CHAPTER VII

SOME ASPECTS OF CELL-CHEMISTRY AND CELL-PHYSIOLOGY

"Les phénomènes fonctionnels ou de dépense vitale *auraient donc leur siège dans le protoplasme cellulaire.*

"Le noyau est un appareil de *synthèse organique, l'instrument de la production, le germe de la cellule.*" CLAUDE BERNARD.[1]

I

A. CHEMICAL RELATIONS OF NUCLEUS AND CYTOPLASM

IT is no part of the purpose of this work to give even a sketch of general cell-chemistry. I shall only attempt to consider certain questions that bear directly upon the functional relations of nucleus and cytoplasm and are of especial interest in relation to the process of nutrition and through it to the problems of development. It has often been pointed out that we know little or nothing of the chemical conditions existing in living protoplasm, since every attempt to examine them by precise methods necessarily kills the protoplasm. We must, therefore, in the main rest content with inferences based upon the chemical behaviour of dead cells. But even here investigation is beset with difficulties, since it is in most cases impossible to isolate the various parts of the cell for accurate chemical analysis, and we are obliged to rely largely on the less precise method of observing with the microscope the visible effects of dyes and other reagents. This difficulty is increased by the fact that both cytoplasm and karyoplasm are not simple chemical compounds, but mixtures of many complex substances; and both, moreover, undergo periodic changes of a complicated character which differ very widely in different kinds of cells. Our knowledge is, therefore, still fragmentary, and we have as yet scarcely passed the threshold of a subject which belongs largely to the cytology of the future.

It has been shown in the foregoing chapter that all the parts of the cell arise as local differentiations of a general protoplasmic basis. Despite the difficulties of chemical analysis referred to above, it has been determined with certainty that some at least of these organs are the seat of specific chemical change; just as is the case in the various organs and tissues of the organism at large. Thus, the nucleus is

[1] *Leçons sur les phénomènes de la vie*, I., 1878, p. 198.

characterized by the presence of nuclein (chromatin) which has been proved by chemical analysis to differ widely from the cytoplasmic substances,[1] while the various forms of plastids are centres for the formation of chlorophyll, starch, or pigment. These facts give ground for the conclusion that the morphological differentiation of cell-organs is in general accompanied by underlying chemical specializations which are themselves the expression of differences of metabolic activity; and these relations, imperfectly comprehended as they are, are of fundamental importance to the student of development.

1. *The Proteids and their Allies*

The most important chemical compounds found in the cell are the group of *protein substances*, and there is every reason to believe that these form the principal basis of living protoplasm in all of its forms. These substances are complex compounds of carbon, hydrogen, nitrogen, and oxygen, often containing a small percentage of sulphur, and in some cases also phosphorus and iron. They form a very extensive group of which the different members differ considerably in physical and chemical properties, though all have certain common traits and are closely related. They are variously classified even by the latest writers. By many authors (for example Halliburton, '93) the word "*proteids*" is used in a broad sense as synonymous with *albuminous substances*, including under them the various forms of *albumin* (egg-albumin, cell-albumin, muscle-albumin, vegetable-albumins), *globulin* (fibrinogin vitellin, etc.), and the *peptones* (diffusible hydrated proteids). Another series of nearly related substances are the *albuminoids* (reckoned by some chemists among the "proteids"), examples of which are gelatin, mucin, and, according to some authors also, *nuclein*, and the *nucleo-albumins*. Some of the best authorities however, among them Kossel and Hammarsten, follow the usage of Hoppe-Seyler in restricting the word *proteid* to substances of greater complexity than the albumins and globulins. Examples of these are the nucleins and nucleo-proteids, which are compounds of nucleinic acid with albumin, histon, or protamin. The nucleo-proteids, found only in the nucleus, are not to be confounded with the nucleo-

[1] It has long been known that a form of "nuclein" may also be obtained from the nucleo-albumins of the cytoplasm, *e.g.* from the yolk of hens' eggs (vitellin). Such nucleins differ, however, from those of nuclear origin in not yielding as cleavage-products the nuclein bases (adenin, xanthin, etc.). The term "paranuclein" (Kossel) or "pseudo-nuclein" (Hammarsten) has therefore been suggested for this substance. True nucleins containing a large percentage of albumin are distinguished as *nucleo-proteids*. They may be split into albumin (or albumin radicals) and nucleinic acid, the latter yielding as cleavage-products the nuclein bases. Pseudo-nucleins containing a large percentage of albumin are designated as *nucleo-albumins*, which in like manner split into albumin and paranucleinic or pseudo-nucleinic acid, which yields no nuclein bases. (See Hammarsten, '94.)

albumins, which are compounds of pseudo-nucleinic acid with albumin and yield no nuclein-bases (xanthin, hypoxanthin, adenin, guanin) as decomposition products.

The distribution of these substances through the cell varies greatly not only in different cells, but at different periods in the life of the same cell. The cardinal fact always, however, remains, that *there is a definite and constant contrast between nucleus and cytoplasm.* The latter always contains large quantities of nucleo-albumins, certain globulins, and sometimes small quantities of albumins and peptones; the former contains, in addition to these, *nuclein* and *nucleo-proteids*, which form its main bulk, and its most constant and characteristic feature. It is the remarkable substance, nuclein, — which is almost certainly identical with chromatin, — that chiefly claims our attention here on account of the physiological *rôle* of the nucleus.

2. *The Nuclein Series*

Nuclein was first isolated and named by Miescher, in 1871, by subjecting cells to artificial gastric digestion. The cytoplasm is thus digested, leaving only the nuclei; and in some cases, for instance pus-cells and spermatozoa, it is possible by this method to procure large quantities of nuclear substance for accurate quantitative analysis. The results of analysis show it to be a complex albuminoid substance, rich in phosphorus, for which Miescher gave the chemical formula $C_{29}H_{49}N_9P_3O_{22}$. The earlier analysis of this substance gave somewhat discordant results, as appears in the following table of percentage-compositions:[1] —

	Pus-cells. (Hoppe-Seyler.)	Spermatozoa of Salmon. (Miescher.)	Human Brain. (v. Jaksch.)
C	49.58	36.11	50.6
H	7.10	5.15	7.6
N	15.02	13.09	13.18
P	2.28	5.59	1.89

These differences led to the opinion, first expressed by Hoppe-Seyler, and confirmed by later investigations, that there are several varieties of nuclein which form a group having certain characters in common. Altmann ('89) opened the way to an understanding of the matter by showing that "nuclein" may be split up into two substances; namely, (1) an organic acid rich in phosphorus, to which he

[1] From Halliburton, '91, p. 203. [The oxygen-percentage is omitted in this table.]

gave the name *nucleinic acid*, and (2) a form of albumin. Moreover, the nuclein may be synthetically formed by the re-combination of these two substances. Pure nucleinic acid, for which Miescher ('96) afterward gave the formula $C_{40}H_{54}N_{14}P_4O_{27}$,[1] contains no sulphur, a high percentage of phosphorus (above 9%), and no albumin. By adding it to a solution of albumin a precipitate is formed which contains sulphur, a lower percentage of phosphorus, and has the chemical characters of "nuclein." This indicates that the discordant results in the analyses of nuclein, referred to above, were probably due to varying proportions of the two constituents; and Altmann suggested that the "nuclein" of spermatozoa, which contains no sulphur and a maximum of phosphorus, might be uncombined nucleinic acid itself. Kossel accordingly drew the conclusion, based on his own work as well as that of Liebermann, Altmann, Malfatti, and others, that "what the histologists designate as *chromatin* consists essentially of combinations of nucleinic acid with more or less albumin, and in some cases may even be free nucleinic acid. The less the percentage of albumin in these compounds, the nearer do their properties approach those of pure nucleinic acid, and we may assume that the percentage of albumin in the chromatin of the same nucleus may vary according to physiological conditions."[2] In the same year Halliburton, following in part Hoppe-Seyler, stated the same view as follows. The so-called "nucleins" form a series leading downward from nucleinic acid thus: —

(1) Those containing no albumin and a maximum (9–10%) of phosphorus (pure nucleinic acid). Nuclei of spermatozoa.
(2) Those containing little albumin and rich in phosphorus. Chromatin of ordinary nuclei.
(3) Those with a greater proportion of albumin — a series of substances in which may probably be included *pyrenin* (nucleoli) and *plastin* (linin). These graduate into
(4) Those containing a minimum (0.5 to 1%) of phosphorus — the nucleo-albumins, which occur both in the nucleus and in the cytoplasm (vitellin, caseinogen, etc.).

Finally, we reach the globulins and albumins, especially characteristic of the cell-substance, and containing no nucleinic acid. "We thus pass by a gradual transition (from the nucleo-albumins) to the other proteid constituents of the cell, the cell-globulins, which contain no phosphorus whatever, and to the products of cell-activity, such as the proteids of serum and of egg-white, which are also principally

[1] Derived from analysis of the salmon-sperm. [2] '93, p. 158.

phosphorus-free."[1] Further, "in the processes of vital activity there are changing relations between the phosphorized constituents of the nucleus, just as in all metabolic processes there is a continual interchange, some constituents being elaborated, others breaking down into simpler products." This latter conclusion has been well established; the others, as stated by Halliburton, require some modification, on the one hand, through the results of later analyses of chromatin, on the other, because of the failure to distinguish between the nucleo-proteids and the nucleo-albumins. First, it has been shown by Miescher ('96), Kossel ('96), and Mathews ('97, 2) that the chromatin of the sperm-nuclei (in fish and sea-urchins) is not pure nucleinic acid, as Altmann conjectured, but a salt of that acid, with histon, protamin, or a related substance. Thus, in the spermatozoa of the salmon, Miescher's analyses give 60.56% of nucleinic acid and 35.56% of protamin ($C_{16}H_{28}N_9O_2$). In the herring the chromatin is a compound of nucleinic acid (over 63%) and a form of protamin called by Kossel "clupein" ($C_{30}H_{57}N_{17}O_6$). In the sea-urchin *Arbacia* Mathews finds the chromatin to be a compound of nucleinic acid and "arbacin," a histon-like body. Kossel finds also that chromatin (nuclein) derived from the thymus gland, and from leucocytes, is largely a histon salt of nucleinic acid, the proportion of the latter being, however, much less than in the sperm-chromatin, while albumin is also present. In these cases, therefore, the greater part of the nucleinic acid is combined not with albumin but with a histon or protamin radical. Second, the nucleo-albumins of the cytoplasm are in no sense transitional between the nucleins and the albumins, since they contain no true nucleinic acid, but only pseudo-nucleinic acid.[2] The fact nevertheless remains that the nucleins and nucleo-proteids, though confined to the nucleus, form a series descending from such highly phosphorized bodies as the sperm-chromatin toward bodies such as the albumins, which are especially characteristic of the cytoplasm; and that they vary in composition with varying physiological conditions. The way is thus opened for a more precise investigation of the physiological *rôle* of nucleus and cytoplasm in metabolism.

3. *Staining-reaction of the Nuclein Series*

In bringing these facts into relation with the staining-reactions of the cell, it is necessary briefly to consider the nature of staining-reactions in general, and especially to warn the reader that in the whole field of "micro-chemistry" we are still on such uncertain ground that all general conclusions must be taken with reserve.

First, it is still uncertain how far staining-reactions depend upon chemical reaction and how far upon merely physical properties of

[1] '93, p. 574. [2] *Cf.* p. 331.

the bodies stained. The prevalent view that staining-reactions are due to a chemical combination of the dye with the elements of the cell has been attacked by Gierke ('85), Rawitz ('97), and Fischer ('97, '99), all of whom have endeavoured to show that these reactions are of no value as a chemical test, being only a result of surface-attraction and absorption due to purely physical qualities of the bodies stained. On the other hand, a long series of experiments, beginning with Miescher's discovery ('74) that isolated nucleinic acid forms green insoluble salts with methyl-green, and continued by Lilienfeld, Heidenhain, Paul Mayer, and others, gives strong reason to believe that beyond the physical imbibition of colour a true chemical union takes place, which, with due precautions, gives us at least a rough test of the chemical conditions existing in the cell.[1]

Second, *similarity of staining-reaction is by no means always indicative of chemical similarity*, as is shown, for example, by the fact that in cartilage both nuclei and inter-cellular matrix are intensely stained by methyl-green, though chemically they differ very widely.

Third, colour in itself gives no evidence of chemical nature; for the nucleus and other elements of the same cell may be stained red, green, or blue, according to the dye employed, and to class them as "erythrophilous," "cyanophilous," and the like, is therefore absurd.

Fourth, *the character of the staining-reaction is influenced and in some cases determined by the fixation or other preliminary treatment*, a principle made use of practically in the operations of mordaunting, but one which may give very misleading results unless carefully controlled. Thus Rawitz ('95) shows that certain colours which ordinarily stain especially the nucleus (saffranin, gentian-violet), can be made to stain only the cytoplasm through preliminary treatment of object with solutions of tannin, followed by tartar-emetic. In like manner Mathews ('98) shows that many of the "nuclear" tar-colours (saffranin, methyl-green, etc.) stain or do not stain the cytoplasm, according as the material has been previously treated with alkaline or with acid solutions.

The results with which we now have to deal are based mainly upon experiments with tar-colours ("aniline dyes"). Ehrlich ('79) long since characterized these dyes as "acid" or "basic," according as the colouring matter plays the part of an acid or a base in the compound employed, showing further that, other things equal, the basic dyes (methyl-green, saffranin, etc.) are especially "nuclear stains" and the acid (rubin, eosin, orange, etc.) "plasma stains." Malfatti ('91), and especially Lilienfeld ('92, '93), following out Miescher's earlier work ('74), found that albumin stains preëminently in the acid stains, nucleinic acid only in the basic; and, further, that artifi-

[1] *Cf.* Mayer, '91, '92, '97; Lilienfeld, '93; Mathews, '98.

cial nucleins, prepared by combining egg-albumin with nucleinic acid in various proportions, show a varying affinity for basic and acid dyes according as the nucleinic acid is more or less completely saturated with albumin. Lilienfeld's starting-point was given by the results of Kossel's researches on the relations of the nuclein group, which are expressed as follows:[1]—

Nucleo-proteid (1% of P or less),
by peptic digestion splits into

Peptone — *Nuclein* (3–4% P),
by treatment with acid splits into

Albumin — *Nucleinic acid* (9–10% P),
heated with mineral acids splits into

Phosphoric acid — *Nuclein bases* (adenin, guanin, etc.). — (*A carbohydrate.*)

Now, according to Kossel and Lilienfeld, the principal nucleoproteid in the nucleus of leucocytes is *nucleo-histon*, containing about 3% of phosphorus, which may be split into a form of *nuclein* playing the part of an acid, and an albuminoid base, the *histon* of Kossel; the nuclein may in turn be split into albumin and nucleinic acid. These four substances — albumin, nucleo-histon, nuclein, nucleinic acid — thus form a series in which the proportion of phosphorus, which is a measure of the nucleinic acid, successively increases from zero to 9–10%. If the members of this series be treated with the same mixture of red acid fuchsin and basic methyl-green, the result is as follows. Albumin (egg-albumin) is stained red, nucleo-histon greenish blue, nuclein bluish green, nucleinic acid intense green. "We see, therefore, that the principle that determines the staining of the nuclear substances is always the nucleinic acid. All the nuclear substances, from those richest in albumin to those poorest in it, or containing none, assume the tone of the nuclear (*i.e.* basic) stain, but the combined albumin modifies the green more or less toward blue."[2] Lilienfeld explains the fact that chromatin in the cell-nucleus seldom appears pure green on the assumption, supported by many facts, that the proportions of nucleinic acid and albumin vary with different physiological conditions, and he suggests further that the intense staining-power of the chromosomes during mitosis is probably due to the fact that they contain a maximum of nucleinic acid. Very interesting is a comparison of the foregoing staining-reactions with those given by a mixture of a *red basic* dye (saffranin) and a *green acid* one ("light green"). With this combination an effect is given which reverses that of the Biondi-Ehrlich mixture; *i.e.* the nuclein

[1] From Lilienfeld, after Kossel ('92, p. 129).

[2] *l.c.*, p. 394.

is coloured red, the albumin green, which is a beautiful demonstration of the fact that staining-reagents cannot be logically classified according to colour, but only according to their chemical nature, and gives additional ground for the view that staining-reactions of this type are the result of a chemical rather than a merely physical combination.

These results must be taken with some reserve for the following reasons: Mathews ('98) has shown that methyl-green and other basic dyes will energetically stain albumose, coagulated egg-albumin, and the cell-cytoplasm in or after treatment by alkaline fluids; while conversely the acid dyes do not stain, or only slightly stain, these substances under the same conditions. This probably does not affect the validity of Heidenhain's results,[1] since he worked with acid solutions. What is more to the point is the fact that hyaline cartilage and mucin, though containing no nucleinic acid, stain intensely with basic dyes. Mathews probably gives the clue to this reaction, in the suggestion that it is here probably due to the presence of other acids (in the case of cartilage a salt of chondroitin-sulphuric acid, according to Schmiedeberg); from which Mathews concludes that the basic dyes will, in acid or neutral solutions, stain any element of the tissues that contains an organic acid in a salt combination with a strong base.[2] Accepting this conclusion, we must therefore recognize that, as far as the cytoplasm is concerned, the basic or "nuclear" stains are in no sense a test for nuclein, but only for salts of organic acids in general. In case of the nucleus, however, we know from direct analysis that we are dealing with varying combinations of nucleinic acid, and hence, with the precautions indicated above, may draw provisional conditions from the staining-reactions.

Thus regarded, the changes of staining-reaction in the chromatin are of high interest. Heidenhain ('93, '94), in his beautiful studies on leucocytes, has correlated some of the foregoing results with the staining-reactions of the cell as follows. Leucocytes stained with the Biondi-Ehrlich mixture of acid fuchsin and methyl-green show the following reactions. Cytoplasm, centrosome, attraction-sphere, astral rays, and spindle-fibres are stained pure red. The nuclear substance shows a very sharp differentiation. The chromatic network and the chromosomes of the mitotic figure are green. The linin-substance and the true nucleoli or plasmosomes appear red, like the cytoplasm. The linin-network of leucocytes is stated by Heidenhain to consist of two elements, namely, of red granules or microsomes suspended in a colourless network. The latter alone is called "linin" by Heidenhain. To the red granules is applied the term "oxychromatin," while the green substance of the ordinary chromatic network,

[1] See below.

[2] '98, pp. 451–452.

forming the "chromatin" of Flemming, is called "basichromatin."[1] Morphologically, the granules of both kinds are exactly alike,[2] and in many cases the oxychromatin-granules are found not only in the "achromatic" nuclear network, but also intermingled with the basichromatin-granules of the chromatic network. Collating these results with those of the physiological chemists, Heidenhain concludes that basichromatin is a substance rich in phosphorus (*i.e.* nucleinic acid), oxychromatin a substance poor in phosphorus, and that, further, "basichromatin and oxychromatin are by no means to be regarded as permanent unchangeable bodies but may change their colour-reactions by combining with or giving off phosphorus." In other words, "the affinity of the chromatophilous microsomes of the nuclear network for basic and acid aniline dyes is regulated by certain physiological conditions of the nucleus or of the cell."[3]

This conclusion, which is entirely in harmony with the statements of Kossel and Halliburton quoted above, opens up the most interesting questions regarding the periodic changes in the nucleus. The staining-power of chromatin is at a maximum when in the preparatory stages of mitosis (spireme-thread, chromosomes). During the ensuing growth of the nucleus it always diminishes, suggesting that a combination with albumin has taken place. This is illustrated in a very striking way by the history of the egg-nucleus or germinal vesicle, which exhibits the nuclear changes on a large scale. It has long been known that the chromatin of this nucleus undergoes great changes during the growth of the egg, and several observers have maintained its entire disappearance at one period. Rückert first carefully traced out the history of the chromatin in detail in the eggs of sharks, and his general results have since been confirmed by Born in the eggs of *Triton*. In the shark *Pristiurus*, Rückert ('92, 1) finds that the chromosomes, which persist throughout the entire growth-period of the egg, undergo the following changes (Fig. 157): At a very early stage they are small, and stain intensely with nuclear dyes. During the growth of the egg they undergo a great increase in size, and progressively *lose their staining-capacity*. At the same time their surface is enormously increased by the development of long threads which grow out in every direction from the central axis (Fig. 157, *A*). As the egg approaches its full size, the chromosomes rapidly diminish in size, the radiating threads disappear, and the staining-capacity increases (Fig. 157, *B*). They are finally again reduced to minute, intensely staining bodies which enter into the equatorial plate of the first polar, mitotic figure (Fig. 157, *C*). How great the change of volume is may be seen from the following figures. At the beginning the chromosomes measure, at most, 12 μ (about $\frac{1}{2000}$ in.) in length and

[1] '94, p. 543. [2] *l.c.*, p. 547. [3] *l.c.*, p. 548.

$\frac{1}{2}$ μ in diameter. At the height of their development they are almost eight times their original length and twenty times their original diameter. In the final period they are but 2 μ in length and 1 μ in diameter. These measurements show a change of volume so enormous, even after making due allowance for the loose structure of the large chromosomes, that it cannot be accounted for by mere swelling or shrinkage. The chromosomes evidently absorb a large amount of

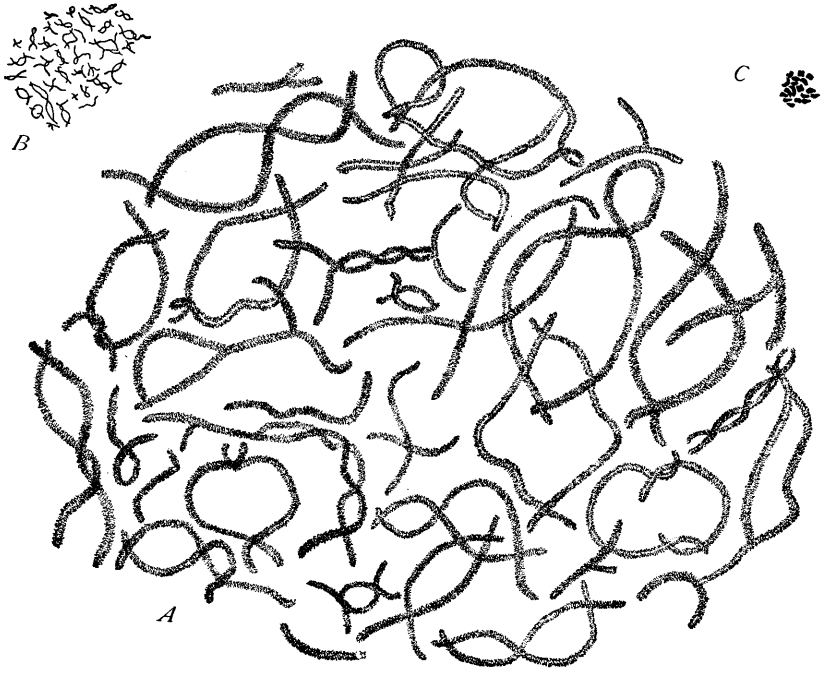

Fig. 157. — Chromosomes of the germinal vesicle in the shark *Pristiurus*, at different periods, drawn to the same scale. [RÜCKERT.]

A. At the period of maximal size and minimal staining-capacity (egg 3 mm. in diameter). *B*. Later period (egg 13 mm. in diameter). *C*. At the close of ovarian life, of minimal size and maximal staining-power.

matter, combine with it to form a substance of diminished staining-capacity, and finally give off matter, leaving an intensely staining substance behind. As Rückert points out, the great increase of surface in the chromosomes is adapted to facilitate an exchange of material between the chromatin and the surrounding substance; and he concludes that the coincidence between the growth of the chromosomes and that of the egg points to an intimate connection between the nuclear activity and the formative energy of the cytoplasm.

If these facts are considered in the light of the known staining-reaction of the nuclein series, we must admit that the following conclusions are something more than mere possibilities. We may infer that the original chromosomes contain a high percentage of nucleinic acid; that their growth and loss of staining-power is due to a combination with a large amount of albuminous substance to form a lower member of the nuclein series, probably a nucleo-proteid; that their final diminution in size and resumption of staining-power is caused by a giving up of the albumin constituent, restoring the nuclein to its original state as a preparation for division. The growth and diminished staining-capacity of the chromatin occurs during a period of intense constructive activity in the cytoplasm; its diminution in bulk and resumption of staining-capacity coincides with the cessation of this activity. This result is in harmony with the observations of Schwarz and Zacharias on growing plant-cells, the percentage of nuclein in the nuclei of embryonic cells (meristem) being at first relatively large and diminishing as the cells increase in size. It agrees further with the fact that of all forms of nuclei those of the spermatozoa, in which growth is suspended, are richest in nucleinic acid, and in this respect stand at the opposite extreme from the nuclei of the rapidly growing egg-cell.

Accurately determined facts in this direction are still too scanty to admit of a safe generalization. They are, however, enough to indicate the probability that chromatin passes through a certain cycle in the life of the cell, the percentage of albumin or of albumin-radicals increasing during the vegetative activity of the nucleus, decreasing in its reproductive phase. In other words, a combination of albumin with nuclein or nucleinic acid is an accompaniment of constructive metabolism. As the cell prepares for division, the combination is dissolved and the nuclein-radicle or nucleinic acid is handed on by division to the daughter-cells. A tempting hypothesis, suggested by Mathews on the basis of Kossel's work, is that nuclein, or one of its constituent molecular groups, may in a chemical sense be regarded as the formative centre of the cell which is directly involved in the process by which food-matters are built up into the cell-substance. Could this be established, we should have not only a clear light on the changes of staining-reactions during the cycle of cell-life, but also a clue to the nuclear "control" of the cell through the process of synthetic metabolism. This hypothesis fits well with the conclusions of other physiological chemists that the nucleus is especially concerned in synthetic metabolism. Kossel concludes that the formation of new organic matter is dependent on the nucleus,[1] and that nuclein in some manner plays a leading *rôle* in this process; and he makes

[1] Schiefferdecker and Kossel, *Gewebelehre*, p. 57.

some interesting suggestions regarding the synthesis of complex organic matters in the living cell with nuclein as a starting-point. Chittenden, too, in a review of recent chemico-physiological discoveries regarding the cell, concludes: "The cell-nucleus may be looked upon as in some manner standing in close relation to those processes which have to do with the formation of organic substances. Whatever other functions it may possess, it evidently, through the inherent qualities of the bodies entering into its composition, has a controlling power over the metabolic processes in the cell, modifying and regulating the nutritional changes" ('94).

These conclusions, in their turn, are in harmony with the hypothesis advanced twenty years ago by Claude Bernard ('78), who maintained that the cytoplasm is the seat of destructive metabolism, the nucleus the organ of constructive metabolism and organic synthesis, and insisted that the *rôle* of the nucleus in nutrition gives the key to its significance as the organ of development, regeneration, and inheritance.[1]

B. Physiological Relations of Nucleus and Cytoplasm

How nearly the foregoing facts bear on the problem of the morphological formative power of the cell is obvious; and they have in a measure anticipated certain conclusions regarding the *rôle* of nucleus and cytoplasm, which we may now examine from a somewhat different point of view.

Brücke long ago drew a clear distinction between the chemical and molecular composition of organic substances, on the one hand, and, on the other hand, their definite grouping in the cell by which arises *organization* in a morphological sense. Claude Bernard, in like manner, distinguished between *chemical synthesis*, through which organic matters are formed, and *morphological synthesis*, by which they are built into a specifically organized fabric; but he insisted that these two processes are but different phases or degrees of the same phenomenon, and that both are expressions of the nuclear activity. We have now to consider some of the evidence that the power of morphological, as well as of chemical, synthesis centres in the nucleus, and that this is therefore to be regarded as the especial organ of inheritance. This evidence is mainly derived from the comparison of nucleated and non-nucleated masses of protoplasm; from the form,

[1] "Il semble donc que la cellule qui a perdu son *noyau* soit stérilisée au point de vue de la génération, c'est à dire de la synthèse morphologique, et qu'elle le soit aussi au point de vue de la synthèse chimique, car elle cesse de produire des principes immediats, et ne peut guère qu'oxyder et détruire ceux qui s'y étaient accumulés par une élaboration antérieure du noyau. Il semble donc que le *noyau* soit le *germe* de nutrition de la cellule; il attire autour de lui et élabore les matériaux nutritifs" ('78, p. 523).

position, and movements of the nucleus in actively growing or metabolizing cells; and from the history of the nucleus in mitotic cell-division, in fertilization, and in maturation.

1. *Experiments on Unicellular Organisms*

Brandt ('77) long since observed that enucleated fragments of *Actinosphærium* soon die, while nucleated fragments heal their wounds and continue to live. The first decisive comparison between nucleated and non-nucleated masses of protoplasm was, however, made by Moritz Nussbaum in 1884 in the case of an infusorian, *Oxytricha*. If one of these animals be cut into two pieces, the subsequent behaviour of the two fragments depends on the presence or absence of the nucleus or a nuclear fragment. The nucleated fragments quickly heal the wound, regenerate the missing portions, and thus produce a perfect animal. On the other hand, enucleated fragments, consisting of cytoplasm only, quickly perish. Nussbaum therefore drew the conclusion that the nucleus is indispensable for the formative energy of the cell. The experiment was soon after repeated by Gruber ('85) in the case of *Stentor*, another infusorian, and with the same result (Fig. 159). Fragments possessing a large fragment of the nucleus completely regenerated within twenty-four hours. If the nuclear fragment were smaller, the regeneration proceeded more slowly. If no nuclear substance were present, no regeneration took place, though the wound closed and the fragment lived for a considerable time. The only exception — but it is a very significant one — was the case of individuals in which the process of normal fission had begun; in these a non-nucleated fragment in which the formation of a new peristome had already been initiated healed the wound and completed the formation of the peri-

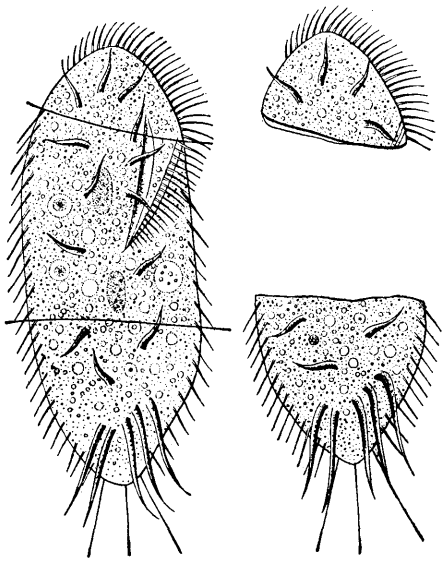

Fig. 158. — *Stylonychia*, and enucleated fragments. [VERWORN.]

At the left an entire animal, showing planes of section. The middle piece, containing two nuclei, regenerates a perfect animal. The enucleated pieces, shown at the right, swim about for a time, but finally perish.

stome. Lillie ('96) has recently found that *Stentor* may by shaking be broken into fragments of all sizes, and that nucleated fragments as small as $\frac{1}{27}$ the volume of the entire animal are still capable of complete regeneration. All non-nucleated fragments perish.

These studies of Nussbaum and Gruber formed a prelude to more extended investigations in the same direction by Gruber, Balbiani, Hofer, and especially Verworn Verworn ('88) proved that in *Polystomella*, one of the Foraminifera, nucleated fragments are able to

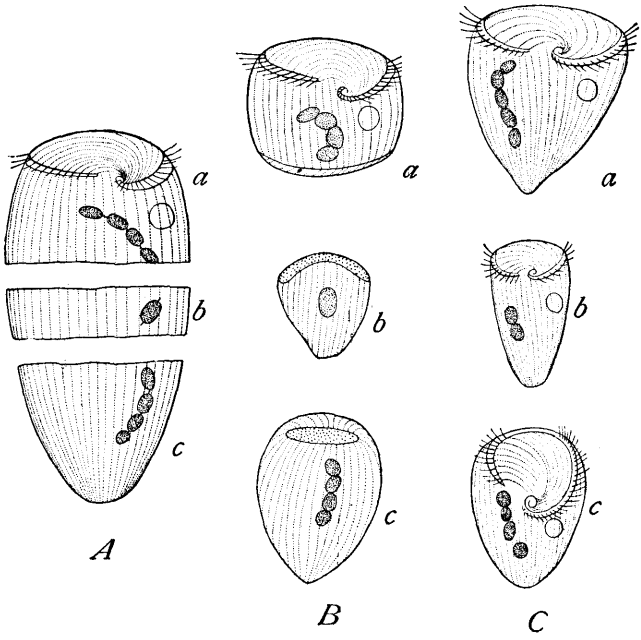

Fig. 159. — Regeneration in the unicellular animal *Stentor*. [From GRUBER after BALBIANI.]

A. Animal divided into three pieces, each containing a fragment of the nucleus. *B*. The three fragments shortly afterward. *C*. The three fragments after twenty-four hours, each regenerated to a perfect animal.

repair the shell, while non-nucleated fragments lack this power. Balbiani ('89) showed that although non-nucleated fragments of Infusoria had no power of regeneration, they might nevertheless continue to live and swim actively about for many days after the operation, the contractile vacuole pulsating as usual. Hofer ('89), experimenting on *Amœba*, found that non-nucleated fragments might live as long as fourteen days after the operation (Fig. 160). Their movements continued, but were somewhat modified, and little by little ceased, but the pulsations of the contractile vacuole were but slightly affected; they lost more or less completely the capacity to

digest food, and the power of adhering to the substratum. Nearly at the same time Verworn ('89) published the results of an extended comparative investigation of various Protozoa that placed the whole matter in a very clear light. His experiments, while fully confirming the accounts of his predecessors in regard to regeneration, added many extremely important and significant results. Non-nucleated fragments both of Infusoria (*e.g. Lachrymaria*) and rhizopods (*Polystomella*, *Thalassicolla*) not only live for a considerable period, but perform perfectly normal and characteristic movements, show the same susceptibility to stimulus, and have the same power of ingulfing food, as the nucleated fragments. *They lack, however, the power of digestion and secretion.* Ingested food-matters may be slightly altered, but are never completely digested. The non-nucleated fragments are unable to secrete the material for a new shell (*Polysto-*

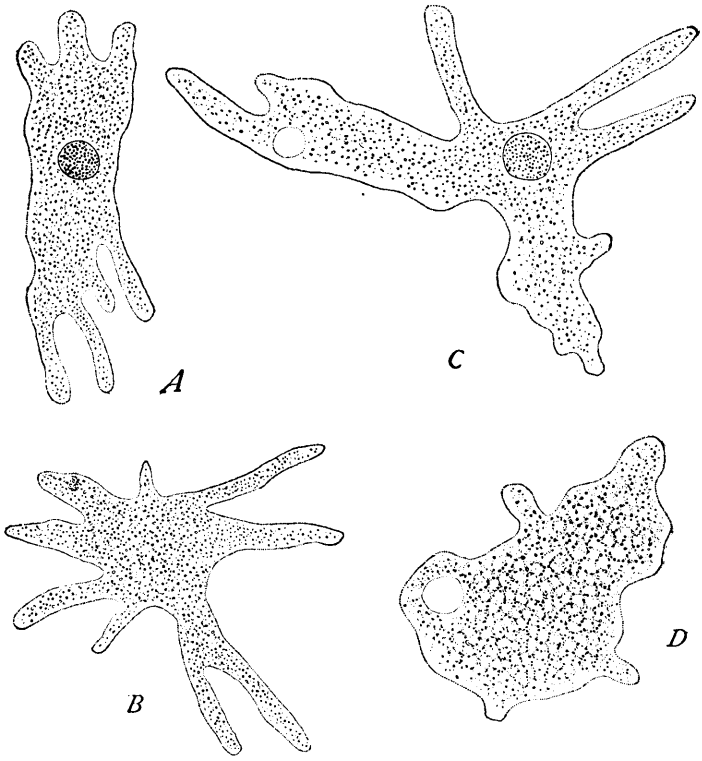

Fig. 160. — Nucleated and non-nucleated fragments of *Amœba*. [HOFER.]

A. B. An *Amœba* divided into nucleated and non-nucleated halves, five minutes after the operation. *C. D.* The two halves after eight days, each containing a contractile vacuole.

mella) or the slime by which the animals adhere to the substratum (*Amœba*, *Difflugia*, *Polystomella*). Beside these results should be placed the well-known fact that dissevered nerve-fibres in the higher animals are only regenerated from that end which remains in connection with the nerve-cell, while the remaining portion invariably degenerates.

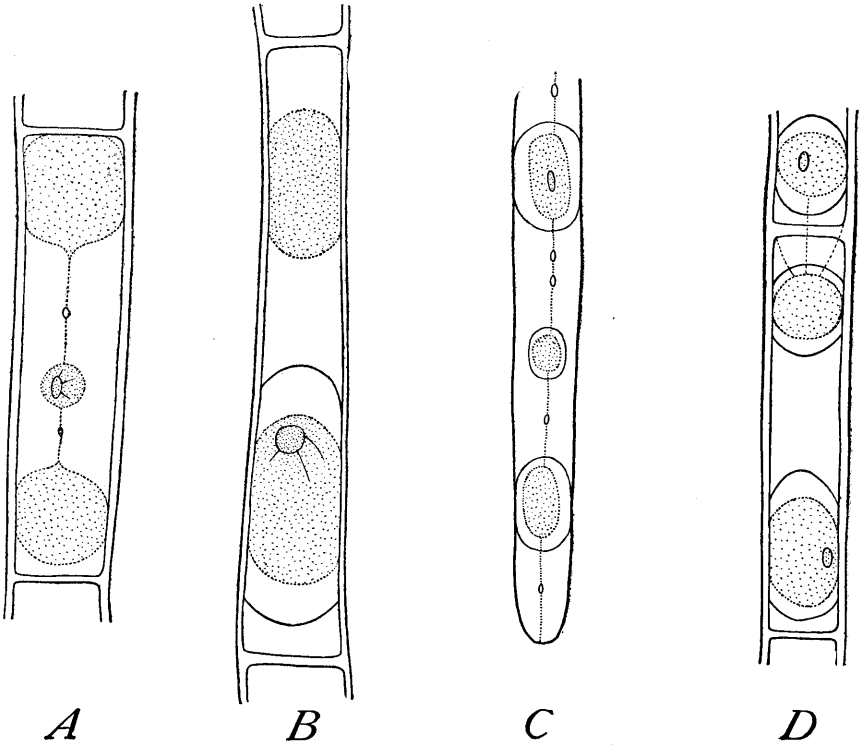

Fig. 161. — Formation of membranes by protoplasmic fragments of plasmolyzed cells. [TOWNSEND.]

A. Plasmolyzed cell, leaf-hair of *Cucurbita*, showing protoplasmic balls connected by strands. *B*. Calyx-hair of *Gaillardia;* nucleated fragment with membrane, non-nucleated one naked. *C*. Root-hair of *Marchantia;* all the fragments, connected by protoplasmic strands, have formed membranes. *D*. Leaf-hair of *Cucurbita;* non-nucleated fragment, with membrane, connected with nucleated fragment of adjoining cell.

These beautiful observations prove that destructive metabolism, as manifested by coördinated forms of protoplasmic contractility, may go on for some time undisturbed in a mass of cytoplasm deprived of a nucleus. On the other hand, the building up of new chemical or morphological products by the cytoplasm is only initiated in the presence of a nucleus and soon ceases in its absence. These facts form a complete demonstration that the nucleus plays an essential