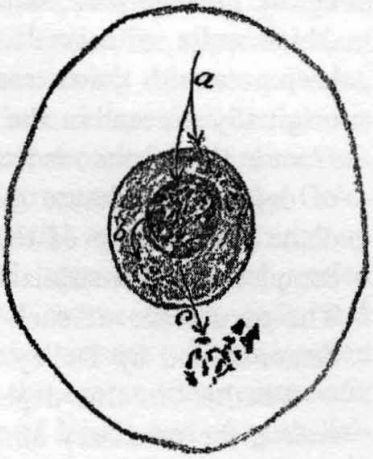
TEXT-FIGURE 4.

and the two processes balance each other. Text-figure 3 represen the same series of events as Text-figure 2, except that cellular fun tion is much more active, larger amounts of nuclear materials a necessary in the cytoplasm for the very active function, and the is a chromatin formation sufficiently active to keep nucleus an plasma in equilibrium. Such a process occurs in many protozo and in certain metazoan cells, notably gland and ganglion cell The increased demand of the cytoplasm for nuclear materia leads to the extrusion from the nucleus of masses and granul which take the chromatin stains, extranuclear chromatin of varie morphology making up the chromidial apparatus of Goldschmi

(1904*a*). Nucleolar substance uncombined with chromatin may also be given off to the cytoplasm, as indicated by the arrow (*d*). In Text-figures 2 and 3 we are dealing with cells with a normal nucleus-plasma relation because of the perfect balance maintained between the using up of nuclear materials (*c*) and the formation of chromatin (*a*). The remaining text-figures illustrate the mechanism in the disturbance of the nucleus-plasma relation due to abnormalites in one or both links of the chain. If in such a cell as is represented by Text-figure 2 the passage of nuclear materials into the cytoplasm (*c*) remains normal, or is decreased by loss of func-



TEXT-FIGURE 5.



TEXT-FIGURE 6.

tion, while the passage of chromatin forming materials into the nucleus is increased (*a*), the latter becomes loaded with chromatin, as shown in Text-figure 4. Such hyperchromatic nuclei are indicative of an upset of the nucleus-plasma relation in favor of the nucleus, a disturbance characteristic of the depression seen in protozoa, in certain stages of the development of sex cells, in thymus cells and in tumor cells. If now, as indicated in Text-figure 5, the passage of nuclear materials into the cytoplasm (*c*) can be increased to such a degree as to approximate almost or wholly the increased chromatin formation (*a*), the hyperchromatism decreases, the nucleus approaches the normal in appearance, and the depression is more or less completely overcome. One of the evidences of this increased passage of nuclear materials into the cytoplasm is the

presence of chromidia. Chromidiosis as a regulatory mechanism, a process for the reëstablishment of a normal nucleus-plasma relation, occurs in depressed protozoan, thymus and egg cells and, as we shall show, in tumor cells. In the production of depression characterized by hyperchromatism, actual or relative increase in chromatin formation is the active link in the chain of events. There is, however, the possibility of depression due to increased chromatin destruction, as shown in Text-figure 6. If a cell is thrown into a condition of functional hyperactivity the cytoplasm makes an increased demand upon the nucleus for nuclear materials. If the passage into the nucleus of those cytoplasmic substances which unite with nucleolar substance to form chromatin cannot keep pace with the excessive chromatin consumption, the chromatin originally present in the nucleus and in the karyosome disappears from both and the nucleus becomes pale. The cell is in a condition of depression because the nucleus-plasma relationship is markedly disturbed in favor of the cytoplasm. The end of the process is a complete dechromatinization of the nucleus and death of the cell. The occurrence of such a series of phenomena has recently been demonstrated by Dolley (1909) in hyperstimulated ganglion cells.

Whether, in this dependence of chromatin upon nucleolar substance, the necessary amounts of the latter are formed only in the nucleus from materials derived from the cytoplasm, or whether nucleolar substance can be preformed as such in the latter situation, cannot be definitely stated. The constant production of nucleolar substance, however, would seem to be a premise required for the explanation of the continuous formation of chromatin and the ceaseless transference of nuclear materials to the cytoplasm. That the production of nucleolar substance may become excessive and exceed all the probable demands of the cell we shall point out later in tumor cells.

THE PREVENTION OF AND RECOVERY FROM DEPRESSION. REGULATORY PROCESSES.

The Avoidance of Depression.

It has been pointed out in the foregoing pages that cells in general tend to fall into depression. In normal cells with a normal divi-

sion rate, the functional activities of which are not interfered with, depression is avoided by the regularly repeated divisions and by the using up of nuclear and cytoplasmic materials in differentiation and functional activity. When, in cells readily capable of division, functional activity is increased, due to overfeeding in protozoa, for instance, there is marked increase of nuclear material and the nucleus-plasma relation is upset in favor of the nucleus. Division, as long as it can occur, tends to restore the nucleus-plasma balance. Calkins and Lieb (1902) have shown that the addition of certain salts to cultures of *Paramœcium* results in avoiding depression in this animal for many generations. Gland, muscle and ganglion cells find in functional activity a marked outlet for superabundant nuclear material. The extranuclear functioning nuclear material, which plays such an important rôle in these cells, called by various authors Nebenkerne, ergastoplasm, pseudochromosomes, mitochondria, chromidial net, etc., and correctly grouped together by Goldschmidt (1904*a*) under the term chromidial apparatus, is used up in cellular activities. This chromidial apparatus is of varied morphology, such as networks, fine and coarse fibrils, coils, and large and small round, oval and irregular masses. The secretion granules of pancreas and poison cells, the extranuclear chromatic substance of ganglion cells (the granules of Nissl) and of epithelial-muscle cells, all examples of chromidial apparatus, are used up in functional activity and renewed during rest.

Recovery from Depression.

As cells may avoid depression by the use of certain regulatory processes, just so may they recover under favorable conditions from a depression otherwise fatal. Its nucleus-plasma relation once disturbed, the cell strives to restore it to the normal condition. The methods employed vary with the capacities and states of the cell. In cells fatigued short of exhaustion, the nucleus-plasma relation being in favor of the cytoplasm, the nuclear material is restored during a period of rest. This is well illustrated by the changes which occur in pancreas, ganglion and certain muscle cells.

When the nucleus-plasma relation in depressed cells is in favor of the nucleus, four methods of regulation are available: (1) cell

creased to a very great degree. Marcus (1908), it will be recalled, found that when after rapidly repeated divisions the cells of the thymus have fallen into marked depression, and the organ is composed of "lymphoid" cells, the cytoplasm of some of these cells grows markedly, thus forming the "epithelioid" cells, which may divide.

It has been shown by the studies of Calkins, R. Hertwig, and the latter's pupils, that the conjugation epidemics of certain protozoa occur during periods of marked depression, and if conjugation is prevented the animals finally die. Sex cells, though they preserve or regain divisional capacity through chromidiosis and other means, finally die unless fertilization ensues. In animals in which the type of conjugation is isogamous, as in *Actinosphærium eichhorni*, in deep depression even this opportunity for regulation and a fresh start may be debarred, for on account of the massive development of nuclear material the animal may not be able to encyst, or, if encystment is possible, the conjugation elements in the cysts may be so abnormal that the process is abortive.

R. Hertwig (1889, 1902*b* and 1903*b*) holds that conjugation is essentially a phenomenon of cell regulation without which the cell must finally perish. So far as is known, protozoa and sex cells are the only cells which have been able to preserve this most important regulatory process for the avoidance of and recovery from depression. In metazoa the soma perishes ultimately from physiological death; only the sex cells, which conjugate, have the capacity to live on indefinitely. In the nature of things, therefore, cells debarred from conjugation, although protected from the effects of accident, infection, toxemia, starvation, overwork and various harmful physical agents and the like, are destined ultimately to death from physiological degeneration. With the exception of prolonged overwork, the most distinctive mark and the apparent constant cause of physiological degeneration is excessive growth of nuclear material at the expense of the protoplasm.

SUMMARY OF GENERAL CELLULAR BIOLOGY.

In the foregoing pages we have indicated and emphasized the main features in the structure of nucleus and cytoplasm and the

known laws of cell growth, division and differentiation, and have shown, (1) that in order to prevent or postpone depression, physiological degeneration and death, cells must reorganize and regulate themselves; (2) that depression and physiological death in growing cells and tissue are associated with and dependent upon overgrowth of the nuclear material at the expense of the cytoplasm, that is, to an upset of the nucleus-plasma relation in favor of the nucleus; (3) that the change occurs especially in rapidly multiplying cells; (4) that the nucleus-plasma relation is intimately associated with cell division; and, finally, (5) that cells have developed several methods of regulation.

These regulatory processes are: (1) growth of cytoplasm, with differentiation, secretion and other methods of cell activity in which excessive nuclear material is used up; (2) cell division; (3) nuclear resorption and chromidiosis, often followed by cell division; (4) dissolution and extrusion of nuclei; (5) karyogamy.

III. DESCRIPTION OF TUMORS.

MATERIAL AND METHODS.

The material upon which this study of tumor cells is based has been collected over a period of several years from operations and autopsies. The operation material was fixed within a short time, often only a few minutes, after removal from the body, and for the most of it we are indebted to our colleagues, Professors Allen, Crile, Hamann and Robb.

As fixing agents, saturated aqueous sublimate solution, saturated aqueous sublimate solution and formalin, Flemming's solution and Zenker's fluid have been used. Surprisingly good fixation has been often obtained with formalin and with Orth's fluid. All material which did not show perfect fixation has been discarded. Great care has been taken to prevent shrinkage and other artefacts by the avoidance of rapid hydration and dehydration, and the use of graded alcohols has been scrupulously adhered to. Chloroform has been used for clearing and chloroform paraffin for embedding. As small, thin specimens have been used, the material has usually been kept in the paraffin incubator (50° to 55° C.) not longer than

two hours. Only paraffin sections have been used, and the sections have been fixed to the slides by the water method and, therefore, never incubated.

As routine stains we have used Grenacher's alcoholic borax-carmin, either alone or followed by dilute Delafield's hematoxylin, Mayer's acid hemalum, Delafield's hematoxylin and aqueous eosin, and Heidenhain's iron hematoxylin, with and without eosin. As special stains, Mallory's fibril stains and the Biondi-Heidenhain triple stain have been resorted to.

OUTLINE OF WORK.

The present work embraces a study of tumor cells as individuals as well as tissue forming cells. Therefore, it has been concerned especially with the study of the growth rate and other growth habits, and the differentiation capacity of tumor cells, the occurrence or non-occurrence of physiological degeneration among them and the regulatory processes by which the latter are avoided or recovered from. In short, the problem which we have set out to answer is, to what degree do tumor cells adopt and follow the same general biological laws which have been ascertained for protozoa, sex, thymus and certain other metazoan cells?

It is necessary to recall that different types of cells vary very widely in their capacity for division, the very highly differentiated cells like nerve cells, striated muscle cells and certain gland cells having either no divisional capacity, or a relatively slight one. In differentiation their idiochromatin has been completely or nearly completely lost, or perhaps on the same account their protoplasm has lost the power of performing its share in the division process. The various types of epithelium have very different capacities for division, germinal and covering epithelia having much greater power of division than that of glands, and certain glandular epithelia divide much more frequently and readily than others. Of the supporting tissues, fibrous tissues, vascular endothelium, glia, bone, cartilage and smooth muscle seem to respond to division incitants in the order named.

CLASSIFICATION.

As growth is the most fundamentally important and universally applicable of the known attributes of tumor cells, our tumors are classified according to their rate of growth, as follows: (1) slow, (2) moderately rapid, and (3) rapid.

1. *Tumors of Slow Growth.*

To avoid unnecessary details, descriptions of the well known structural characters of the ordinary benign tumors of fibrous, smooth muscle, cartilage, bone and epithelial tissues will be omitted. Speaking generally, these tumors are of slow growth and the differentiation of their cells is good and often perfect. Inter- and intracellular fibrils and ground substance are produced as in normal tissues. Careful study shows that in many such tumors, especially in the areas where the cell divisions have been more active, the cells are somewhat larger, both as regards cytoplasm and nuclei, than the cells from which the tumor has sprung; but there are, as a rule, few evidences of depression to be made out. Giant nuclei and giant cell formation rarely occur. The growth rate is so regular and slow and the differentiation capacities of the cells are so well preserved that the normal structure of the tissue is attained. When in such tumors the growth rate becomes accelerated, and differentiation is imperfect, evidences of depression are met with. Examples of this type are seen in fibromata and leiomyomata which become sarcomatous. In certain slowly growing fibrosarcomata, the bulk of the tissue is composed of cells rather than of fibrils, and many or even most of the cells are of the giant cell type, with a single nucleus, often in the process of budding. Here the divisions are more rapid, depression ensues, the cells do not form fibrils (fail to differentiate properly), and there may be a heavy mortality of the tumor cells from depression. A peculiar type of slowly growing sarcoma, with almost complete failure of differentiation, is illustrated by Tumor No. 1, a submucous tumor of the lower jaw of a boy of eight years.

Tumor No. 1. Epulis of the Lower Jaw.—The tumor, a small, ill-defined and unencapsulated growth, had been present for over two years before the excision of the portion submitted. Since it attained the size which attracted attention

it has remained practically stationary. The clinical diagnosis was epulis, and at the first hurried microscopic examination the tumor seemed to be composed of large multinucleated giant cells lying in a nucleated, vascular stroma. Closer examination shows, however, that the ground substance is a protoplasmic mass containing rather large, oval, vesicular nuclei (Plate I, Fig. 1). The great vascularity is due, not to definite blood vessels, but to irregularly shaped, unlined blood spaces running throughout the syncytial tissue (Plate I, Fig. 2). The nuclei vary in size and show transverse diameters of 3.3 to 6.7 micra, and longitudinal diameters of 6.4 to 13.5 micra. They contain a rather wide-meshed reticulum upon which are deposited fine chromatin granules. Many nuclei show also two or several larger chromatin masses, and a considerable proportion are supplied with rather large karyosomes containing relatively little chromatin in the form of one or more granules.

The giant cells present bear a very definite relationship to the blood spaces and have been produced apparently in a mechanical way by the cutting off of multinucleated portions of protoplasm (Plate I, Fig. 3). The cell illustrated in this figure has a size of 58.1 by 37.1 micra. The giant cells thus produced do not appear to be capable of independent existence. They show all degrees of degeneration from irregularity of nuclear outline, condensation of chromatin and vacuolization of protoplasm (Plate I, Fig. 4) to nuclear fragmentation and destruction of the entire protoplasmic mass. In these degenerative changes the cytoplasm takes a faint, diffuse hematoxylin tinge. Individual, large mononucleated cells are also produced. These also seem to have been cut off mechanically by the blood spaces (Plate I, Fig. 2). Some lie free in the spaces. They are irregularly polygonal in shape, but do not take on the spindle form associated with advancing differentiation. They vary in length from 8.7 to 18.9 micra and even more, and in breadth from 3.7 to 8.7 micra. Their nuclei vary from 3.0 by 6.4 micra to 5.4 by 8.7 micra. In some of these cells the nuclei are hyperchromatic and irregular, and the cells seem to be in an early stage of degeneration. In very rare instances the protoplasm about a nucleus embedded in the syncytial mass stains in a slightly different tone and there is faintly outlined what appears to be the beginning of a cell—a process not due to the mechanical action of the blood spaces (Plate I, Fig. 2). From a few of the individualized cells fine fibrils extend. Nuclear figures are extremely rare—only two or three have been met with in a rather large number of sections studied. Histologically as well as clinically the tumor is one of very slow growth. It extends superficially to just beneath the mucosa of the gum, without invading the papillae and without having caused any flattening or atrophy of them.

The point of chief interest in this tumor is the extreme loss of the power of differentiation, a condition upon which is largely dependent the slow rate of growth. Because the formation of individual cells is interfered with and to a large extent prohibited, there is not attained such a relationship between nucleus and cytoplasm as permits a regular rate of cell multiplication. This tumor has also a very important bearing upon the origin of certain mixed

tumors and syncytiomata and upon dermoid cysts, as will be indicated later.

The slowly growing malignant tumors of epithelial origin resemble in many respects benign tumors. In our experience, the slow growth depends upon (*a*) capacity of the cells to differentiate properly, (*b*) imperfect capacity of cell regulation and (*c*) slight divisional capacity, and (*d*) perhaps to the presence of the incitants to cell growth in only small amounts.

The tumors arising from stratified epithelia furnish numerous examples of this class. Arising from the layer of basal or germinative cells of the part which gives rise normally to cells which show a certain degree of morphological differentiation—fibril formation—and produce pigment, but are otherwise devoid of secretory activity and which have a considerable degree of division capacity, these tumors show great variation in growth rate and in differentiation capacity.

The common type of epithelioma of slow growth resembles an inverted skin papilloma and shows a reproduction of true differentiated epidermis with the various layers of morphologically differentiated cells with fibril formation, the central cells undergoing keratinization. The divisions here are not numerous at any one time and occur always in the peripheral or basal cells. The older cells are pushed towards the centre of the alveoli. Such tumors rarely form metastases and do not invade deeply. The division factors are inactive, the cells divide slowly and differentiate very much as in normal skin. The nucleus-plasma relation is disturbed to the advantage of the nucleus in many of the cells.

The so-called basal cell epitheliomata grow slowly, show but few division figures and form long thin or thick masses of cells with relatively very large hyperchromatic nuclei and a very small amount of cytoplasm. They differentiate but poorly morphologically, either in size or fibril formation, and, with their relatively large hyperchromatic nuclei, bear all the marks of depressed cells. They represent tumor cells acting under mild division incitants and not having the capacity efficiently to use either ordinary or acquired methods of regulation.

A third group of slowly growing epitheliomata springing from

stratified epithelia is characterized by closely packed alveoli of the carcinomatous type and entire absence of differentiation. There is no attempt at the formation of epidermis and no difference between the characters of the cells at the margins and those in the center of the alveoli. Contrary to what obtains in the first group above described, cell division is not limited to the cells of the external layers, but may occur in any of the cells of the alveoli. Keratinization and "pearl" formation are absent. The majority of the cells are in marked depression, as shown by hypertrophy (often giant nucleus formation), hyperchromatism and various degenerations of the nuclei. Giant cells with a single large or with many small nuclei are numerous. The mortality of cells from irrecoverable depression is a marked feature of these tumors. Nuclear budding, the importance of which as a regulatory process will be developed later, is the only discernible evidence of regulation. In these tumors the incitant to cell division is evidently strong, but increase in bulk of tumor tissue is slow, because, on account of failure to develop satisfactory regulatory processes, a large proportion of the cells fall into hopeless depression and finally die. For the same reason metastases do not occur. Tumor No. 2 well illustrates this group.

Tumor No. 2. Epithelioma of the Mucosa of the Right Cheek.—The patient, a man of 60 years, was admitted to the service of Dr. G. W. Crile, September 2, 1907. Some months before, he had noticed three small papillomatous growths of the mucosa of the right cheek, at a point where the membrane was irritated by a broken tooth. The growths and the tooth were removed. On admission, on the inner aspect of the right cheek there was a small hard growth with an ulcerated, crater-like center. The tumor was removed at operation.

The growth consists of larger and smaller alveoli of oval and polygonal epithelial cells supported by a well-marked and vascular connective tissue stroma. The alveoli are not of uniform size and are usually elongated. The cells within the alveoli vary in size, but show no evidence of differentiation. The marginal and the central cells show no distinctive differences, and there is no keratinization and no formation of "pearls."

The type cell of the tumor, as found in the growing areas among cells showing mitoses, measures 17.0 by 15.0 micra, is round, oval or polygonal, with a finely alveolar cytoplasm and a rather large round or oval vesicular nucleus, whose meshwork is quite wide. The nucleus contains one fairly large, deeply staining karyosome, and numerous small chromatin granules scattered over the reticulum. The sections include the whole depth of the growth. In the more superficial portion, the alveoli and the tumor cells are much larger than in the

deeper portion. In the latter, which is evidently the younger growing portion, there are fairly numerous mitoses. They are bipolar, but very rich in chromatic material. Even in this portion of the growth, the cells vary very much in size. In the older portion, there are few or no mitoses. Nearly all the cells are large, with large single hyperchromatic nuclei, or multiple nuclei, the result of budding. Types of these large or giant cells measure as follows: 21.5 by 16.0, 30.0 by 16.0, 38.0 by 25.0, 51.0 by 23.5, and 61.5 by 28.0 micra. (Plate I, Figs. 5 and 6, Plate II, Figs. 7, 8 and 10.) All gradations of nuclear hypertrophy and hyperchromatism can be traced up to large giant nuclei containing numerous large and small round and oval chromatic masses (Plate II, Fig. 8). The giant hyperchromatic nuclei may undergo direct division with the formation of two nuclei of equal size, budding with the formation of one or more daughter nuclei (Plate I, Fig. 6, Plate II, Fig. 7) or various forms of nuclear degeneration. The chromatic material may become diffused and present a homogeneous mass, with or without vacuolization (Plate II, Fig. 9), or break up into a large number of small round or oval masses which may become vacuolated (Plate II, Fig. 10). In such depressed cells vacuolization and hyaline degeneration of the cytoplasm are frequent and the cells perish. There is no evidence of chromidiosis.

Tumors similar to the above are found in the skin and the cervix uteri.

The slow growth of tumors springing from glandular epithelium, especially in the pancreas, is often due to the marked depression and lack of regulatory processes of their cells.

Many slowly growing malignant tumors, especially of the skin, are, doubtless, kept going, when otherwise they would stand still or even die out, by renewed beginnings of growth from new foci in the neighboring original tissue—pluricentric origin.

2. *Tumors of Moderately Rapid Growth.*

To this class belong (1) tumors that grow slowly at the start but that, through the development of regulatory processes and, perhaps, through an increase in the incitants to division, take on more rapid growth, and (2) tumors that grow moderately rapidly from the beginning.

In the first division belong certain sarcomata starting as fibrosarcomata, and carcinomata arising from slowly growing epitheliomata, which have developed nuclear reduction by amitosis, budding and chromidiosis; and certain sarcomata starting from fibromata and leiomyomata and certain carcinomata springing from benign epithelial tumors, in which the growth acceleration is best

explained by assuming the accession of an increase in the growth incitants. The future history of such tumors is governed by the degree of regulation that they can accomplish.

In the second division, composed of tumors whose growth rate is moderately rapid from the start, certain types of regulation are, in our experience, always present. In a certain group of these, notably the adenocarcinomata of the intestines and uterus, the tumor cells do not fall into marked depression, probably because, as is suggested by the morphology of the individual cells, there is retained a degree of differentiation compatible with a considerable amount of functional activity—mucous secretion, etc. In these growths it is evidently only the increased divisional activity that distinguishes them from benign adenomata. We have, however, occasionally met with adenocarcinomata of the intestine in which many of the tumor cells were in fatal depression. In another group, in which many cells fall into marked depression and many die, the most marked regulatory processes are amitosis and nuclear budding. The latter is often followed by both direct and indirect division. Chromidiosis and nuclear resorption are comparatively rare, but when they occur, a higher degree of regulation and less well marked depression are met with.

Tumor No. 3. Metastasizing Giant Cell Sarcoma of the Esophagus.—Of the tumors giving clinical and microscopic evidence of moderately rapid growth, the first to be detailed is a metastasizing giant cell sarcoma of the esophagus, which shows certain interesting phenomena associated with amitotic division and nuclear budding.

The patient had exhibited clinical symptoms referable to the tumor for about one year previous to death.

At autopsy, the esophagus showed a large fungating tumor mass, most prominent at a point opposite the tracheal bifurcation and extending from here to the lower end of the esophagus. The tumor showed numerous areas of necrosis and of hemorrhage. The bronchial lymph glands were involved and the majority were necrotic. Numerous nodules of a pinkish gray color were present in the liver. Both adrenals were enlarged by the presence of tumor metastases, situated chiefly in the cortical portions of the organs. In the adrenals the tumor tissue contained small areas of brownish pigment.

The tumor is composed of closely packed cells of varying size and shape, showing no tendency toward alveolar arrangement. There is a very slight amount of intercellular connective tissue stroma. Wide, thin-walled blood vessels are numerous.

In the older portions of the tumor the cells vary in size from those measuring

10.1 by 14.5 micra to giant cells even larger than the one shown in Fig. 12 (Plate II). The latter measures 52.0 by 58.1 micra. In the smaller cells the nucleus has average measurements of 7.7 by 9.1 micra. It has a rather coarsely meshed reticulum and a definite membrane. Upon the former and upon the inner surface of the latter are deposited fine chromatin granules. In the nuclei of cells of medium size there occur, as well, larger round chromatin masses (Plate II, Fig. 11). The nucleus of a cell of the smaller size contains a single karyosome, usually loaded with chromatin and of an average diameter of 2.0 micra. Occasionally the nucleus may contain two karyosomes and, under conditions to be pointed out later, even more than two. The majority of these nuclei are vesicular and appear well regulated. Hyperchromatism occurs in the smaller cells, but is never very marked. Mitoses are very numerous.

In the larger cells the cytoplasm is more coarsely meshed than in the smaller ones and the nuclei are often hyperchromatic and lobulated. Mitoses among these larger cells are numerous, but in many the nuclear figure is disrupted and the chromosomes are in part fused with each other and in part widely separated throughout the cell. Almost all the larger cells show evidences of nuclear budding, but only rarely does one see a cell with even the slightest amount of extranuclear chromatin. Cells with budding nuclei are well supplied with cytoplasm—the large size of the cell is not due entirely to nuclear hypertrophy. Many of the exceptionally large giant cells are invaded by polymorphonuclear leukocytes.

That the nuclear budding so frequently seen in tumor cells is a purposeful process whose aim is a decrease in hyperchromatism is shown in Plate II, Figs. 12 and 13, and Plate III, Figs. 14 and 15. Giant nuclei which show no evidence of budding may be so loaded with chromatin that no finer structure is visible. With the beginning of budding the nucleus and its buds stain less deeply (Plate II, Fig. 12), and the greater the degree of budding, the more vesicular do the buds and the mother nucleus become (Plate II, Fig. 13, and Plate III, Fig. 14). The process leads to a marked increase in the surface area of the nucleus. If, at the same time, the amount of chromatin is not increased, the hyperchromatism must be decreased or overcome. The process leads finally to the formation of mutiple nuclei of the primitive vesicular type met with in the advancing margins of the tumor. In giant cells with multiple nuclei of this type, degeneration of individual nuclei may occur (Plate III, Fig. 15). Such an end phenomenon in the process of nuclear budding leads not only to a decrease in the amount of chromatin in the individual daughter nuclei, but also to an actual decrease in the amount of chromatin present in the cell.

At the advancing margins of the tumor, the cells are small, 11.4 by 14.1 micra, and have nuclei measuring 7.4 by 8.7 micra. The nuclei are vesicular and have a coarse reticulum upon which is deposited a small amount of finely granular chromatin (Plate III, Fig. 16). Each nucleus possesses one, very often two, and sometimes several karyosomes. In their structure and in certain phenomena relating to the karyosome these nuclei bear a striking resemblance to certain primitive protozoan nuclei, as will be pointed out later. At this point we wish merely to indicate the occurrence of changes in which the karyosome plays a part.

In a small number of cells the nucleus contains a chromatin-free, plastin

karyosome (Plate III, Fig. 17). Various stages of chromatin deposition within the karyosome can be seen, until finally the nucleolar substance becomes almost entirely hidden by chromatin (Plate III, Figs. 16 and 18). The average diameter of such chromatin-laden karyosomes is 2.0 micra. The relationship of the karyosome to chromatin production may become abnormal in two directions. Some nuclei show hypertrophied chromatin-free karyosomes, which may be vacuolated (Plate III, Fig. 19). Others contain several hypertrophied plastin nucleoli (Plate III, Fig. 20). In such cells the cytoplasm is usually vacuolated. Abnormality in the opposite direction is met with when the hypertrophy of the karyosome is associated with chromatin formation. Such a process leads to the production of large, intensely stained intranuclear bodies (Plate III, Fig. 21). Continuation of the process leads to a diffusion throughout the nucleus of chromatin in the form of fine granules (Plate III, Fig. 22) and finally to a condition of hyperchromatism so extreme that the nucleus becomes transformed into an almost solid mass of chromatin (Plate III, Fig. 23). These evidences of progressive hyperchromatism are associated with gradual increase in the size of the affected cell.

A striking thing in these cells of the advancing portions of the tumor is the presence in many of the nuclei of two or several chromatin-containing karyosomes of equal or unequal size. At first glance such a condition seems to be purely fortuitous. Closer study shows, however, that these nuclei can be grouped in definite series. There occur karyosomes which show binary equal division of the contained chromatin mass (Plate III, Fig. 24). Still others are elongated and dumb-bell shaped, the two knob-like ends being united by a distinct band (Plate III, Figs. 25 and 26). The ends draw apart from each other, but even after the separation has proceeded to a considerable degree the daughter karyosomes may remain united by fine strands (Plate III, Fig. 27). There occur, finally, complete separation of the two karyosomes and gradually increasing indentation of the nuclear membrane (Plate III, Figs. 28 and 29). The end result is the production of two nuclei of equal size, each with a central, chromatin-containing karyosome (Plate IV, Fig. 30).

The process becomes slightly modified when the binary division of the karyosome is not an equal one. One sees nuclei with a larger and smaller body united to each other with fine strands (Plate IV, Fig. 31), other nuclei with two unequal karyosomes widely separated (Plate IV, Fig. 32), and finally a larger nucleus with a smaller bud (Plate IV, Fig. 33).

Still another modification of the process is characterized by multiple division of the karyosome, the daughter products remaining united by fine strands for some time (Plate IV, Fig. 34). After separation of the daughter karyosomes (Plate IV, Fig. 35) the nuclear membrane becomes indented in several places (Plate IV, Fig. 36) and there are formed as many buds as there are karyosomes present.

In the advancing portions of the tumor those cells whose nuclei are undergoing the processes of binary fission and of single budding outlined above are well regulated and have a fair amount of cytoplasm as compared with the nuclear volume. Those cells whose nuclei show multiple budding are all of larger size than the type cells. Cells with hypertrophied plastin karyosomes are also above the average size.

In this tumor differentiation does not occur, and there is no chromidiosis. Depression, toward which there is a marked tendency, is overcome by nuclear budding, and regulation is accomplished by mitosis and direct division.

Tumor No. 4. Epithelioma of the Cervix Uteri.—The growth involved a considerable part of the vaginal portion of the cervix uteri.

Material for examination was excised on two occasions; the first, four months after the first clinical symptoms appeared, and the second, three months later.

The material obtained at both operations consists of numerous elongated or oval alveoli containing from one to four or five rows of rather flat, round or polygonal cells of the epithelial type. The interstitial tissue is comparatively scanty. Differentiation is poor. Keratinization and "pearls" are absent. Unlike what obtains in the more slowly growing epidermoid carcinomata with considerable differentiation, there are no distinctive marks between the size and appearances of the cells at the margins of the alveoli (basal cells) and those internal to these, and mitotic division occurs indiscriminately among the cells of the various rows in the alveoli. There are no appearances suggestive of attempts to reproduce tissue of the epidermal type so commoly seen in epidermoid carcinomata.

The type cell (Plate IV, Fig. 37) of this growth measures 17.0 by 13.8 micra and has a round or oval, rather vesicular nucleus, and finely reticular, faintly eosin staining cytoplasm. In these cells the nuclear chromatin is often in finely divided masses and granules scattered over the widely meshed reticulum. The majority of the cells are well regulated and evidently capable of active proliferation. Examples of depression, cytoplasmic and nuclear degeneration and necrosis, are, however, fairly numerous both in large giant cells and in small cells.

Nuclear hypertrophy with marked hyperchromatism and considerable growth of the cytoplasm are frequently met with. Many such nuclei (Plate IV, Fig. 38) show large central karyosomes, with numerous smaller chromatin staining masses scattered on the nodes of the nuclear meshwork. These nuclei may make abortive attempts at mitotic division, which may end in degeneration of the chromosomes in the spireme stage (Plate IV, Fig. 39), or they may divide by amitosis (Plate IV, Fig. 40) or by nuclear budding (Plate IV, Figs. 41 and 42). In nuclei which undergo multiple budding, the chromatic material does not continue to increase, for the nuclei produced by budding are always less hyperchromatic than the original mother nucleus from which they spring. The number of giant cells is considerable. Some have a single giant nucleus, but in most there are multiple nuclei, as many as twenty being not uncommon, the result of active budding from the mother nucleus (Plate IV, Figs. 42 and 43). The cytoplasm of these giant cells may be normal (Plate IV, Figs. 41 and 42) or vacuolated (Plate IV, Fig. 43). There occur both mononuclear and multinuclear giant cells in further stages of degeneration. Fig. 44 (Plate IV) shows a giant cell with two nuclei. One-half of the cell is dead, the cytoplasm completely hyalin and its hyperchromatic nucleus is in advanced vacuolar degeneration. The other end of the cell shows vacuolar degeneration of both cytoplasm and hyperchromatic nucleus. More or less complete hyalin degeneration with death of the cell and karyorrhexis with the dispersion of nuclear fragments throughout the hyalin cytoplasm

are not of infrequent occurrence (Plate V, Fig. 45). Fig. 46 (Plate V) represents a huge giant cell, 74.5 by 345.0 micra, whose original nucleus, after sending off numerous buds, has undergone marked degeneration and is disintegrating. Most of the cytoplasm is hyalin and dead. The three pale staining areas containing nuclear buds are still unchanged. There is evidence that such nucleated areas may separate off from the mother cell and from independent cells.

Besides the giant cells there are many small cells, often smaller than the type cell, which are in depression, with relatively large hyperchromatic nuclei and a small amount of hyalin cytoplasm (Plate V, Fig. 47). Altogether the mortality of cells from depression is considerable in this comparatively well regulated tumor.

In other tumors (Tumors Nos. 2 and 3) it has been made apparent that nuclear budding represents a partially successful effort at nuclear reduction in cells whose nuclear material is too great to be divided by the ordinary division methods. Certain cells of this tumor offer evidence even more striking of the active purposeful nature of the budding process. Fig. 48 (Plate V) shows a cell containing three nuclear buds and a part of a mitotic figure in cross section; in Fig. 49 (Plate V) another cell with a cross section of a mitotic figure shows at one side a large number of degenerated nuclear buds. Fig. 50 (Plate V) shows a cell in the early anaphase of mitotic division with a mass of fused degenerated nuclear buds being cast from one pole of the cell. It is evident that in these cells the original nucleus has so decreased its bulk and its hyperchromatism by budding divisions that it has been able to accomplish mitosis, and that the cytoplasm is able to rid itself of this excessive nuclear material by extrusion. This hitherto undescribed regulatory process plays an important rôle in this tumor.

As usually obtains in the epidermal carcinomata, the cells of this tumor do not form chromidia.

Tumor No. 5. Hypernephroma of the Adrenal of a Steer.—A tumor of the adrenal of a steer is placed among the tumors of moderately rapid growth, although we have no knowledge of the clinical duration of the neoplasm. The tumor, a large mass, had entirely replaced an adrenal, without invading the kidney or surrounding structures. The tumor mass extended along the lumen of the adrenal vein and into the lumen of the inferior cava for a considerable distance. It was nowhere attached to the wall of the cava, and the endothelium of the latter was everywhere normal.

Histologically the neoplasm is a typical hypernephroma. It is composed of

irregularly spheroidal cells lying in a reticulum formed by compressed capillaries. Most of the nuclei are round and vesicular. We believe that the tumor was one of moderately rapid growth because of its size and of the well regulated condition of the majority of its cells. Absence of invasion of surrounding tissues and the absence of metastases further indicate that the rate of growth was not very rapid.

In this tumor regulation occurs chiefly by the extrusion of chromatin. In this it is an exception to the rule that tumors of moderately rapid growth do not form chromidia. The reason for this lies in the fact that the tumor is derived from cells which normally have the property of transforming chromatin into pigment, and it is to illustrate this process that the tumor is included here. Certain cells with somewhat hyperchromatic nuclei show both extranuclear chromatin and granules of brown pigment (Plate V, Fig. 51). In other cells the nucleus stains more faintly and the amount of pigment in the cytoplasm is increased (Plate V, Figs. 52 and 53). Finally, cells in which no nuclei can be seen, but which are loaded with pigment are not unusual (Plate V, Fig. 54). The pigment occurs in the form of fine granules and larger round and oval masses. In the pigmented cells increase of pigmentation and decrease in the amount of nuclear chromatin go hand in hand.

3. *Tumors of Rapid Growth.*

In this division, as in the second, two groups are to be distinguished: (1) tumors which, starting with slow or moderately rapid growth, develop rapid growth, and (2) tumors whose growth is rapid *ab initio*.

We have met with a number of examples of the first group. Invariably many or even most of the cells in the older parts of the growth are in depression and incapable of mitosis. Often there is a high percentage of death from physiological degeneration among the cells in these areas. A variable proportion of the cells show one or more regulatory processes, direct division, budding and chromidiosis. In the rapidly growing areas of such tumors the picture is reversed; comparatively few cells are in depression, and most of the cells are well regulated. The most conspicuous regulatory processes in evidence here are chromidiosis and nuclear resorption.

Examples of this group are furnished by carcinomata springing from slowly growing epitheliomata of the skin and tongue which, after moderately rapid growth, suddenly grow rapidly and metastasize freely in the tissues and lymph glands of the region. In these, however, chromidiosis has not been observed, and amitosis and

nuclear budding are the chief regulatory processes. In numerous sarcomata and in certain carcinomata of glandular organs, chromidiosis (sometimes with pigment formation) and nuclear resorption, as well as amitosis and nuclear budding, are prominent regulatory mechanisms. Tumors Nos. 6 and 7 illustrate these points.

Tumor No. 6. A Recurring, Metastasizing Carcinoma of the Thyroid.—A male patient, aged 38 years, was admitted to the Lakeside Hospital, service of Dr. Dudley P. Allen, with a tumor of the neck which had been first noticed twenty years previously as a small mass in the left thyroid region. There had been no symptoms of exophthalmic goiter. At the time of the first operation, November 10, 1905, a tumor mass measuring 33.0 by 22.0 by 15.0 cm., situated in the region of the left lobe of the thyroid, was readily shelled out. In June, 1906, another mass was noticed in the position of the original tumor. This grew rapidly and was removed on December 11, 1906. It was easily removed except at the base, where removal was not complete. Recurrence was noticed soon after leaving the hospital. The tumor grew rapidly, and in July, 1907, made its appearance on the right side of the neck. Growth was rapid until readmission to the hospital on November 21, 1907, shortly after which the patient died.

At autopsy the entire anterior neck region was occupied by a large, lobulated tumor mass of a dark red color. The cervical lymph glands were markedly enlarged. The tumor showed many large areas of necrosis. In the lungs were numerous metastases varying in size from two to thirty millimeters.

Material removed at the first operation shows a tumor composed of alveoli varying in size and separated from each other by a small amount of vascular stroma. The alveoli are composed of closely packed cells which have a small amount of granular cytoplasm and whose nuclei have diameters of 5.4 to 6.7 micra. Such nuclei are vesicular, with a closely meshed reticulum upon which is deposited finely granular chromatin. A fair proportion of nuclei reach a diameter of 9.1 micra, and very hyperchromatic giant nuclei measuring as much as 15.2 by 20.2 micra are not infrequent. In the latter there are no evidences of budding. In the cells of medium size some finely granular extranuclear chromatin is seen, but chromidiosis is not very active. Mitoses are not numerous.

In the recurrence removed on December 11, 1906, the alveoli are, in general, smaller than in the primary tumor and there is less interalveolar stroma. The alveoli have no lumina. There is greater variation in the size of the nuclei, many having a diameter of only 3.0 micra. Giant nuclei are present, but do not reach so large a size and are not so numerous as in the primary tumor. Hyperchromatism, particularly of small and medium sized nuclei, is more marked. Little extranuclear chromatin is seen. Mitoses are present in small numbers.

In the local tumor removed at autopsy the general architecture is the same as above, except that at the peripheral portions of the mass, particularly on the right side, there is the formation of alveoli which approach more nearly normal thyroid acini.

In the final local recurrence the tumor is covered with a thin fibrous capsule. Next to this the tissue is composed of closely placed alveoli which show con-

siderable variation in size and some irregularity of outline. There are large areas of necrosis, in which the stroma has lost all structure, but in which widely separated acini are still well preserved. Many of these are surrounded by a clear space. The tumor is very vascular. The alveoli contain a small amount of finely granular, unstained material, indicating some secretory activity in those acini which have lumina. The lining cells are cuboidal and contain considerable cytoplasm. Those which appear well regulated measure 14.4 by 10.04 micra and have nuclei with dimensions of 7.1 by 5.7 micra (Plate V, Fig. 55*a*). The nuclei of such cells are round and vesicular, with an easily seen reticulum upon which are deposited very fine chromatin granules, as well as a small number of larger ones. The majority of the nuclei are of this type. The cytoplasm of the acinar cells is rather coarsely meshed and usually considerably vacuolated, particularly about the nucleus. A rather large proportion of the nuclei are intensely stained and are irregular in outline (Plate V, Fig. 55*b*). The hyperchromatism of such nuclei is due chiefly to an increase in finely granular chromatin. Chromidiosis, leading to a decrease in hyperchromatism, is frequent (Plate V, Fig. 55*c*). No mitoses are seen.

In the cervical lymph gland invasions, necrosis is also extensive and complete, only the invaded marginal zones of the glands being spared. The lymphoid tissue is completely replaced by tumor tissue, except for a few small islands of lymphocytes just beneath the capsule. The tumor tissue is composed of very small irregular alveoli, most often in the form of round or elongated solid cell nests. The very small amount of stroma is richly supplied with large capillaries. Individual cell outlines are often difficult to determine and the nuclei are closely placed because of the small amount of cytoplasm. The predominating cells have a size of 7.4 by 6.4 micra, with nuclear diameters of 5.5 micra (Plate V, Figs. 56*a* and 56*b*). Giant nucleus formation leads to cells measuring 9.8 by 12.5 micra, whose nuclei measure 8.4 by 10.8 micra (Plate V, Fig. 56*c*). Hyperchromatism is much more marked than in the recurrence—the majority of the nuclei showing varying degrees of this condition. Many of the hyperchromatic nuclei are irregular in shape. Chromidiosis, while present, is not so active as in the local tumor. Only a few mitoses are seen.

In the lung metastases no lung tissue is evident. The surrounding lung tissue is slightly compressed, but shows no inflammatory reaction. The metastases are well defined, but in places there is actual invasion of the tissue of the alveolar septa. The tumor nodule is composed of cells arranged in large, irregularly shaped areas bounded by wide capillaries. Only here and there is there a slight indication of alveolus formation, without the production of any acini which even remotely approach those of the normal thyroid. There is no stroma except the minimal amount associated with the blood vessels. The cells show considerable variation in size. The average diameter of the majority of the nuclei is slightly less than in the recurrence or the lymph gland invasion—5.7 micra as compared with 6.4 micra. The cytoplasm forms a just barely visible rim about the nucleus. Moderate nuclear hypertrophy leads to the production of a considerable number of nuclei with an average diameter of 8.6 micra. In general, the cells of the metastases are well regulated, although scattered about there occur small, elongated cells with condensed, intensely stained nuclei (Plate V,

Fig. 57). In the peripheral portions of the nodules a fair number of very hyperchromatic, slightly hypertrophied nuclei are found. There is some chromidiosis, which may, occasionally, be associated with vacuolization of nuclei (Plate V, Fig. 58). Mitoses are numerous. In many of the cells in various stages of karyokinesis there occur granules or rods of chromatin not included in the nuclear figure (Plate V, Figs. 59*a* and 59*b*).

Tumor No. 7. A Lymphendothelioma, Primary in the Lumbar Vertebrae, with Extensive Metastases.—The patient was a male, 45 years of age; he was admitted to the Lakeside Hospital, service of Dr. E. F. Cushing, June 16, 1901. He had been in good health until seven weeks before admission, when he was taken ill with pain in the abdomen, left side and back. On admission he walked with great difficulty and pain and a shuffling gait. Physical examination was negative, except for a considerable prominence at the side of the spinous processes of the third and fourth lumbar vertebrae. On July 20, 1901, a painful swelling at the sternal end of the left clavicle was first noted. Death occurred July 29, 1901. The clinical diagnosis was malignant tumor, primary in the lumbar vertebrae.

The autopsy was performed two hours after death. The growth was evidently primary in the third or fourth lumbar vertebra, the bony tissue of which was almost entirely replaced by a firm, greyish, translucent tissue. The growth also involved the body of the second lumbar vertebra and extended into the surrounding tissues, including the left psoas muscle.

There were extensive metastases involving the lumbar, mesenteric, gastrohepatic and bronchial lymph glands, liver, lungs, heart, left clavicle, sternum and three ribs. There were also smaller subcutaneous nodules in the skin over the chest, abdomen, back and left buttock. The metastatic growths were softer and evidently more cellular than the original growth, but otherwise they presented the same appearances.

In the primary growth the bone tissue has been almost entirely replaced by a rather dense fibrous supporting tissue, well supplied with blood and lymph vessels, and elongated alveoli containing very large cells. The cells are, as a rule, in rows of from one to six cells in width. There is no intercellular substance. The cell groups are, usually, sharply delimited from the supporting tissue, though in many places one or more cells are seen invading the latter. The cells in general are large, an average cell being 21.5 by 16.0 micra; some are huge giant cells, 65.0 by 30.0 micra and 90.0 by 20.0 micra being not unusual dimensions. Nearly all the cells of this, the oldest, portion of the tumor show marked increase of the nuclear material, even in proportion to the largely increased amount of cytoplasm. The nuclei of the smaller cells are, as a rule, single and hyperchromatic, with widely meshed reticulum and with one large or several smaller karyosomes. In some the chromatic material is in the form of fine granules scattered over the reticulum. Plastin nucleoli are present in some of the nuclei. Nuclear degenerations are common, and consist in chromatin diffusion and vacuolization. Giant nucleus formation is frequent. Ordinary direct division and nuclear budding are common. Mitoses are rare.

The huge giant cells show either active budding or the presence of numerous smaller nuclei, the result of budding. The cytoplasm, unless degenerated, is finely granular. In many large cells with nuclear hypertrophy and giant nucleus

formation, in addition to nuclear degeneration, there is hyalin degeneration of the cytoplasm in whole or in part. Chromidiosis and nuclear resorption are not evident. The evidences of extension by means of the lymphatics are unmistakable. No tumor cells are to be seen in the blood vessels.

In the metastases the characters of the growth and of the cells differ with the age of the metastases. In the older lymph gland metastases and in the direct extensions of the tumor they are, in general, identical with those of the original tumor. In the smaller lymph nodes, omentum, clavicle, lungs, and liver, the characters of the growth and of the cells are quite different. That is, in the younger, more actively growing metastases the characters of the tumor are changed. The supporting tissue is much less developed, often quite cellular, the alveoli are more numerous, closer together, larger, round or oval in outline and of a more constantly uniform size. The cells are smaller (17.5 by 20.0 micra, 20.0 by 23.0 micra), oval in shape, with nuclei relatively but not excessively large. The nuclei are vesicular, with well marked membranes and linin meshwork. The chromatic material is usually in small masses and rarely in large karyosomes. Many nuclei contain one or more large plastin nucleoli. Very markedly hypertrophied and hyperchromatic nuclei, amitosis and nuclear budding are comparatively rare. Mitoses, contrary to what obtains in the primary growth and the old metastases, are numerous. Free in the cytoplasm in many cells are from two to ten large and small chromatic masses well without the division spindle—apparently dropped chromosomes, for there is no evidence of chromidiosis in resting cells. A relatively small proportion of the cells are of the giant cell type, with nuclei and cytoplasm showing all the changes described in the original growth. In certain areas multipolar mitoses are numerous.

Examples of rapidly growing tumors of the second group occur chiefly among carcinomata of glandular organs and sarcomata. The cells of these tumors exhibit regulatory processes in high degree. The most prominent are nuclear resorption, chromidiosis, amitosis and nuclear budding. We have noted a strong tendency for the cells of a given tumor to adopt and develop to a high degree a single method of regulation, sometimes, but not always, largely to the exclusion of others. Thus, in Tumors Nos. 8 and 9 the chief regulatory processes are chromdiosis and nuclear resorption, in Tumor No. 11 amitosis and nuclear budding, without chromidiosis, and in Tumor No. 10 all of these methods are prominent.

Tumor No. 8. A Rapidly Growing Carcinoma of the Right Breast with Extensive Metastases in the Right Axilla.—A woman, aged 31 years, was operated on February 26, 1908, by Dr. G. W. Crile at the Lakeside Hospital. Six months before, she had noticed a small mass in the right breast. This had increased in size very markedly in the past four weeks. On admission to the hospital the right breast was twice the size of the left.

The growth occupied a large portion of the breast and there were extensive

but small metastases in the axillary lymph glands. Material was fixed in Flemming's solution and in saturated sublimate solution within five minutes after removal from the body.

In the breast tumor the growth is composed of closely placed alveoli containing oval and polygonal cells of the epithelial type, evidently of glandular origin, and supported by a rather cellular fibrous tissue stroma well supplied with blood vessels. The alveoli are usually oval, and in the older parts of the growth are rather large and completely filled with cells. In the younger parts, particularly at the extending margins, the alveoli are smaller, further apart and often lined by from one to three rows of cells and show central lumina.

As a whole, the cells have the appearance of being well regulated. Mitoses are numerous, except in the oldest portions of the growth. A type cell among dividing cells has a finely reticulated cytoplasm and an oval vesicular nucleus with a well marked reticulum. The nucleus has a fairly large karyosome and usually fine chromatin granules are present on the nodes of the reticulum. The cytoplasm of such a cell measures 15.0 by 10.8 micra, the nucleus, 10.0 by 6.75 micra. Cells with proportionately larger nuclei, both with and without increased amounts of cytoplasm, show a number of interesting phenomena.

In the cells somewhat larger than the type cell, the nucleus often possesses a very large karyosome, either round, oval or elongated. In these, various stages of karyosomic division, identical with those described in Tumor No. 3, are evident. The karyosome divides into two and sometimes three parts which are usually equal. If these karyosomes remain in the nucleus, the latter shows evidences of division and budding as in Tumor No. 3. In many nuclei, however, a young karyosome, or even two, often of considerable size, may be extruded from the nucleus as chromidia. In some cells, the karyosome extends from the nucleus through the cytoplasm and beyond the latter.

Cells of a size often smaller than the type cell show marked nuclear hyperchromatism. These nuclei may reduce themselves by chromidiosis, or the nuclei may degenerate with marked vacuolization of the enlarged karyosomes, leaving finally, after hyalin degeneration of the cytoplasm, free, shrunken and vacuolated karyosomes, as one of us (Howard, 1908) has described for *Actinosphærium*, and as so often occurs in Tumor No. 2.

In many cells with hypertrophied, hyperchromatic nuclei, the protoplasm reaches considerable size. The cytoplasm of a cell of this variety measures 20.0 by 13.5 micra, the nucleus 15.0 by 10.8 micra. Such cells may reduce their nuclear material very greatly by chromidiosis (Plate V, Figs. 60, 61 and 62*a*), after which mitosis may occur (Plate V, Fig. 62*b*). The whole nucleus may dissolve into chromidia (Plate V, Fig. 63). The nucleus may become of giant size (as in a cell whose cytoplasm measures 22.0 by 10.0 micra, and whose nucleus measures 19.25 by 8.4 micra), with or without budding. The nuclei and cytoplasm of such cells often show degenerative changes and even necrosis. Chromidiosis does not occur in cells with giant nuclei. The throwing off of nuclear buds by hypertrophied, hyperchromatic or even giant nuclei may be followed by mitosis of the mother nucleus, as in Tumor No. 4. The growth of the cytoplasm in cells with large nuclei may be so marked as to produce a cell measuring 41.5 by 30.4 micra.

The mitoses are usually normal in appearance, but in many the amount of

chromatic material in the division figure is excessive—hyperchromatic mitosis. In many such cells the division is abortive and the chromosome mass breaks up into larger and smaller masses. Multipolar mitoses are occasionally found. Though the cells in the main are fairly well regulated, there are evidences of considerable nucleus-plasma upset on the part of some cells, as shown by the nuclear hypertrophy, hyperchromatism, giant nucleus formation, nuclear budding and various degenerations of nuclei, hyalin and vacuolar degeneration, overgrowth and necrosis of the cytoplasm of some of the cells.

In the lymph gland metastases the alveoli are, as a rule, large, closely packed together, with relatively little supporting tissue. There are but few alveoli with central lumina. The growth is evidently rapid; there are very numerous mitoses, which are, as a rule, normal. The type cell is of the same size and structure as in the original growth. Nuclear hypertrophy, hyperchromatism and giant nucleus formation are present, but not so frequent. The cells do not reach such large size, and there is not so great a mortality from physiological degeneration. Giant cells are rare. Direct division and budding are not infrequent and are always preceded by very definite binary and trinary divisions of the karyosome. Chromidiosis is very frequent and here, too, the same relation to karyosomic division is also seen, and one or more of the products of this division may be cast out. There is a considerable number of cells with large plastin nucleoli in various stages of growth. Many of the cells in mitotic division contain chromidia. There are some hyperchromatic mitoses, and in many of these the division is abortive and the figure may disintegrate. On the whole, in the metastases the growth is rapid, the regulation is good and the mortality of the cells is low. The marked influence of chromidiosis as a factor in regulation is the most important feature of this tumor.

Tumor No. 9. A Metastasizing Carcinoma of the Lung.—A colored woman, aged 37 years, gave a history of having "caught cold." There was some vomiting during the early weeks of her illness. Death occurred seven weeks after the earliest symptoms.

At autopsy the greater portion of one lung was found transformed into grayish red tissue. The other lung and the pleuræ of both sides were involved. All the abdominal organs showed numerous small metastases and both layers of the peritoneum were studded with small nodules.

Microscopically the tumor tissue of the lung showing most involvement is composed of very irregular, gland-like alveoli embedded in a dense fibrous stroma, which is well supplied with blood vessels. In most alveoli the lining cells are heaped up in several layers, forming short papillomatous ingrowths into the alveolar lumina. The alveoli vary in size and in outline.

The individual cells of the primary tumor vary considerably in size. A cell diameter of 10.2 micra, with a nuclear diameter of 6.6 micra, may be taken as about the average. Of cells differing in size from this average the greater portion are slightly larger. In such cells the nucleus has a fine reticulum upon which are deposited very fine chromatin granules, with an occasional larger chromatin mass. The cytoplasm is closely meshed. The greater portion of the cells in the primary tumor have hyperchromatic nuclei whose outlines are often somewhat irregular. Depression, as evidenced by hyperchromatism and giant nucleus formation, is marked, although the tumor shows at the same time great

regulatory powers, as indicated by chromidiosis, nuclear budding and normal mitosis. Many cells, however, are unable to overcome the depression and die with cytoplasmic vacuolization and fragmentation of hyperchromatic nuclei.

In the metastases the stroma is quite prominent, but varies somewhat in amount in the various organs. The acini are smaller than in the primary growth and show much more intra-acinar papillary ingrowth of the epithelium. Mitoses are numerous. Although there is considerable variation in the size of the cells, not so large a proportion of cells is hyperchromatic as is the case in the primary tumor. Type cells (Plate V, Fig. 64) measure 15.0 by 7.8 micra and have nuclei measuring 6.4 by 7.8 micra. Large cells with well regulated, vesicular nuclei are, however, numerous. Hyperchromatism is seen in nuclei of the type cell size (Plate V, Fig. 65) and in giant nuclei (Plate V, Fig. 66). Such nuclei may be so loaded with fine chromatin granules that the reticulum is almost completely hidden (Plate V, Figs. 65 and 66). In other cases there are, in addition, larger chromatin masses. More rarely the excess of chromatin is not distributed uniformly throughout the nucleus, but is deposited in a small number of larger bodies (Plate V, Fig. 67). Budding is not so frequent in the metastases as in the primary tumor, but chromidiosis is very active. The amount of chromatin extruded may exceed that still remaining within the nucleus (Plate V, Fig. 68) or almost the entire nucleus may be transformed into chromidia (Plate VI, Fig. 69). In some cases chromatin extrusion, although present, is unable to restore the proper balance and the cytoplasm becomes markedly vacuolated. An occasional hyperchromatic nucleus is vacuolated (Plate VI, Fig. 70).

Tumor No. 10. A Giant Cell Sarcoma of the Neck.—A white male, aged 43 years, three months before admission to the Lakeside Hospital began to be troubled with neuralgic pains in the left arm. Five weeks before admission he noticed a small swelling on the left side of the neck. The mass grew rapidly and there had been a loss in weight of over twenty pounds since the beginning of symptoms. An extensive operation was necessary for the removal of the tumor.

The tumor is composed of irregularly shaped cells, running between which is a small amount of connective tissue stroma. Broader bands of fibrous tissue carrying thin-walled blood vessels divide the tumor tissue into areas of varying size and shape. Even with very low magnifications one is struck by the variation in the size of the nuclei and by the intense hyperchromatism of the larger ones.

The nuclei of the smaller cells do not contain an excessive amount of chromatin. Such well regulated cells vary in size from 18.2 by 9.5 micra to 12.8 by 9.5 micra. The nuclei show corresponding variations, 11.4 by 4.0 to 7.4 by 5.4 micra, so that the individual cells contain considerable cytoplasm. The distribution of the chromatin within the nuclei varies. In some, small granules distributed about upon a closely meshed reticulum bring the latter into view. Finely and more coarsely granular chromatin is also deposited upon the inner surface of the nuclear membrane (Plate VI, Fig. 71). In other nuclei the reticulum can hardly be distinguished and the chromatin occurs in the form of large masses applied upon the membrane and lying free within the nucleus (Plate VI, Fig. 72). In the hyperchromatic giant nuclei similar differences also occur. Some nuclei are loaded with finely granular chromatin (Plate VI, Fig. 73). In others the excessive amount of chromatin is gathered into very large round or oval

masses (Plate VI, Fig. 74). Chromidiosis is marked and occurs in most of the cells which show even only a slight increase in the amount of nuclear chromatin. The extranuclear chromatic material may be in the form of very minute granules, often present near and upon the outer surface of the nucleus (Plate VI, Fig. 74), or in the form of large as well as small granules scattered throughout the cytoplasm (Plate VI, Fig. 75). The larger chromidia may be vacuolated. In a few cells the cytoplasm has a diffuse, faint brownish tinge due to the presence of minute granules of pigment. In such cells the nucleus is usually not hyperchromatic and the presence of fine particles of chromatin in the cytoplasm indicates that the nucleus has reduced itself by chromatin extrusion (Plate VI, Fig. 76).

In spheroidal cells with an average diameter of 12.8 micra the cytoplasm is coarsely vacuolated and both relatively and actually large in amount (Plate VI, Fig. 77). The nuclei, which measure 4.3 micra in diameter, are so intensely hyperchromatic as to look almost like solid balls of chromatin. When chromidiosis occurs in such cells (Plate VI, Fig. 78) the nucleus stains more faintly, but we have never seen nuclei of this type in which there was a complete return to the vesicular character of well regulated nuclei.

Actual resorption of chromatin, with or without solution of the nuclear membrane, also occurs in this tumor. In a nucleus which must have had originally a structure like that of Fig. 72 (Plate VI), the membrane is intact, but the chromatin masses stain very faintly and portions of the cytoplasm take a diffuse chromatin stain (Plate VI, Fig. 79). This same process occurs also in cells with small, solid, round, hyperchromatic nuclei (Plate VI, Fig. 80). In other nuclei, in addition to the decrease in stainability of the chromatin, there is partial disappearance of the nuclear membrane (Plate VI, Figs. 81 and 82).

Examples of amitosis (Plate VI, Fig. 83) and of nuclear budding (Plate VI, Fig. 84) are very numerous. Some cells may show chromidia as well as buds (Plate VI, Fig. 84). Before the process of budding has reached so advanced a stage as that shown in Fig. 84, the giant nuclei in which the process has just begun are still very hyperchromatic.

In this tumor are found nuclei of a kind which we have seen in no other neoplasm. They may show one or two large protuberances covered with a distinct nuclear membrane (Plate VI, Figs. 85 and 86). Because of the retention of the outlines of the original nucleus (Plate VI, Fig. 85) these bud-like outgrowths give the impression of having been very suddenly formed. This is further accentuated by the streaming of the chromatin within the buds, leading to the formation of chromosome-like strands. Concerning the end result and purpose of this process we are not quite sure. Mitotic figures occur, but are not numerous.

The chief points of interest in this tumor may be summarized as follows: There is marked depression of a large proportion of the cells, as evidenced by the intense nuclear hyperchromatism. The depression does not, however, lead to any very striking cell mortality. Very little evidence of cellular degeneration and death is seen, except the excessive nuclear resorption illustrated by Figs. 79 to 82 (Pate VI). That the depression does not have more serious consequences for the individual cells is explained by the very active regulation by

means of marked chromidiosis, nuclear budding, chromatin resorption, direct division and mitosis.

Tumor No. 11. A Rapidly Growing Carcinoma Occurring in a Nursing Breast, Occupying the Whole Breast and Invading the Underlying Muscle; Large Metastases in the Axilla.—Three months after the birth of a child, and while nursing the latter, the right breast became painful and swollen. It was treated as a mammary abscess. A week before removal of the breast, and two months after the first symptoms, incision emitted a considerable amount of puriform material.

The growth, which occupied the whole breast, had a central cavity about the size of a small orange. The cavity had firm walls and contained a small quantity of puriform material. The axillary glands varied from 3.0 to 10.0 cm. in diameter.

Sections taken from the older portions of the growth are composed of very large and long alveoli of round, oval cells of the epithelial type, supported by a relatively small amount of fibrous tissue stroma. There is no semblance of an attempt at the formation of glands or ducts. The tumor cells vary much in size. For the most part they are large, often giant in size, with hypertrophied and often giant nuclei. Some of the giant cells have a large diameter. The nuclei are for the most part vesicular, with one or more large karyosomes. Amitosis and nuclear budding are present in nearly all of the cells. Many cells are filled with from three to twenty or thirty nuclear buds. Degeneration and extrusion of nuclear buds are not infrequent.

In some giant cells, after nuclear budding, mitosis of the mother nucleus, as described in Tumor No. 4, may occur. In some of the giant cells containing nuclear buds, with a large nucleus still throwing off buds, several, in one case four, nuclear buds are in various stages of mitosis, with definite spindles.

While most of the giant cells are evidently the result of excessive growth of the cytoplasm of individual cells, some are clearly due to plasmogamy. A large proportion of the cells are in hopeless depression, and show vacuolar and hyalin degeneration of nuclei and cytoplasm. The mortality from physiological degeneration is high in this portion of the growth.

Mitosis is infrequent and, where present, the mitotic figures are hyperchromatic and the chromosomes are large. A large proportion of the mitotic figures are multipolar. Dropping of chromosomes is not infrequent. Degenerated mitotic figures, sometimes with vacuolar degeneration of some of the chromosomes, are met with.

There is no inflammatory exudation in the tumor. The large area of necrosis is sharply marked and evidently vascular in origin.

In the peripheral portions of the growth and in the metastases, the picture is quite different. The alveoli are smaller, and the supporting tissue is more in evidence. The tumor cells are smaller and much better regulated. Giant cells and nuclear hypertrophy and hyperchromatism are less common. Mitoses are very numerous and the division figures are of a more normal type. They are present in every stage. Some multipolar mitoses are met with, but relatively much less frequently than in the older portions of the growth.

IV. THE CORRELATION OF THE GROWTH PHENOMENA OF TUMOR CELLS WITH CERTAIN FACTS OF GENERAL CELLULAR BIOLOGY.

GROWTH AND DIFFERENTIATION. ANAPLASIA AND METAPLASIA.

One of the most striking characteristics of protoplasm is its inherent capacity for growth and differentiation. In normal growth and development, the growth rate and differentiation of protoplasm follow definite laws. With proper food and environment, growth proceeds at definite rates which vary for different stages of development and may be modified experimentally, as has been shown. Protozoan cells grow to a certain size and divide, and the daughter cells repeat these phenomena. In the developing metazoön, growth and cell divisions are rapid, with a minimum amount of differentiation and, after repeated haltings and startings, the animal and its organs attain a certain rather definite size, when growth and cell divisions are arrested as if automatically, and differentiation corresponding to the function of the various component cells is perfected. Thenceforth, further growth and cell divisions occur only under certain definite conditions: (1) for renewals, to replace cells and parts; (2) under physiological stimulation, producing the hypertrophies and hyperplasias, the repair of wounds, etc., which latter may, in essence, be regarded as renewals; (3) proliferations incited by certain stimuli from without, resulting in inflammatory new formations; (4) the formation of sex cells; and (5) tumor formation.

The bulk of evidence is in favor of the idea that growth is slowed, curbed or completely arrested by certain factors residing in the individual cells and to some degree associated with cell differentiation, which cause, in effect, a hindrance to further growth. This hindrance may be almost complete, as in the case of nerve and striated muscle cells, or more or less readily overcome, as in epithelia and mesenchymal cells.

Each variety of cell leaves division with a given amount of dynamic energy, the production of which is due to cellular metabolism and the expenditure of which is divided in varying proportions between growth and functional activity. In the normal cell, there

are limits within which the amount of dynamic energy which it can produce varies. In the cells of hypertrophies, hyperplasias and benign tumors, while the amount of dynamic energy is increased to a maximum within normal limits, its distribution between growth and function remains in approximately the same proportion as in the normal cell. In the cells of malignant tumors, on the other hand, the dynamic energy is not only increased in amount and does not wear out, but is diverted largely to growth rather than to specialized functional activity.

The setting aside of the limits of energy production and the disturbance of its normal proportionate distribution and expenditure between growth and function constitute the main and essential differences between the cells of malignant tumors and other cells. This conception is partly reached by Hansemann (1893) in his anaplasia theory and by R. Hertwig (1904) in his idea of the difference between cytotypic and organotypic growth in relation to tumor cells.

According to our conception, the essential factor in the restraint or hindrance to continuous growth on the part of individual cells is the inherent tendency of protoplasm to preserve the proper or normal balance between the distribution of the dynamic energy produced by the cell between growth and functional activity, as exhibited chiefly in differentiation and secretion.

In the renewals of lost parts, in the physiological hypertrophies and hyperplasias, and in the inflammatory new formations, in short, in all examples of renewed and accelerated rates of growth due to known growth stimuli, this law applies. In all these processes growth is usually rapid, but, as a general rule, in inverse proportion to its rapidity, differentiation is perfect or imperfect. There is a marked tendency for the cells to breed true and to fully differentiate into normal tissue, and to recover the proper balance in the distribution of their dynamic energy. In exceptional instances this recovery is incomplete or even perverted. In the latter condition, metaplasias arise.

The thyroid furnishes a remarkable illustration of the application of this law to the variations in the characters of growth under different influences. In this gland, whose cells are under

good control, after renewals for wear and tear and after the excision of parts, growth halts with the achievement of perfect differentiation when renewal is complete.

The studies of our colleague, Marine (1907 *a* and *b*, 1908, 1909 *a*, *b*, *c* and *d*), have shown that, under conditions otherwise constant, the withdrawal of a single substance, iodin, from the diet, is followed by active growth of the glandular tissue of this organ in direct proportion to the amount of this element withheld. A slight deficiency of iodin is followed by a true hypertrophy, with an increase in the size of the acini and of the individual epithelial cells, and a greater deficiency of iodin by a marked true hyperplasia with newly formed acini with relatively large cells. In the hyperplasia there may be produced an enormous amount of new tissue, the physiological value of whose secretion to the organism is inverse to the degree of hyperplasia. The absence of iodin, relative or absolute, acts as a powerful growth stimulus under the influence of which the cell increases markedly its production of dynamic energy, which is, however, diverted from normal physiological secretory functional activity to growth and division. The active growth and division of the thyroid cells under these conditions may result in profound depression, in which so many of the cells are lost that myxedema results (personal communication from Doctor Marine). Marine has further shown that the administration of iodin in proper doses during any period of thyroid hypertrophy and hyperplasia, short of profound depression, is followed by arrest of growth and resumption of normal functional secretory activity, colloid production, and the reversion of the gland to a condition approaching the normal structure, colloid goiter, with a return of the cells to their normal shape and size, and the restoration on the part of the tissue of an iodin content as great as or greater than normal.

Proceeding from thyroid hyperplasias, as they occur in nature, in which the cells recover from or avoid depression by various regulatory processes, and grow rapidly, there occur adenomata and carcinomata, over which iodin has no control.

In the case of the hyperplasias, the absence of a single substance, iodin, and the attempt of the gland as a whole to produce as efficient as possible a secretion in spite of the lack of this necessary element,

act in conjunction as a very powerful stimulus, as the result of which there is a marked increase of the cellular dynamic energy. The continuous action of the stimulus, due to the persistent deficiency of iodin, leads to the diversion of an abnormal proportion of this energy from physiological function to growth. Removal of the stimulus at any stage of the hyperplasia, by the administration of iodin, results in the restoration of a more or less perfect balance in the distribution of this energy between growth and functional activity. In proportion to the degree to which this resumed functional activity follows normal lines, growth is arrested.

When, however, the stimulus acts to an excessive degree or for too long a time, the balance in the distribution of the increased dynamic energy between growth and functional activity is permanently altered, growth becomes unlimited and the result is a malignant tumor over which iodin has no effect.

And, likewise, in tumors elsewhere, the initiation and progress of the process are to be explained by the accession on the part of the cells of increased energy production and by an improper correlation of the expenditure of the latter between growth and functional activity.

Hitherto, in our discussion of tumor growth, we have purposely left out of consideration cell division, which we think the work of R. Hertwig (1903 *a* and *b*) and of Popoff (1908) has proved to be the direct result of growth. When, as the result of growth, a cell reaches a certain size and the proper nucleus-plasma tension is attained, division occurs. Division, as we have indicated, is a regulatory process. The present problem is not so much, why does division occur? as, what are the causes of the growth of protoplasm that lead to division and make it important or even necessary to the life of the cells?

The varying capacity for division possessed by different cells when incited to growth has a profound influence upon the course and fate of tumors. Unless the division capacity of the cells is great and the division factors powerful, the growth, however great the stimulus, is limited. These properties, as we have already indicated, vary with different cells.

When the incitant necessary to stimulate excessive growth acts

upon the cells of a part and is accepted, the course and fate of the resulting tumor must depend upon: (1) the divisional capacity of the cells; (2) the nutrition; and (3) the avoidance of depression and the regulatory processes available to and utilized by its cells.

It is evident that beyond a certain point the growth rate is determined by the division rate, for unless the cells can divide, growth becomes arrested. The division rate is dependent upon the division factors of the cell itself. These may rise or fall in strength in a given tumor. If they are not powerful enough to produce division of some type, the cell vegetates or goes into depression and often dies. The mortality of cells from depression is often great, even in rapidly growing tumors. Death of large foci of tumor tissue is usually traceable to vascular changes and is, therefore, due to lack of nutrition. It is probable that retrogression of tumors is due to these two factors, the death of individual cells from depression and of masses of cells from lack of nutrition, together with certain others, such as hemorrhage and the production of lytic and other antibodies.

Tumor formation is associated with varying degrees of loss of differentiation of the constituent cells. As a general rule, rapidity of growth and increasing loss of differentiation go hand in hand. Whether this association means that slowly growing tumors show greater degrees of differentiation because the time between divisions of individual cells is long enough to permit of differentiation or that the acquiring of an increased divisional capacity has destroyed, to a certain degree, the potentiality of differentiation, is difficult to decide. The rule that growth rate and loss of differentiation go hand in hand is not an inflexible one. Krompecher's basal cell epitheliomata of the skin usually have a slow rate of growth, yet the individual cells tend to retain the morphological characteristics of the basal cells of the epidermis. Other epidermal tumors, of a much more rapid rate of growth, may show considerable differentiation of individual cells. In very rapidly growing malignant tumors, the cells, so long as individual cells are formed, rarely lose all differentiation. The cells may be morphologically young, but are rarely true, undifferentiated embryonic cells. The work of Mallory (1908) and others has shown that the

cells of such tumors form fibrils characteristic for the adult normal cell from which the tumor was derived. Gland cell tumors may show a certain degree of secretory activity.

Differentiation, then, seems to be the morphological expression of specialized cell function or, at least, of functional potentiality. Structural modifications are the mechanism by means of which the nucleus controls cell activity. Differentiation is a process through which nuclear energy finds an outlet and by means of which cell depression due to nuclear overgrowth may be in part prevented.

No matter how rapid the rate of tumor growth and how great the loss of differentiation, individualized cells are produced. Mall (1902) has shown, however, that connective tissues are derived from a syncytium. He leaves open the question as to whether the mesenchyme was ever composed of individual cells or whether it was not from its very beginning a syncytial structure. From the syncytium the individual cells and the intercellular substances of differentiated connective tissues are derived. Although the connective tissue tumors with which we are acquainted are composed of individual cells, which may show very great loss of differentiation, there arises the question of the theoretical possibility of the occurrence of tumors in which the power of differentiation has been lost to such an extreme degree that not even individual cells are produced—tumors, other than those derived from the placenta, testis, or ovary, composed of syncytium. Associated with this question is the further one as to the fate and the rate of growth of such a tumor. According to Mall, in the syncytium from which normal connective tissues are derived, the rate of protoplasm growth exceeds that of nuclear growth. According to our interpretation of this observation, it is not until the greater bulk of the plasma has been used up, by the process of differentiation, in the production of intercellular substances that the cells, now individualized, show such a relation between nucleus and cytoplasm as permits them to attain their normal division rate.

Since connective tissue cells do often revert to so young a condition as that met with in round cell and spindle cell sarcomata, one must grant the possibility of even further reversion and of the occurrence of a syncytial connective tissue tumor. And if this

syncytial tumor mass is incapable of a degree of differentiation sufficient to produce intercellular substance or individual cells, it ought, theoretically, to have an extremely slow rate of growth. One would then have a striking exception to the rule of the association of rapid growth and loss of differentiation—an exception in which extreme loss of differentiation is associated with very slow growth. Tumor No. 1 is an example of such a neoplasm.

The future possibilities of such a tumor are interesting and would seem to be dependent very largely upon the differentiation capacity of the tumor tissue. If a slight degree of differentiation is attainable with the formation of individual cells with the nucleus plasma relation of spindle sarcoma cells, there is given the possibility of the transformation of a slowly growing, relatively benign tumor into a rapidly growing malignant one. A greater degree of differentiation along more normal lines might lead to the formation of intercellular connective tissue which might have the structure of fibrous tissue, cartilage or bone. The result might be a benign connective tissue tumor or a nodule of normal tissue with a structure either similar to or different from that of the surrounding tissue. There is the final possibility of complete inability to produce individual cells or intercellular substance. The end result of such a process would be atrophy of the tumor tissue.

The tumor under consideration is especially important because of the emphasis that it gives to the loss of the power of differentiation. In most tumors anaplasia is only relative—there is rarely the formation of true embryonic tissue. Moreover, the anaplasia may be only a temporary condition in the life of any individual tumor cell. In most tumors comparison of the youngest and oldest cells shows that the cells possess to a considerable degree the property of advancing differentiation. By a loss of differentiation in a connective tissue tumor so extreme as that in this tumor, there is given the possibility of advancing differentiation which may lead to the formation of a connective tissue of a type different from that from which the tumor originally came.

It is well established that different degrees of lack of differentiation, that is, different grades of anaplasia, are associated with cell depression. From this state of anaplasia, after recovery from de-

pression and upon the resumption of growth and cell division, metaplasias often proceed. A familiar example of this is found in certain chronic inflammatory processes in the choroid, lungs, lymph glands, arteries and heart valves, in which in the newly formed fibrous tissue, after depression and anaplasia, there is a period of renewed growth with aberrant differentiation or metaplasia, resulting in the formation of cartilage and bone. Several authors have described the formation of mucous cells, striated muscle and even ganglion cells in the thymus, and Marcus (1908) has pointed out that this occurs at a period when some of the thymus cells are recovering from profound depression and are anaplastic. Marcus further correlates these phenomena of the thymus cells—their capacity for metaplasia after recovery from depression—with the various histological differentiations found in dermoid cysts. In the same manner, from such a tumor as the one under discussion (Tumor No. 1), or from any similar completely anaplastic tissue, on the accession of renewed growth and division capacity, there might arise not only a complicated mixed tumor, such as are so common in the urogenital tract, but dermoid tumors and even the so-called chorio-epitheliomata of the testis. In the light of the foregoing, it is entirely unnecessary to assume the origin of such tumors from hypothetical embryonic rests, either simple or compound. The existence of the latter, in adult tissues, R. Hertwig (1904) has shown, on theoretical grounds, to be impossible.

Therefore, in conclusion, it may be said that the growth rates of tumors vary not only with the growth capacities of the tissues from which they spring and the capacity of the cells of these tissues to divide, but must be influenced further by the release from hindrances to growth and by the action of new growth stimuli; and that after the tumor growth has been initiated, the growth rate must be governed by the capacity of the cells for differentiation and for other regulatory processes, as well as by nutrition.

VARIATIONS IN GROWTH RATE IN THE DIFFERENT TUMOR GROUPS.

Benign Tumors.

The slowly growing benign tumors, springing from smooth and striated muscle, from the supporting tissues in general and from

epithelial tissues, are chiefly characterized by differentiation of their cells, which is perfect or nearly perfect. The cells of these tumors differentiate as do the tissues in hypertrophies and hyperplasias. The growth and division rates of the cells are uniformly more rapid than those of normal tissues, differentiation is good and evidences of depression are lacking. The nucleus-plasma relation is permanently fixed, so long as the tumor remains benign, at a level different from that of normal tissues. They seem to bear the same relation to normal tissues that *Frontonia* individuals cultured at warm temperatures bear to those grown in the cold. These tumors are to a certain degree self limited and do not, as a rule, form metastases, except by accident, that is, by invasion of a vein and by implantation in contiguous tissues.

Malignant Tumors of Slow Growth.

In the slowly growing malignant tumors, especially in the sarcomata composed of smooth muscle and fibrous connective tissue, and in certain epithelial tumors, especially those springing from stratified epithelia, differentiation is often well marked, but usually short of perfect. In such tumors there are commonly evidences of depression from imperfect cell regulation. It is also likely that the incitants to cell division are not very active.

Study of Tumor No. 1 shows, among other things, that even lack of differentiation does not necessarily result in rapid growth. Imperfect cell regulation may be a potent cause of slow growth, as is shown by the findings in Tumor No. 2, in which there is a heavy mortality among the tumor cells from depression and arrest of mitotic division, without proper differentiation and other modes of regulation.

Malignant Tumors of Moderately Rapid Growth.

In tumors of moderately rapid growth, in our experience, the growth rate is either uniform with well marked differentiation of the cells, often accompanied by secretory activity—as in the typical adenocarcinomata of the intestines and uterus; or a slow growth rate is followed by a more rapid growth rate, in which there are evidences of the accomplishment of regulation by chromidiosis, ami-

tosis and nuclear budding (the latter associated with degeneration and extrusion of nuclear buds), not infrequently followed by mitosis; or lastly, with considerable evidence of regulation and rapid division, many of the cells fall into fatal depression. In tumors of the latter category, a large part of the gain in cells is lost by depression, physiological degeneration, and death of their cells.

Malignant Tumors of Rapid Growth.

The tumors of rapid growth spring from the same tissues which give rise to tumors of slow and moderately rapid growth. The variations in the strength of the unknown incitants to growth and division cannot, of course, be estimated, but *a priori,* they must play a large part in the determination of the rate of growth. As has long been known, in these tumors differentiation is poor, and evidences of physiological secretion are absent. Both of these factors, while giving greater freedom to the cells for growth, on the other hand favor the occurrence of depression. Hence, though the cells of these tumors are in general well regulated, with a nucleus-plasma relation within physiological limits, cell depression occurs. On this account, in many cells division is no longer possible and the mortality of individual cells, isolated and in groups, usually in the older portions of the tumors, from physiological degeneration is often heavy.

The most conspicuous characteristics of these tumors are the large numbers of mitoses and amitoses, indicative of great growth and division energy and of the regulatory capacity of their cells. Taking an individual tumor as a whole, while hypernucleosis may be common, giant cells with single giant nuclei and multiple small nuclei are comparatively rare. The cells dying of depression are usually small cells with hypertrophied and hyperchromatic nuclei similar to thymus cells.

The cytoplasm shows marked evidences of a well developed capacity to reduce nuclear material by nuclear resorption and chromidiosis, followed by extrusion or breaking down of the chromatic material in the cytoplasm, occasionally with pigment formation. The dropping of chromosomes from hyperchromatic division figures is not of unusual occurrence.

Rapidly growing tumors which are exceptions to the general rule and have numerous giant cells are not infrequently met with. These are well illustrated by Tumors Nos. 10 and 11, the first being a sarcoma, and the second, a carcinoma. In Tumor No. 10, in which growth was very rapid, there is a very considerable development of giant cells and depression is marked. But regulatory processes, amitotic division, budding and chromidiosis, are conspicuous and are sufficient to save many of the cells from death, though few recover the capacity for mitosis. In Tumor No. 11, though chromidiosis does not occur, amitosis, budding, with nuclear extrusion, and mitosis of mother nuclei thereafter, are common.

DEPRESSION IN TUMOR CELLS.

A review of the tumors described in this paper makes it evident that depression may and often does occur in the cells of many tumors.

In the slowly growing benign tumors with perfect or nearly perfect differentiation, depression is not in evidence.

In some of the slowly growing malignant tumors it is often marked. For instance, in the so-called basal cell carcinomata with very poor differentiation it is great. In many slowly growing fibrosarcomata and in carcinomata, especially in those springing from stratified epithelia, the slow growth is evidently due largely to the marked depression of the cells (Tumor No. 2). The depression is so marked that division is arrested.

In many tumors of moderately rapid growth depression of many or even most of the cells, especially in older areas, is often the most prominent feature; while in tumors of rapid growth many of the cells show evidences of varying degrees of depression.

The depression of tumor cells shows all the marks and characteristics of depression of protozoa, sex cells, thymus cells and of certain other metazoan cells, as described by R. Hertwig and his pupils. In so far as the nature of tumor cells coincides with that of the above mentioned cells, our findings are in complete accord with theirs.

Rapidly repeated divisions are followed in certain protozoa, in sex cells, in thymus cells and in many tumor cells by a depression

in which the most marked feature is an upset of the nucleus-plasma relation in favor of the nucleus. In certain protozoa, other causes, such as overfeeding or change of temperature, may bring about the same result. In protozoa whose functional activity and growth and division rates are increased (overfeeding), the same overgrowth of nuclear material and degenerations of nucleus and cytoplasm are produced. Therefore, the causes of depression in all these types of cells are doubtless closely related, if not identical; at any rate they produce the same effects. Often in tumors of moderately rapid and of rapid growth, just as in protozoa in overfed cultures, the cells undergo alternate waves of depression and recovery with renewed capacity for multiplication. In some of the cells of certain tumors (Tumor No. 10) in which chromidiosis and nuclear resorption are very well marked, we have observed a form of depression quite the reverse of the common type and in which, due to the excessive elimination and destruction of nuclear material, the nucleus-plasma relation is upset in favor of the cytoplasm (Plate VI, Figs. 77 to 82). This brings about a condition of the affected cell analogous to that caused by hyperfunction, but the essential cause here is probably the development on the part of the cytoplasm of an excessive capacity for breaking down nuclear material.

The two most conspicuous results of depression in tumor cells are curbing or arrest of capacity for mitotic division and the occurrence of physiological degeneration and death unless depression is relieved.

Apparently the most important factor concerned in the loss of the capacity for mitotic division in tumors of moderately rapid and of rapid growth is the inability of the division factors of the cells to accomplish successfully indirect division of the excessively large and hyperchromatic nuclei; that is, to inaugurate division and to divide the hypertrophied chromosomes. It is evident that Marcus' explanation of the causation of heterotypical mitoses in sex and thymus cells applies to tumor cells as well.

The nuclear overgrowth in tumor cells leading to depression is due, as in overfed *Actinosphærium eichhorni,* partly to excessive growth of nucleolar substance and partly to the taking up by the nucleus of an excessive amount of fluid from the cytoplasm. The

influence of the latter factor, especially in markedly hypertrophied and giant nuclei, is clearly shown by the increase in the size of the spaces of the nuclear meshwork and by the frequent rupture of the latter. Of these two factors, the first is undoubtedly the primary and most important in tumor cells, for in the typically depressed nucleus, the great excess of nucleolar substance, with or without chromatin, is the striking feature.

THE REGULATORY PROCESSES IN TUMOR CELLS.

Chromidiosis and Nuclear Resorption. The Fate of Chromidia.

In the foregoing pages, the influence of a slow and regular growth rate, of cell division, of differentiation, and of functional activity as regulatory factors in tumor cells have been elaborated. It has been pointed out further, that chromidiosis and nuclear resorption are often resorted to by tumor cells, and that tumor cells which can attain to these two regulatory processes have a distinct advantage. They occur chiefly in rapidly growing tumors, in the metastases and at the spreading borders of tumors of moderately rapid growth. These two processes play the same effective rôle in tumor cells as in protozoan, sex and thymus cells, in preventing and relieving depression by restoring a proper nucleus-plasma relation, which may and often does allow mitotic division to follow.

The fate of chromidia is extrusion or disintegration in the cytoplasm, with or without pigment formation. Our observations on the formation of pigment from chromidia in tumor cells confirm those of R. Hertwig (1904) and of Howard (1908) on pigment formation in *Actinosphærium eichhorni*, and of Rössle (1905) on pigment formation in the cells of melanomata. We have not, however, found evidence of pigment formation from nuclear material within hyperchromatic nuclei of tumor cells. The breaking down of chromidia into pigment in tumor cells is of comparatively rare occurrence, except in those that spring from cells normally capable of pigment formation, that is, chromatophores. Perhaps it is largely on account of the power of their cells to break down excessive nuclear material with pigment formation that the rapid growth so characteristic of melanotic tumors is due.

The Dropping of Chromosomes.

Besides the extrusion of chromidia from the nuclei of resting cells, we have observed the occurrence during mitosis of a phenomenon which must, we think, be ascribed to the same category as the chromidiosis of the resting cell, and classed as a regulatory process, though accomplished in a passive way. We have observed in rapidly growing tumors, in a large number of cells in the prophase and in the metaphase of mitotic division, the occurrence of fine granules and coarse, often irregular masses, of chromatic material, that is, portions of chromosomes lying entirely without the planes of the spindle. Sometimes, no doubt, these masses represent chromidia extruded from the nuclei before division began. But in other cases, from their situation, size, and general arrangement, there is no doubt that they represent chromosomes and portions of chromosomes which have either been dropped from or not included in the division figure. A nucleus hypertrophied and hyperchromatic within the limits in which division can be inaugurated starts mitotic division with hypertrophied chromosomes. The energy of the division factors is not powerful enough to pull the whole mass of chromosomes into the figure and, as division progresses, chromosomes or portions thereof are left out (as described in Tumors Nos. 6 and 8, Plate V, Figs. 59*a*, 59*b*, and 62*b*). There is no reason for thinking that these chromatic masses represent accessory chromosomes in the usual sense.

Multipolar Mitosis.

It may be suggested in this connection that the phenomenon of multipolar mitosis is to be regarded in its results, at least, not as a pathological, but, in a passive fashion, as a regulatory process. Its occurrence in tumors is limited to cells with hypertrophied and hyperchromatic nuclei and a considerable amount of cytoplasm, in other words, to cells with a nucleus-plasma relation markedly in favor of the nucleus, but not so excessively so as to preclude the initiation of mitotic division. It may well be imagined that in such cells the centrosomes are stimulated to growth and to multiple division, instead of to the usual single division, in order to divide the mass of hypertrophied chromosomes. Two explanations are

offered for the multipolar mitotic divisions in such cells: (1) that the centrosomes are incited to multiple division by some external agent, as occurs in certain egg cells as the result of the influence of various chemicals; and (2) that in the rapid growth of these anaplastic cells the centrosomes as well as the other structures grow excessively and undergo multiple instead of normal single division.

Amitosis (Primitive Mitosis).

The rôle of amitosis as a factor in the proliferation of tumor tissue merits consideration. In such a consideration, however, one is confronted by the difficulty of determining just what constitutes direct division. Under this type of division are included simple constriction of the nucleus into two halves, nuclear fission following division of the karyosome, and even that form in which a ring-shaped attraction sphere seems to be the active agent in constricting the nucleus into two parts (salamander spermatogonia, Meves (1891)). The essential characteristic of amitosis, then, seems to be the absence of a spindle figure and of individualized chromosomes. Wilson (1897), in reviewing the literature of the subject, points out the rarity of direct division in metazoan cells and concludes that amitosis is a secondary process which indicates the beginning of the end of a series of divisions. We wish to point out the occurrence, in tumor cells, of a mode of division strikingly like amitosis, not as an argument against the correctness of the above conclusion as to the secondary nature of direct division, but as proof of the reversion by tumor cells to a very primitive form of mitosis. We believe it must be granted that such cells can and do employ biological processes which may differ from those utilized by normal tissue cells of the kind from which the tumor cells are derived. If the cell which is to give rise to a tumor could exhibit only those phenomena characteristic of its normal neighbors, it would run the same life cycle as these latter and would have the same destiny—there could be no tumor.

In the description of Tumor No. 3 we have noted the stages in a form of nuclear division in which the karyosome plays a part, without any evidence of centrosomes or spindle figure. Although the process is very much like many which have been considered

amitotic divisions, we believe that the participation of the karyosome is evidence of a reversion to a primitive mitosis. While a phylogenetic seriation of the various types of karyokinesis is not possible, these types can be arranged in series by selecting species not necessarily related to each other in the scheme of evolution. Calkins (1903) has pointed out that in the most primitive form of division the division material, corresponding to the attraction sphere, is permanently intranuclear. In somewhat more complicated divisions the sphere, intranuclear in the resting nucleus, becomes extranuclear during division. And, finally, there are all the modifications in which the sphere is permanently extranuclear and often manifests itself only during mitosis. Goldschmidt and Popoff (1907) also place under karyokinesis, as its most primitive type, the division which takes place in those nuclei ("centronucleus," Boveri) in which a karyosome ("nucleolo-centrosome," Keuten, Blochmann) inaugurates the division. In such divisions individualized chromosomes may (*Euglena*) or may not (*Amoeba crystaligera,* several species of coccidia) be formed. The kind of division described in Tumor No. 3 (Plates III and IV, Figs. 24 to 30) bears a very marked resemblance to that which occurs in the protozoan nuclei above mentioned. It is met with in greatest frequency in the advancing, invading, and most rapidly growing portions of the tumor and occurs more particularly in nuclei not overladen with chromatin. It is seen in those portions of the tumor in which evidences of degeneration are least marked, and the proportion of cells which show it is so large as to indicate that it plays an important part in the proliferation of the tumor tissue. We have observed it not only in this tumor, but also in a number of others, always in those areas in which the growth is rapid and the individual cells not markedly depressed. We wish to call attention, therefore, to the frequent occurrence, in tumor cells, of a mode of division which closely simulates the direct. We prefer to think, however, that the division is a very primitive mitosis, one in which the karyokinetic center has reverted to its primordial permanent intranuclear situation, the result being a form of division which leads to a marked increase in the division rate of tumor cells.

The relationship of nuclear budding to amitosis has long been

recognized. In protozoan cells it is a true reproductive process; in metazoan cells it is associated most often with functional activity. In Tumor No. 3, we have described the participation of the karyosome in single and multiple nuclear budding (Plate IV, Figs. 31 to 36). Here the process seems to be a modification of the primitive mode of mitosis discussed above. Multiple budding occurs in hyperchromatic nuclei with hypertrophied and hyperchromatic karyosomes. The multiple fission of the karyosome may, perhaps, be due to the same factors which cause the multiple division of the centrosome in multipolar mitosis.

GIANT CELL FORMATION.

We are able to recognize three distinct types of giant cell formation in tumors: (1) separation of giant cells from syncytia; (2) plasmogamy; and (3) excessive growth of single cells.

1. *The Separation of Giant Cells from Syncytia.*—From the syncytial masses of syncytiomata—uterus, testis and Tumor No. 1—large and small cells are split off and lie free as new cells in the tissue or blood spaces. The giant cells thus produced usually possess multiple nuclei of the same type as those of the syncytial tissue.

2. *Plasmogamy.*—The fusion of tumor cells resulting in giant cell formation occurs chiefly among depressed cells in the centers of the alveoli of certain epidermoid carcinomata of slow growth, usually in those in which whorls of cells occur. The cells fuse sometimes by processes, but usually along their flattened sides, thus forming large multinucleated giant cells. The nuclei are usually hyperchromatic and often shrunken. The process is analogous to plasmogamy among depressed protozoa and in the thymus cells, in which, as Marcus (1908) has shown, the Hassall bodies are masses of fused epithelioid cells. These giant cells show various types of degeneration and most of them perish.

While the two modes of giant cell formation mentioned above are to be regarded as degenerative rather than regulatory processes, they are included here for the sake of illustration and completeness.

3. *Excessive Growth of Individual Cells.*—This is the common method of giant cell formation in tumors. Two types are to be

distinguished: (*a*) those cells in which the increase in growth is on the part of the nucleus or at least largely confined to nuclear growth, and (*b*) those in which, in addition to the nuclear growth, cytoplasmic growth is excessive.

(*a*) *Nuclear Overgrowth.*—In this first type, the single nucleus, either round, oval or lobulated (the result of budding), is usually vesicular, but often markedly hyperchromatic, and has increased greatly in size, possibly suddenly. It has stretched the original amount of cytoplasm over its increased volume, with but slight if any growth of the cytoplasm. These are really cells with markedly hypertrophied giant nuclei. They are in marked depression, from which they may, perhaps, recover by accomplishing nuclear budding (of which there is evidence) or by growth of cytoplasm to catch up with the giant nucleus.

(*b*) *Combined Cytoplasmic and Nuclear Overgrowth.*—In this type, which is the usual one, the greatly increased size of the cell is evidently due to a marked growth of the cytoplasm, following or coincident with the growth of the nucleus. Here the growth energy and capacity of the cytoplasm are great. The same growth energy would result in the production of a large number of small cells. But the cytoplasm growth is not accompanied by division energy sufficient to divide the large nucleus by mitosis, or the rates of cytoplasmic and nuclear growth, which in the normal cell bear a definite relation to each other, are so altered that there is not produced the nucleus-plasma tension necessary for division.

The increased growth energy of the cytoplasm may be accompanied by great capacity to break down nuclear material in the same manner in which nuclear hypertrophy and hyperchromatism are prevented or recovered from, that is, by chromidiosis and nuclear resorption, and in this case these processes are marked in the giant cells, as in Tumor No. 10 (Plate VI, Figs. 74, 81 and 84), and with the same results as regards recovery from depression as those described in smaller cells.

BUDDING IN GIANT CELLS.

In many cases, however, the giant cell can neither accomplish mitosis nor inaugurate nuclear resorption and chromidiosis. The

cytoplasm, however, may still possess a large control over the nucleus, for, in many instances, there occurs nuclear budding, in which, as has been shown, we have traced a primitive method of nuclear division, in consequence of which there is produced a large number of daughter nuclei, with evident reduction of nuclear hyperchromatism. And with the nuclear material thus scattered in the cytoplasm rather than gathered in a single hyperchromatic nucleus, the cell may avoid fatal depression, and may preserve its life at least as a multinucleated giant cell, though its power of further division may not be always regained. In such a cell, though the larger part of the cytoplasm may die and the mother nucleus may disintegrate, portions of the cytoplasm about vesicular budded nuclei may be quite normal (Plate V, Fig. 46). In such a cell and in multinucleated giant cells with all the cytoplasm in normal condition, the cytoplasm about some of the nuclear buds may separate off from the mother cell and give rise to two or more cells—a very primitive type of cell division, somewhat analogous to the splitting off of separate cells from syncytia.

The numerous nuclei of the multinucleated giant cell are, in our experience, always the result of nuclear budding and this is in some cases preceded by definite divisions of the karyosome of the mother nucleus (Plate IV, Figs. 34, 35 and 36), and in other cases (Plate VI, Figs. 85 and 86) it is associated with the formation of definite strands of chromatic material—perhaps a primitive type of chromosome formation.

THE DIVISION OF BUDDED NUCLEI AND THE EXTRUSION OF NUCLEAR BUDS.

The cytoplasm of some giant cells with hyperchromatic giant nuclei may so far gain in ascendency as to destroy and extrude nuclear buds, after which there may occur mitosis of the mother nucleus (Plate V, Figs. 48, 49 and 50) or of one or more of the daughter nuclei. There are analogies for this phenomenon of mitosis after budding in other types of cells. Gurwitsch (1904) pictures the occurrence of mitosis following amitosis in the large cells of the triton blastomere. The reduction of nuclear material by the extrusion of the products of nuclear division in the maturation

ivisions of the egg cell is closely related in principle with the cast-ıg out of nuclear buds from the cytoplasm of tumor cells. Though ı the egg cell the direct object of the maturation divisions and the xtrusion of the polar bodies is to reduce the amount of chromatic ıaterial for another purpose, to make room for the chromosomes ɔ be furnished by the spermatozoön, the result is the same in both ases, a reduction of the chromatin content of the nucleus to a egree compatible with the normal destiny of each of these two arieties of cell—as shown, partly at least, by their inherent ten-ency to multiple repeated divisions.

It is in tumors whose division rate is very rapid or at least mod-rately rapid that these various nuclear reduction phenomena as-ociated with marked growth capacity of the cytoplasm occur. The ıuclei of the giant cells in slowly growing tumors—giant-cell sar-omata of the usual type, for instance—often bud and the cytoplasm ıay gradually grow to a very considerable size, but so far as our bservation goes, the cytoplasm does not break down or extrude ıuclear buds and the mother nucleus after the budding process does ıot divide by mitosis. These phenomena are accomplished appar-ntly only in moderately rapidly and in rapidly growing tumors, ı whose cells the cytoplasm has not only great growth capacity, but he property of disposing of nuclear material.

KARYOGAMY.

It has been pointed out above that tumor cells may employ all he regulatory processes utilized by tissue, sex and protozoan cells, xcept the phenomenon of karyogamy, a regulatory process to /hich, in the form of conjugation, copulation or autogamy, proto-oa and sex cells finally resort as their last defense against fatal lepression.

None of the appearances hitherto described as indicative of :aryogamy—the copulation phenomena of Farmer, Moore and Valker (1906) and the conjugation of Bashford (1904)—with-tand critical analysis and no adequate series of morphological ap-ıearances has been brought forward to support the claims made ı this regard. The mere presence of leukocytes within tumor ells is far from being convincing evidence of copulation to those

who are acquainted, on the one hand, with the invasive habits of these cells and the ingestive capacity of protozoan and metazoan cells and, on the other hand, with the phenomena of the various steps and types of the fertilization process. The establishment of the occurrence of heterotypical mitosis in tumor cells would not be of necessity evidence of either copulation in or of the gametoid nature of these cells, for, as Marcus (1908) has shown in his work on the thymus cells, heterotypical mitosis is simply evidence of nuclear hyperchromatism. The appearances described by Bashford (1904) as tumor cell conjugation appear to us to be stages in nuclear budding. Compare, in this regard, our Fig. 87 (Plate VI) with Fig. 32 of Bashford's article. At the present time, it is on theoretical grounds alone that karyogamy in tumor cells has any substantial footing.

The tumor cell is the only known cell capable of indefinite proliferation without fertilization. This or some compensating process must occur in the cells of certain tumors, such as the Jensen tumor, which appear capable of indefinite proliferation. The type of fertilization most likely to occur in such cells would be the autogamous.

V. CONCLUSIONS.

Departure from the simple alveolar structure of protoplasm (morphological differentiation) is an expression of functional specialization.

Nucleus and cytoplasm are mutually interdependent.

For every normal cell there is a definite relation between the amount of cytoplasm and the nuclear mass—the nucleus-plasma relation.

The rates of cytoplasm growth and nucleus growth vary. The maximum disproportion between the two, in the normal cell, leads to a condition of nucleus-plasma tension, which inaugurates rapid divisional growth of the nucleus and results finally in cell division.

Many conditions, among them overfeeding, starvation, hyperactivity, changes in temperature, rapidly repeated divisions, lead to an upset of the nucleus-plasma relation, characterized by nuclear hypertrophy and hyperchromatism, and often nuclear hyperplasia.

Excessive chromatin consumption due to excessive functional activity and, in tumor cells, to an increase in the property of cytoplasm to disintegrate nuclear material, leads to an upset of the nucleus-plasma relation in favor of the cytoplasm, which may cause physiological degeneration and death.

The previously described regulatory processes by means of which cells, other than tumor cells, avoid and recover from depression are: (1) growth of cytoplasm with differentiation and functional activity; (2) cell division; (3) nuclear resorption and chromidiosis, often followed by cell division; (4) dissolution and extrusion of nuclei; (5) the renewal of chromatin during rest; and (6) karyogamy.

Dynamic energy, produced by cellular metabolism, manifests itself in cellular growth and function. The amount of energy production of which cells are capable varies for the different species of cells and, within limits, for cells of the same species. In all normal cells energy production becomes exhausted unless there is provided a rejuvenation of protoplasm and a restoration of its potentiality for the production of energy by some form of fertilization. This exhaustion is manifested by senility, physiological degeneration and death of the cell.

In the malignant tumor cell the maximum limit of energy production for the normal cell is overstepped and the energy produced is abnormally distributed to the advantage of growth rather than of function.

The increased growth rate of tumor cells leads to an increased division rate.

Rapidity of growth is usually accompanied by lack of differentiation. But the loss of differentiation may be so complete that individual cells with a fixed division rate are not produced. Such complete anaplasia might be followed, if an accession of growth energy becomes possible, by metaplasia and the production of mixed tumors.

The rapid division rate of tumor cells is followed, as in protozoan, sex and thymus cells, by depression.

Excessive nuclear hypertrophy leads to the formation of giant cells, in which the increased size of the cell may be due largely to

nuclear growth alone. In other cells nuclear growth is associated with cytoplasmic growth. In such cells attempts at regulation may be active. Nuclear budding is the usual method and leads to the production of multinucleated cells. Giant cells in tumors, in addition to being produced by growth of single cells, may be formed by the splitting off of multinucleated masses from syncytia and by plasmogamy (fusion). Giant cells produced by the two latter processes fall into depression and undergo physiological degeneration and death.

To avoid and overcome depression, tumor cells employ all the regulatory processes used by other cells except, so far as we now know, karyogamy.

In addition to these, we have pointed out the occurrence of nuclear budding and its purposeful nature in reducing superabundant nuclear material, following which cell division may occur with or without mitosis.

After nuclear hyperchromatism has been reduced by budding, buds may be extruded, and there may occur mitosis in the mother nucleus or in some of the buds. Or again, cell division may occur with the separation of masses of cytoplasm about nuclear buds.

We believe that the dropping of chromosomes from hyperchromatic mitoses, and the reversion to a very primitive type of mitosis, are also to be regarded as regulatory mechanisms.

It is evident from our observations that amitosis and primitive mitosis are responsible for much of the cell multiplication in tumors.

Whether the growth rate of any given malignant tumor is slow, moderately rapid or rapid depends upon the growth and divisional capacities of the species of normal cell from which the tumor cells were derived, the strength of the incitants which stimulate growth and division, and the degree to which active regulatory processes are developed and utilized by the cells. The growth rate of a given tumor may be varied by the development of modes of regulation not before used by that tumor.

The utilization of the regulatory mechanisms, which have been mentioned above, helps to explain a large degree of the apparently unlimited proliferative capacity which is so characteristic of the cells of malignant tumors.

Theoretically, the unlimited proliferation of which the cells of ertain malignant tumors seem capable demands the occurrence of ome form of karyogamy as the ultimate means for overcoming /hat must otherwise be a fatal depression. The evidence thus far dduced in favor of copulation and conjugation in tumor cells is ot, however, satisfactory.

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EXPLANATION OF PLATES.

All drawings were made with the Spencer camera lucida. In their preparation, except in the case of Fig. 1, two lens systems were used giving, respectively, magnifications of 1,395 and 1,480 diameters.

Plate I.

Figs. 1 to 4. Tumor No. 1. Epulis of lower jaw. Mayer's acid hemalum and eosin. Fig. 1, Leitz 7 objective, Zeiss 6 compensating ocular; × 990. Figs. 2, 3 and 4, Zeiss 2 mm. objective, 6 compensating ocular; × 1,480. In the reproduction Figs. 1, 3, and 4 have been reduced by ½, and Fig. 2 has been reduced by ⅝.

Fig. 1. Syncytial tissue composing the greater portion of the section. The tissue shows numerous wide blood spaces which do not have an endothelial lining.

Fig. 2. Syncytial tissue. Formation of spaces in the protoplasm. Some spaces contain red blood corpuscles. About some of the nuclei the cytoplasm has become differentiated with the formation of individual cells.

Fig. 3. A multinucleated giant cell, surrounded by blood spaces. The formation of such cells seems to be determined by the blood spaces. A few mononucleated cells have also been produced in the same way.

Fig. 4. Two multinucleated giant cells showing beginning degeneration. The cytoplasm is somewhat vacuolated in places and some areas take the hematoxylin stain. The nuclei are decreased in size, several are irregular in outline and all stain deeply and show little structural detail.

Figs. 5–10. Tumor No. 2. Carcinoma of the mucous membrane of the cheek. Grenacher's alcoholic borax-carmin and dilute Delafield's hematoxylin. Leitz ⅛ objective, 4 ocular; × 1,395.

Fig. 5. A cell with an hypertrophied, hyperchromatic nucleus.

Fig. 6. A cell with an hypertrophied budding nucleus.

Plate II.

Fig. 7. A cell with a giant, budding, hyperchromatic nucleus.

Fig. 8. A cell with a giant, hyperchromatic nucleus and vacuolated cytoplasm.

Fig. 9. A cell with a giant, hyperchromatic nucleus showing condensation and vacuolization of the nuclear material; disintegration of the cytoplasm at one side.

Fig. 10. A giant cell with marked vacuolization of the cytoplasm; the large nucleus contains one large and numerous small masses of vacuolated nuclear material.

Figs. 11–36. Tumor No. 3. Giant cell sarcoma of the esophagus. Heidenhain's iron hematoxylin and eosin. Zeiss 2 mm. objective, 6 compensating ocular; × 1,480.

FIG. 11. The majority of the cells in the older portions of the tumor are of this type.

FIG. 12. Budding, I: a giant nucleus so hyperchromatic that the reticulum is completely hidden. Early amitosis-like division with several early small buds.

FIG. 13. Budding, II: two buds are almost completely separated. The structure of the original nucleus is much like that of Fig. 11. The buds are less hyperchromatic.

PLATE III.

FIG. 14. Budding, III: the process still further advanced than in Fig. 13. There are numerous buds, the hyperchromatism of which and of the original nucleus is greatly reduced.

FIG. 15. Budding, IV: end stage. Formation of separate daughter nuclei of a very primitive type. The nuclei are rich in fluid, with a distinct membrane and widely meshed reticulum. The chromatin is small in amount and confined to the karyosome. Several of the nuclei (at *a, b, c*) show beginning degenerative changes—decrease in size, shrivelling of the membrane and condensation of the nuclear fluid.

FIG. 16. A type cell from the advancing border of the tumor.

FIGS. 17 and 18. Stages in the normal process of chromatin deposition in the karyosome, leading to the formation of a cell like Fig. 16.

FIGS. 19 and 20. Plastin hypertrophy of the karyosome.

FIG. 19. An hypertrophied, vacuolated, chromatin-free karyosome.

FIG. 20. A nucleus with three hypertrophied, chromatin-free karyosomes.

FIGS. 21, 22 and 23. Stages in nuclear hyperchromatism.

FIG. 21. An hypertrophied, hyperchromatic karyosome.

FIG. 22. Beginning chromatin diffusion throughout the nucleus.

FIG. 23. Marked chromatin diffusion throughout the nucleus; the nucleus is so hyperchromatic that the reticulum can not be seen.

FIGS. 24–29. Stages in the equal binary fission of the karyosome, leading to nuclear division.

PLATE IV.

FIG. 30. Stage in the equal binary fission of the karysome, leading to nuclear division.

FIGS. 31–33. Stages in the unequal binary fission of the karyosome, leading to single nuclear budding.

FIGS. 34–36. Stages in the multiple fission of the karyosome, leading to multiple nuclear budding.

FIGS. 37–50. Tumor No. 4. Epithelioma of the cervix uteri. Mayer's acid hemalum and eosin. Zeiss 2 mm. objective, 6 compensating ocular; × 1,480.

FIG. 37. A type cell.

FIG. 38. A cell with an hypertrophied, hyperchromatic nucleus.

FIG. 39. A giant cell in abortive mitosis, showing degeneration of the hypertrophied chromosomes.

FIG. 40. A cell whose hypertrophied nucleus has just undergone amitosis.

FIGS. 41 and 42. Giant cells showing stages of nuclear budding.

FIG. 43. A giant cell in which budding has produced many nuclei. The cytoplasm shows vacuolar and hyalin degeneration.

FIG. 44. A cell with two nuclei. One is completely vacuolated and surrounded by hyalin cytoplasm; the other is less completely degenerated and surrounded by vacuolar cytoplasm.

PLATE V.

FIG. 45. A giant cell dead of depression.

FIG. 46. A very large giant cell. Most of the cytoplasm is hyalin, and the mother nucleus is degenerated and broken into fragments. Four nuclear buds are surrounded by well preserved cytoplasm.

FIG. 47. A small cell dead of depression.

FIG. 48. A large cell showing three nuclear buds and a portion of a mitotic figure in cross section.

FIG. 49. A large cell with a cross section of a mitotic figure, and a chain of degenerated nuclear buds at one side.

FIG. 50. A cell showing a mitotic figure in longitudinal section, and a mass of degenerated nuclear buds extruded from one pole of the cell.

FIGS. 51–54. Tumor No. 5. Hypernephroma. Grenacher's alcoholic borax-carmin and dilute Delafield's hematoxylin. Leitz $\frac{1}{12}$ objective, 4 ocular; × 1,395.

FIG. 51. A cell with a small extranuclear chromatin granule and a pigment granule.

FIG. 52. Cells showing marked pigmentation; the nuclei are vesicular and not hyperchromatic.

FIG. 53. A cell with a large amount of pigment; the nucleus is very small and poor in chromatin.

FIG. 54. A cell showing extreme pigmentation; no nuclear material can be seen.

FIGS. 55–59. Tumor No. 6. Carcinoma of the thyroid. Grenacher's alcoholic borax-carmin and dilute Delafield's hematoxylin. Leitz $\frac{1}{12}$ objective, 4 ocular; × 1,395.

FIG. 55. From the peripheral portion of the recurrence. Cells from the wall of an acinus. The cytoplasm is markedly vacuolated. (*a*) A cell with a well-regulated nucleus. (*b*) A cell with an hyperchromatic nucleus, whose membrane is shrunken. (c) Chromidiosis with beginning reconstruction of the nucleus.

FIG. 56. From the cervical lymph gland invasion. (*a*) and (*b*) Type cells. (*c*) A cell with a giant nucleus.

FIG. 57. Small cells with very hyperchromatic nuclei.

FIG. 58. From the lung metastasis. Chromidiosis; almost all the chromatin has left the nucleus.

FIG. 59. From a lung metastasis. Mitosis. (*a*) Bouquet stage; two chromatin rods not incorporated in the figure. (*b*) Equatorial plate; two chromatin particles have not been drawn into the plate.

FIGS. 60–63. Tumor No. 8. Carcinoma of the breast. Grenacher's alcoholic borax-carmin and dilute Delafield's hematoxylin. Zeiss 2 mm. objective, 6 compensating ocular; × 1,480.

FIG. 60. From the metastasis. Two resting cells which are reducing their chromatic material by chromidiosis.

FIG. 61. From the metastasis. A cell showing partial dissolution of its nucleus into chromidia.

FIG. 62. From the metastasis. (*a*) A resting cell showing chromidiosis. (*b*) A dividing cell in the bouquet stage, showing chromidiosis.

FIG. 63. A cell whose nucleus has been completely transformed into chromidia.

FIGS. 64–70. Tumor No. 9. Carcinoma of the lung. Grenacher's alcoholic borax-carmin and dilute Delafield's hematoxylin. Leitz $\frac{1}{12}$ objective, 4 ocular; × 1,395.

FIG. 64. From a liver metastasis. A type cell.

FIG. 65. From an omental metastasis. Markedly hyperchromatic nuclei with chromidiosis.

FIG. 66. From an intestinal metastasis. A cell with a markedly hyperchromatic giant nucleus.

FIG. 67. From an intestinal metastasis. Nuclear hypertrophy and hyperchromatism.

FIG. 68. From an omental metastasis. Marked chromidiosis, with a return of the nucleus to the vesicular type.

PLATE VI.

FIG. 69. From an omental metastasis. Marked chromidiosis. Breaking up of the hyperchromatic nucleus.

FIG. 70. From an intestinal metastasis. Vacuolization of an hyperchromatic nucleus.

FIGS. 71–86. Tumor No. 10. Giant cell sarcoma of the neck. Grenacher's alcoholic borax-carmin and dilute Delafield's hematoxylin. Zeiss 2 mm. objective, 6 compensating ocular; × 1,480.

FIG. 71. A type cell. The cytoplasm is closely meshed. The nucleus is rather vesicular, with a fine reticulum and small chromatin granules lying within the nucleus and upon the inner surface of the membrane.

FIG. 72. Very moderate hyperchromatism leading to the formation of a vesicular nucleus with several large chromatin masses.

FIG. 73. Marked hyperchromatism and giant nucleus formation.

FIG. 74. An hyperchromatic nucleus which shows the beginning of chromatin extrusion.

FIG. 75. Marked chromidiosis in a small hyperchromatic nucleus.

FIG. 76. A cell showing some finely granular, extranuclear chromatin and a considerable amount of finely granular, faint brown pigment scattered in the cytoplasm. Chromidiosis has resulted in the production of a vesicular nucleus.

FIG. 77. A peculiar type of cell, present in considerable numbers. The nucleus is small, round, dense and hyperchromatic. The cytoplasm is vacuolated.

FIG. 78. Chromidiosis in a nucleus of the type shown in Fig. 77.

FIG. 79. Resorption, without destruction of the membrane, in a nucleus of the type shown in Fig. 72. In places the cytoplasm takes a faint chromatin stain.

FIG. 80. Partial resorption of a nucleus of the type shown in Fig. 77.

FIG. 81. Nuclear resorption in a nucleus of the type shown in Fig. 74. The nuclear membrane, at one side, has disappeared.

FIG. 82. Partial resorption of a nucleus of the type shown in Fig. 73. Disappearance of the membrane at one side.

FIG. 83. Amitosis. The division has resulted in the production of two nuclei which are only slightly hyperchromatic.

FIG. 84. Budding and chromidiosis. The nuclear buds are vesicular.

FIGS. 85 and 86. Nuclear changes due, apparently, to a sudden increase in the amount of the fluid contents of the nuclei, resulting in the production, within the nuclei, of chromosome-like strands.

FIG. 87. From the axillary lymph gland metastasis of a carcinoma of the breast. Delafield's hematoxylin and eosin. Zeiss 2 mm. objective, 6 compensating ocular; × 1,480. A cell showing chromidiosis, nuclear budding and beginning cytoplasmic division.

Figs. 5–10, 37–50, and 60–63 by William Travis Howard.
Figs. 1–4, 11–36, 51–59, and 64–87 by Oscar T. Schultz.


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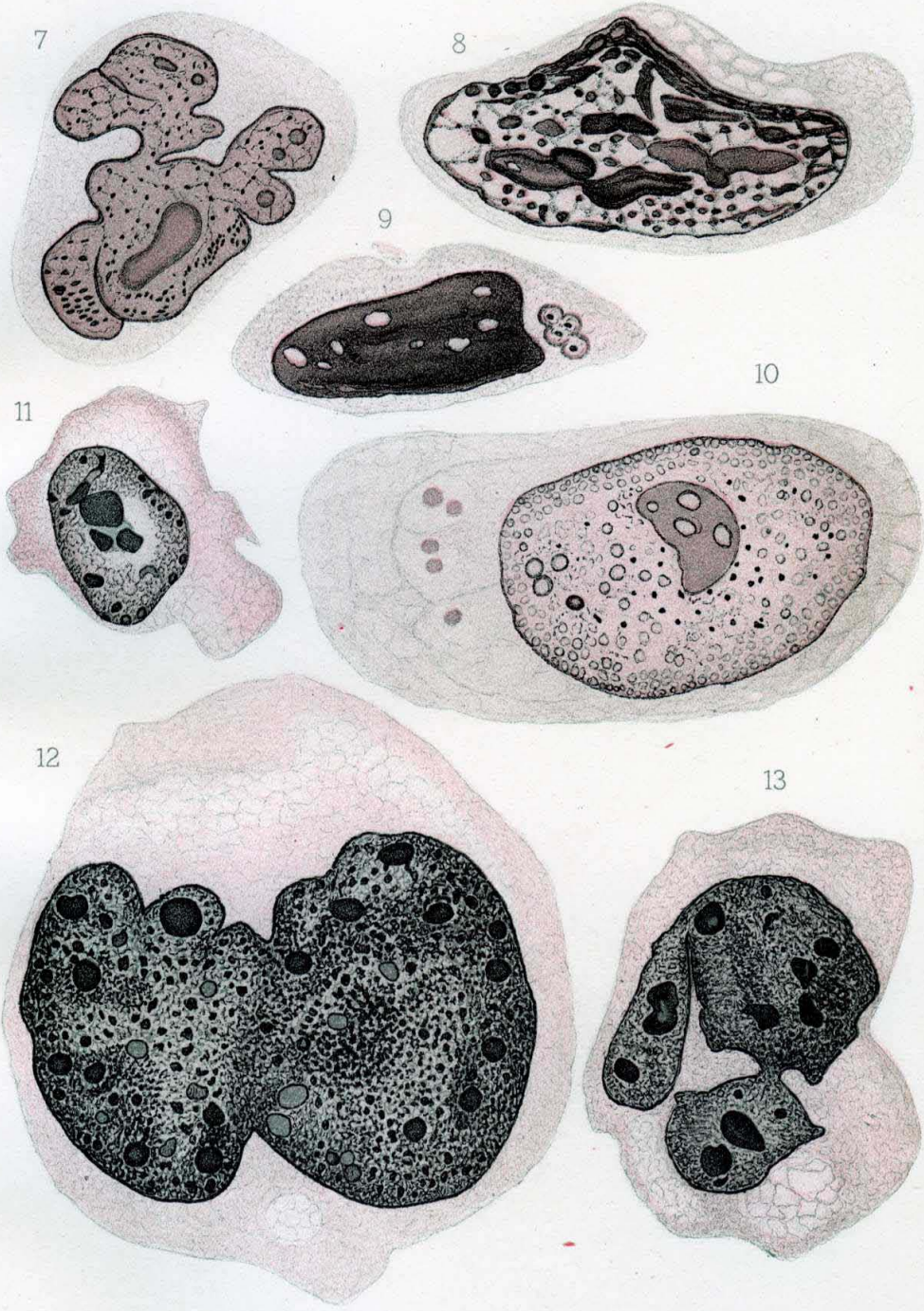
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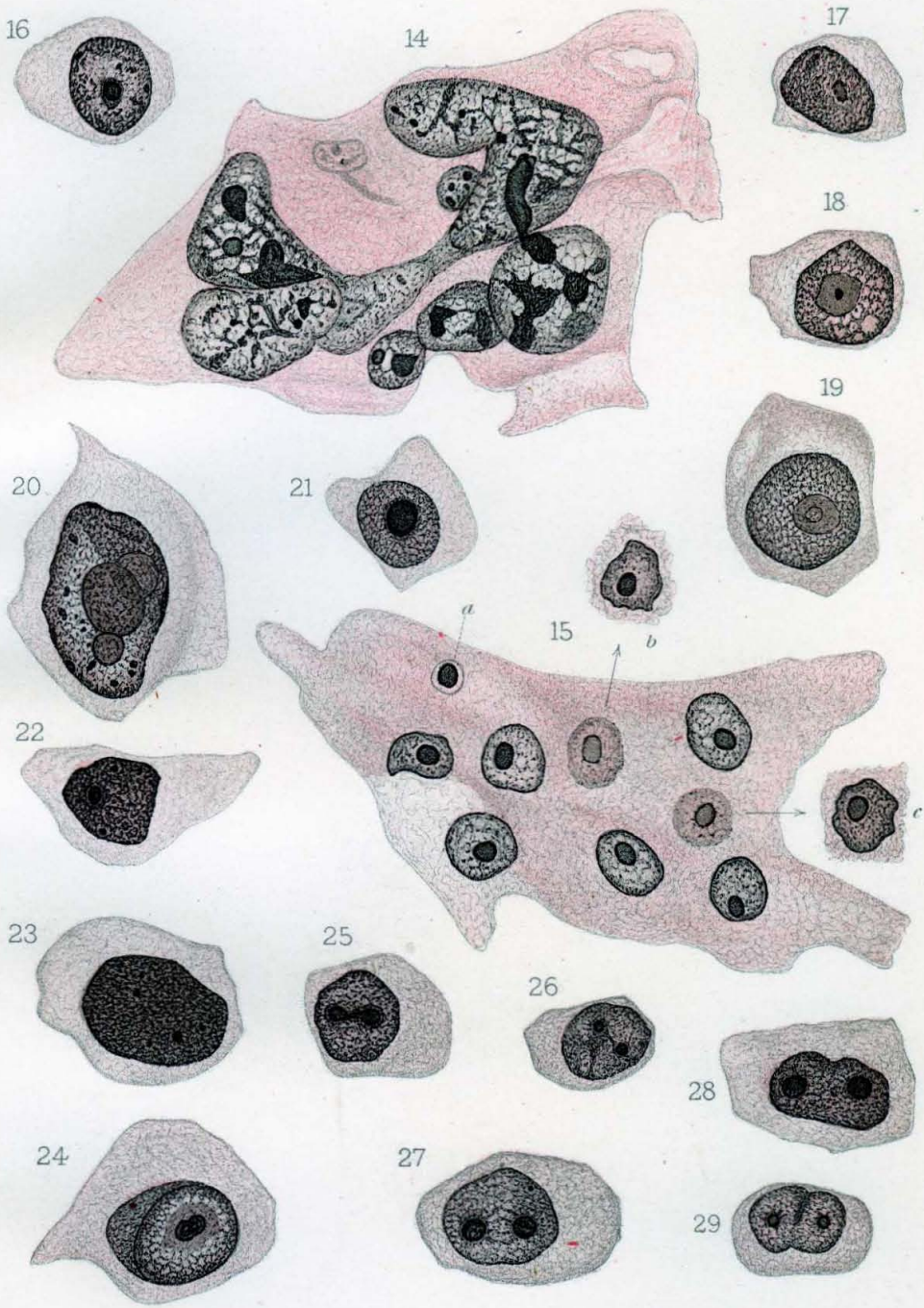
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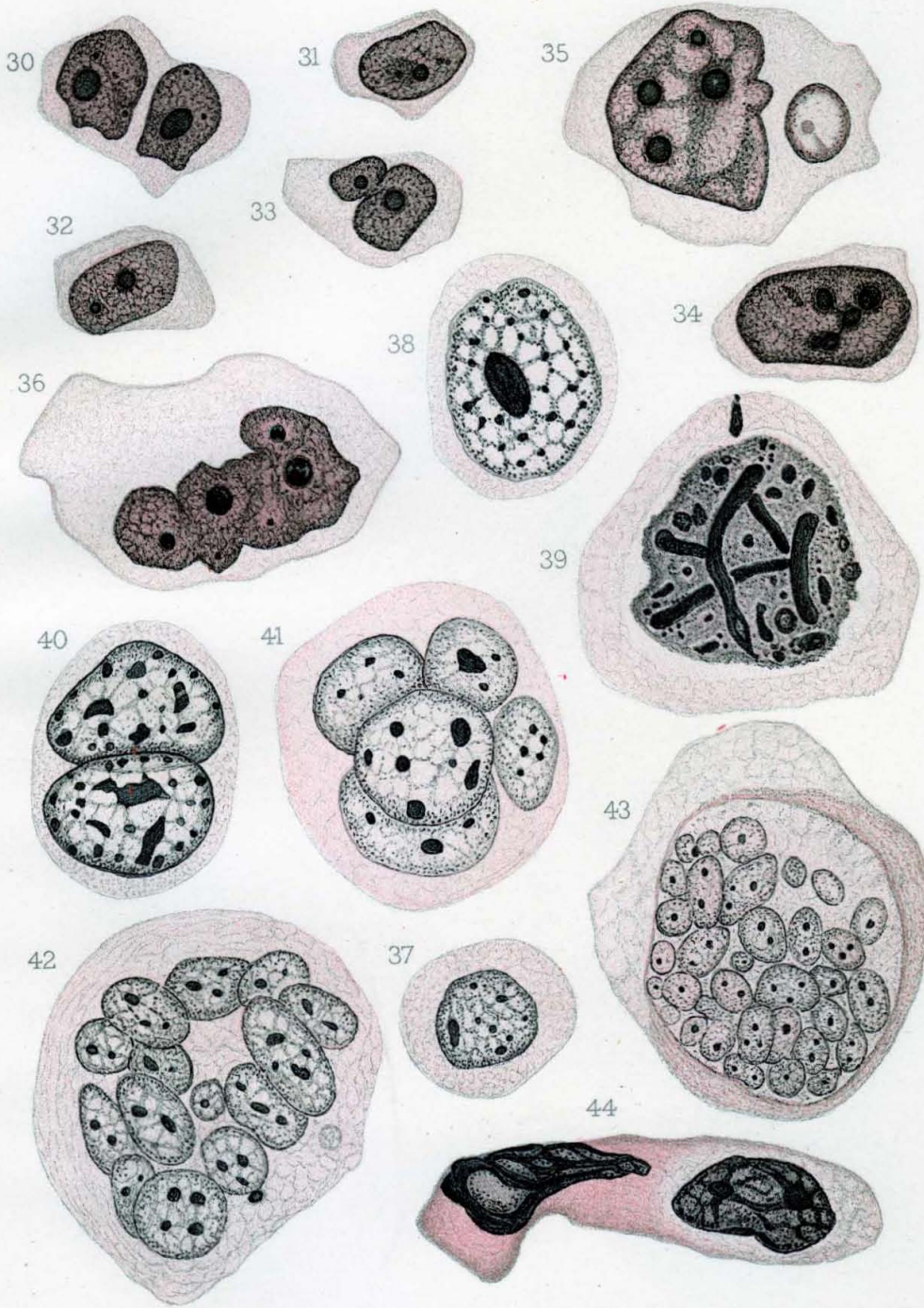
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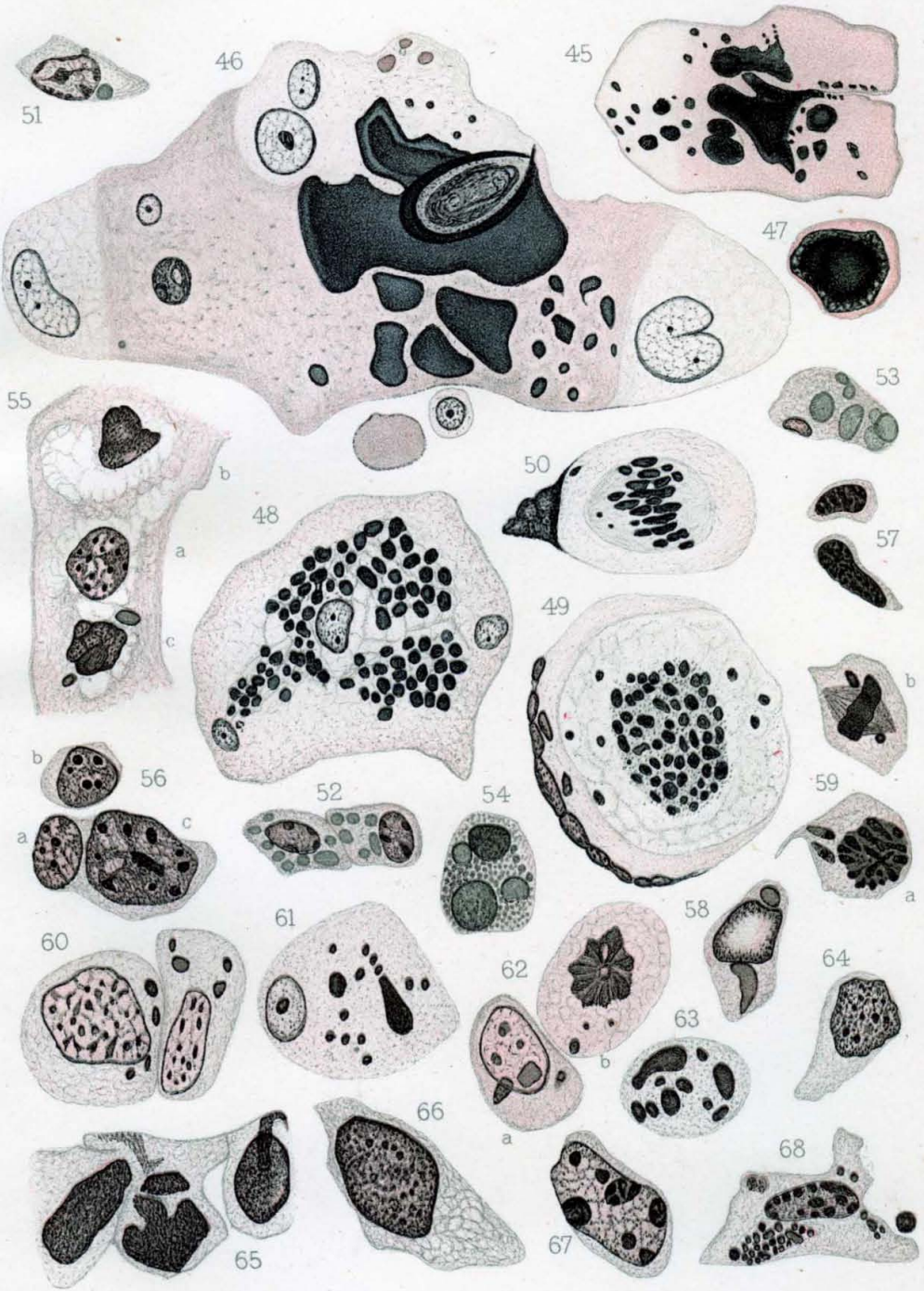
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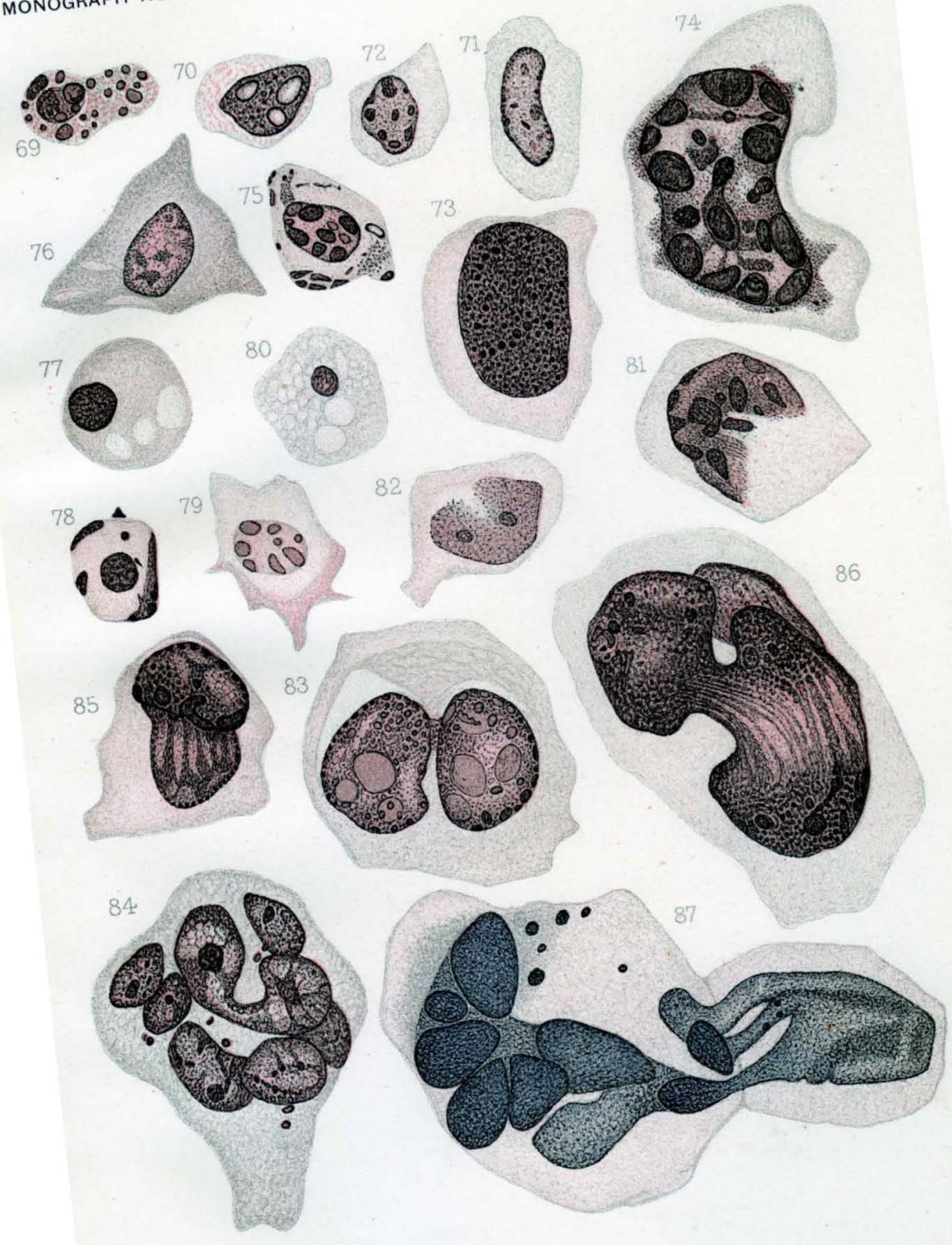
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