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STUDIES ON CELLS: MORPHOLOGY, CHEMICAL CONSTITUTION, AND DISTRIBUTION OF BIOCHEMICAL FUNCTIONS*

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IN 1827 Giovanni Battista Amici, Italian mathematician and astronomer from Modena, came to Paris to demonstrate the microscope that he had just perfected. All those interested in natural sciences went to examine the new instrument and, according to Dutrochet,[1] were considerably impressed. A few weeks later Amici was in London, demonstrating his microscope, among others, to Robert Brown, the man who four years later was to discover the cell nucleus. Soon thereafter the leading microscopists of Europe were in possession of one of Amici's microscopes, or one constructed after his specifications. Amici had finally succeeded in correcting to a large extent the spherical and chromatic aberrations of microscopic lenses. The morphological details in plant and animal tissues were no longer blurred, hopelessly merging as in the old instruments, but appeared sufficiently well defined to convince microscopists that tissues were composed of an ever repeating unit, which has come to be known as the cell.

I have recalled these facts to point out that it is really to Amici and to a relatively slight improvement brought about in the resolving power of the microscope that we owe the concept of the cell as the basic unit of living matter. Had the microscope been somewhat better from the start, the founder of the cell theory might well have been Leeuwenhoeck himself, or Robert Hooke. In the history of cytology it is repeatedly found that further advance had to await, as in the case just mentioned, the accident of technical progress. I hope that I will be forgiven therefore if, in the main part of the

*Lecture delivered January 15, 1948.

presentation that follows, methods and results will be equally emphasized.

Between 1835, with the work of Schleiden and Schwann, and 1860, at the time of Virchow, the principle of the cell theory was defined and firmly established. At the time, however, the cell theory was a wholly morphological concept. It could hardly have been otherwise since chemistry itself was in its infancy and the chemical inventory of living tissues had not started. Indeed it was in 1827, the year of Amici's trip to Paris and London, that Wöhler synthesized urea and opened the field of organic chemistry. What the cell theory did, however, was of great practical significance: it indicated that all the essentials of the living processes were to be found within the narrow boundaries of the cell wall.

One hundred years or so later we find organic chemistry unbelievably wealthy, a long way from the single jar of urea on the shelf. Biochemistry is flourishing and we have gained the assurance that it will be possible, soon or late, to explain cellular life in terms of physics and chemistry. We should not be too hasty in our hope, however, since an important field of cell biology, that dealing with the relation between forms and functions, has hardly been explored. In living cells chemical reactions are not permitted to follow their course down-hill as they would *in vitro,* soon to reach an equilibrium and death, but they are constantly slowed down, inhibited, and re-loaded with energy so that the entire process can again start anew. The maintenance and interplay of such chemical cycles is insured by the existence, in cells, of diverse and evidently specialized structures, and it is in the molecular arrangement of these structures that resides the unique attribute of living matter. If we intend to understand the mechanism of biological processes, therefore, increased attention must be paid to the chemical constitution and the spatial arrangement of the various cell structures, and in that study the application of biochemistry and morphology must proceed hand in hand.

An animal cell is composed of a nucleus, itself containing nucleoli and chromosomes, of mitochondria, one or more centrosomes, and fat or Golgi bodies. This at least is what the light microscope is able

to reveal. The elements just mentioned are found in every active cell and it is apparent that they constitute the indispensable complement of protoplasm. In addition there may be encountered, depending on the type of differentiation or the state of the cell, a variety of elements such as secretory or zymogen granules, pigment granules, or stored products which may have derived from special cellular activity.

How can we get to these cellular elements, microscopic in size, to determine their chemical composition and investigate their biochemical activities? Since Miescher, attempts have been made to apply analytical methods to the study of cell structures and to isolate known cell components mechanically, or through mild chemical manipulations. Separation of nuclei by Miescher[2] led to the discovery of nucleic acids and the identification of certain basic proteins. In 1913, Warburg segregated, but did not isolate, cytoplasmic granules by centrifugation and was able to show that these were responsible for most of the oxygen uptake of cell-free extracts of guinea pig liver.[3] In 1934, Bensley and Hoerr succeeded in concentrating, also by centrifugation, large cellular elements which they identified as mitochondria.[4] In 1938, submicroscopic components of the ground substance referred to later under the term microsomes were isolated in our laboratory, by means of centrifugation at high speed.[5] These results widely scattered over 60 years of microscopic research indicated that, given the proper equipment and a systematic application of the method of centrifugation, it should be possible to separate the various cell components by mechanical means.

The present paper deals with an attempt to characterize and, if possible, to isolate, the elementary constituents of cells and is primarily concerned with the work carried out in our laboratory at the Rockefeller Institute during the past ten years. In the presentation of the results it would be difficult to separate the biochemical work from the morphological observations since the microscope has constantly served as a guide or a check for the chemical and biochemical studies. For the sake of clarity, however, and whenever possible, an effort will be made to present and discuss the analytical data first, taking up later the evidence derived from microscopical observations.

Fig. 1 represents a group of cells, unfixed and unstained.* In the center of these cells the clear area corresponds to the nucleus. Between the nucleus and the cell wall the cytoplasmic area appears filled with relatively large elements, rod-shaped or spherical, representing, for a large part at least, the inclusions usually referred to as mitochondria. The cells shown are liver cells, a material convenient and often used in the type of experiments to be described.

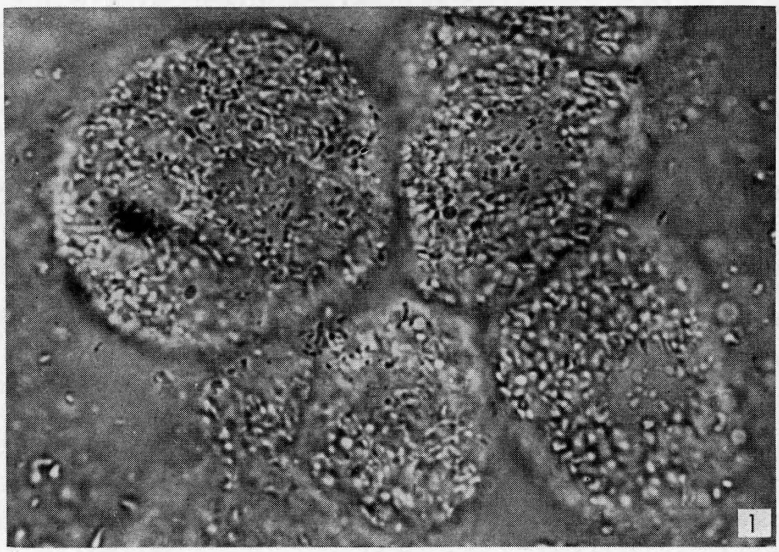

PLATE 1

FIG. 1. *Fresh Liver Cells.* Preparation obtained by dispersing rat liver tissue in 0.88 M sucrose solution, and examining between glass slide and coverslip. Micrograph of cells taken under oil immersion at (1000 x), and enlarged to (1500 x).

Suppose that many of these cells are ground together for only a few minutes. The shearing effect produced at the surface of the cells is sufficient, it is found, to break the cell membrane while most of the nuclei, presumably because of a more resistant wall, are left apparently intact. If the tissue mass is now suspended in a fluid such as an isotonic and neutral salt solution, or a somewhat hypertonic sucrose solution, the various cell constituents will disperse and, under the microscope, nuclei and mitochondria will be seen floating

* This illustration was kindly prepared by Dr. G. E. Palade.

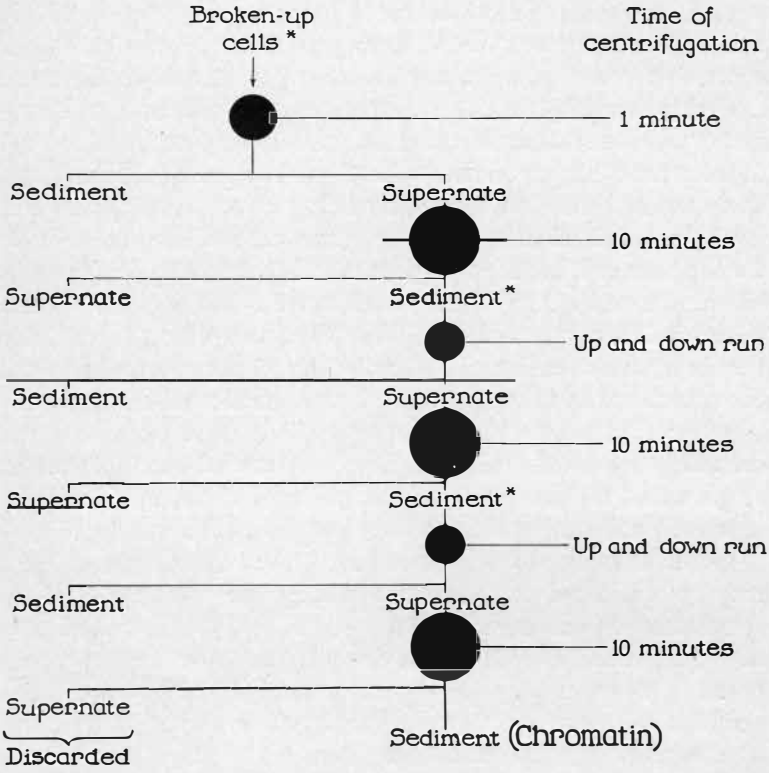

PLATE 2

Fig. 2. Chromatin threads from resting nuclei, isolated by differential centrifugation at (1500 x) g. Diagram of procedure.

freely in the medium. Nuclei are much larger than mitochondria or secretory granules; in turn the latter are much larger than the elements next in size, the microsomes. Because of this disparity in sizes the various cell elements may be expected to sediment at various

speeds under gravity or centrifugal force and, consequently, to segregate at different levels according to their particular size and density. After centrifugation for the appropriate time and speed, the cell elements are now found arranged in the centrifuge tube in successive layers with the nuclei at the bottom, above the nuclei the mitochrondria, then the microsomes, and above these the elements of even smaller size, and the soluble components of the cell. If we started our experiment with 50 or 100 grams of liver tissue, there would be no difficulty in obtaining an ample supply of each of the isolated cell components for chemical and biochemical tests. In this manner it has been possible to isolate currently, in the course of one afternoon, 2 to 3 grams of chromatin, 5 to 6 grams of mitochondria. These quantities should not seem surprising if we realize that in mammalian liver the nuclei represent about 5 to 6 per cent, the mitochondria as much as 15 per cent, of the cell mass. In practice, the cellular elements are not segregated in a single tube but the centrifugation of the tissue suspension is carried out in successive stages and at various speeds, so that each type of element is collected separately and subjected individually to the proper washing.

Once the nuclei have been removed, the remaining suspension may be considered to contain, essentially, the constituents of the cytoplasm.[6] In our experimental work this portion, referred to as the "cytoplasmic extract," is routinely fractionated into three main portions, namely: 1) a *large granule* fraction composed of mitochondria and elements of similar size; 2) a *microsome* fraction, and, 3) a *supernate* fraction containing elements of relatively small molecular weight, after the two preceding fractions have been removed.[6, 7, 8, 9]

It should be pointed out that division of the cell in this manner is not arbitrary and is not based on size differences alone since, as it will be shown later, these various fractions are also distinct in chem-

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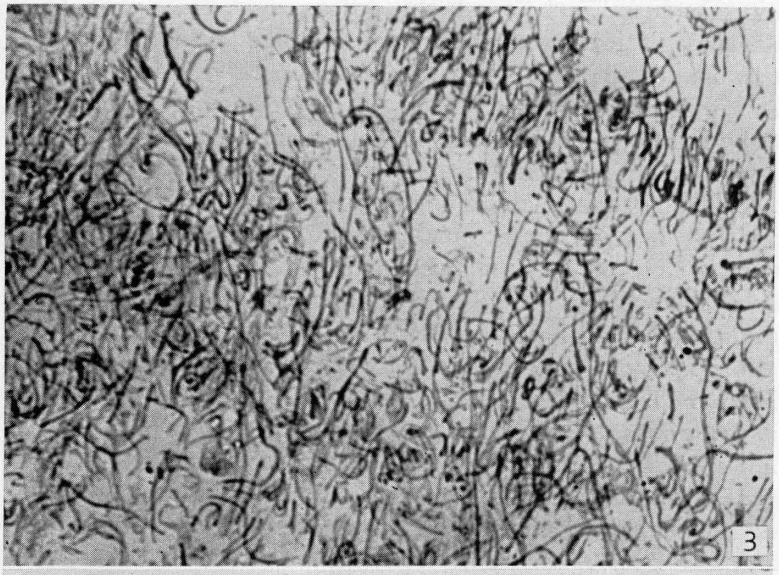
PLATE 3 —→

Isolation of chromatin strands from resting Nuclei.

FIG. 3. The micrograph shows paired, stretched, or broken up strands of chromatin. (1600 x).

FIG. 4. In this preparation, the dried smear of chromatin was treated with chloroform before staining. The chromatin strands, some of them paired, exhibit a beaded, or banded appearance (1600 x). Both preparations were stained by the Feulgen technique.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ical constitution, in biochemical functions, and even in color. Thus, the nuclear fraction is generally snow-white, the mitochondrial fraction usually yellow-brown, and the microsomes red or colorless. It should not be inferred, however, that each of these fractions, especially the large granules and the microsomes, are composed of a single kind of element. It is clear that the homologues of the large granules cannot be identical in organs as dissimilar as the liver, the pancreas, or the adrenal cortex. Likewise, it will be found, I think, that the large granule or mitochondrial fraction of a given organ, let us say the liver, consists of a mixed population of differentiated elements, each with specialized biochemical functions, a situation which would account for the diversified activities normally exhibited by the liver cell. There is evidence also that the microsomes of different organs are not identical. Up to the present stage of this investigation, however, it has been found preferable not to dwell on individual differences but to emphasize first what similar cell constituents have in common, and in this way bring out some of the properties which may have general and fundamental significance.

ISOLATION OF CHROMATIN STRANDS

I have already indicated that, as a rule, the membrane of cells can be broken readily, and that the cell components so liberated can be separated and purified by means of differential centrifugation at appropriate speeds. Experimental conditions can be arranged whereby the nuclear membrane likewise can be caused to break, in the process releasing the nuclear components in the medium, together with the cytoplasmic content. This can be achieved, for example, by grinding the tissue in the presence of sand of definite grain size.* Fig. 2 summarizes the method which was worked out, and which led to the isolation of chromatin strands from the resting nucleus of lymphoid cells.

* In obtaining chromatin strands from resting nuclei two factors, not emphasized in our original paper, are of the greatest importance, namely, 1) the size of the grains of sand used and 2) the amount of fluid present during grinding. The size of sand particles should be as close as possible to that of the nuclei themselves: Flint grade "0000" as provided by the Charles B. Crystal Co., New York, has been found satisfactory. Sand or Flint should be added dry, or with just enough fluid to moisten it slightly. The fluid required for suspension should be added, dropwise at first, at the end grinding. If the sand is too coarse, or if the mixture is too fluid, most of the nuclei may escape injury.

TABLE I

Chemical Composition of Chromatin

SOURCE OF MATERIAL	EXPERIMENT NO.	CHROMATIN	N	P	C	H	S	Cl (FROM SALINE)
			per cent	*per cent*	*per-cent*	*per cent*	*per cent*	*per cent*
Rat leukemia	4	Complete	15.30	3.79				
	4	Lipoid-free	15.62	3.83	43.88	6.61	1.62	
Mouse leukemia	7	Complete	15.79	3.70	46.00	6.41		0.52
	8	Complete	15.59	3.56	46.94	6.46	1.78	0.08
	9	Nuclei present	14.74	3.16	45.85	6.28	1.62	0.13

The material isolated in this manner is a snow-white mass composed of slender strands, often paired, and occasionally beaded, as shown in Figs. 3 and 4. These elements contain about 3.7 per cent phosphorus (Table I) and chemical tests indicate that as much as 40 per cent of their mass is represented by nucleic acid of the desoxyribose type. Evidence has been presented to show that the material consists of chromatin, and that the isolated chromatin strands are morphologically related to chromosomes.

The work concerned with the isolation of chromatin strands from resting nuclei was carried out in collaboration with J. S. Potter, of the Carnegie Institution. The results were first presented in 1941,[10, 11] and have been confirmed by Mirsky and Pollister,[12] Marshak and Walker,[13] Gopal-Ayengar,[14] and others. The method, with but slight modifications, was adopted by Mirsky and associates[15] who have since applied it to the isolation of nucleoproteins, and the study of the constitution of chromosomes.

ISOLATION OF MELANIN GRANULES[16]

In tissue extracts elements of nearly the same size, but of different constitution, can nevertheless be separated readily by means of differential centrifugation if they happen to vary sufficiently in density. This is the case for the melanin granules which occur in abundance in amphibian livers (Fig. 5). These elements are slightly elongated, measuring approximately 0.5 by 1.0μ, and in size and shape are in the range of the so-called liver large granules; their greater density, and to some extent their failure to absorb water, causes them to sediment ahead of the large granules so that their separation is possible, and their purification greatly facilitated.[10] Isolated melanin granules from amphiuma liver, as seen in the light, and the electron microscopes are illustrated in Figs. 6 and 7, respectively. Isolation of melanin granules from a mouse melanoma offered greater diffi-

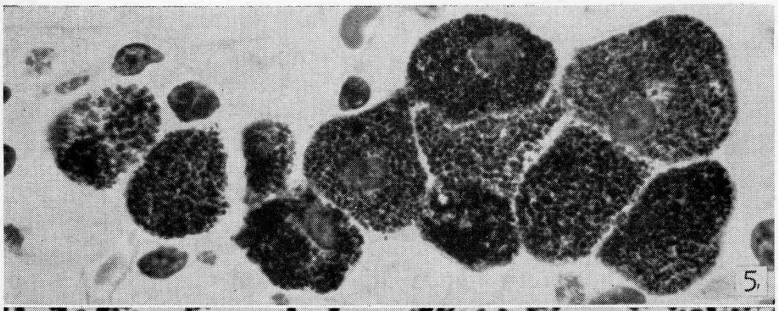
PLATE 4 →

Isolation of Melanin Granules from Amphiuma Liver.

FIG. 5. Section of Amphiuma Liver, showing cells filled with clusters of melanin granules. (375 x).

FIG. 6. Isolated melanin granules; unstained. Micrograph taken under oil immersion at (1000 x), and enlarged to (2000 x).

FIG. 7. Isolated melanin granules; unstained. Electron micrograph taken at (10,000 x), and enlarged to (20,000 x).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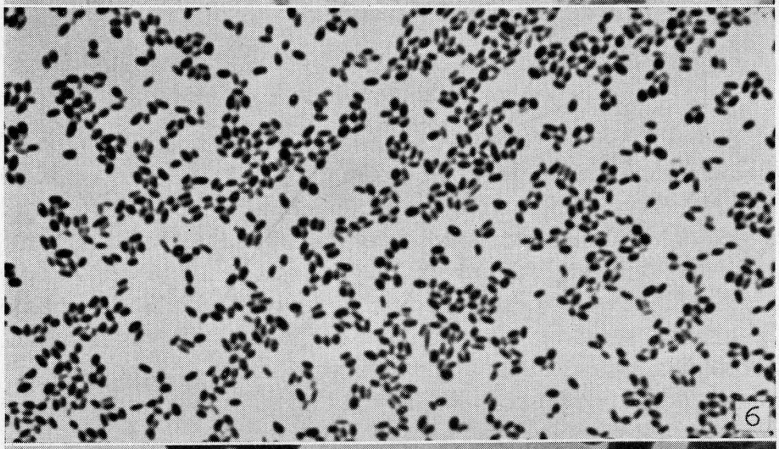
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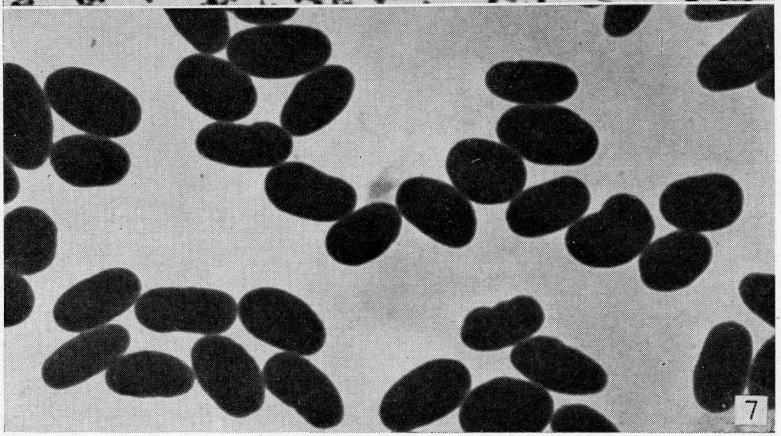
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culties because of smaller size and of a weight nearly that of the large granules of the cell.

The results of elementary analysis, shown in Table II, indicates that the melanin granules isolated are rather complex structures. The presence of phosphorus is doubtful. Characteristic features are the abundance of sulfur and an unusually high ash content, although the preparation, and repeated washing, of the granules was carried out in distilled water. Obviously more work will be needed to elucidate the constitution of these pigment granules, and the mode of their deposition in the cell.

COMPOSITION AND FUNCTIONS OF MITOCHONDRIA (LARGE GRANULES)

Every cell type so far investigated has yielded a fraction composed of elements in the approximate size range of 0.5 to 2.0μ in diameter and which have been referred to under the general term, large granules.[8, 9] The basic constituents of this fraction are mitochondria, elements which appear to be a constant part of active protoplasm. As already noted, the large granule fraction obtained from a variety of organs cannot be expected to be of uniform composition since it contains, in addition to mitochondria, secretory or zymogen granules in unknown proportion.[9] In the case of the liver, the proportion of these granules is apt to vary according to the time of feeding. Nevertheless, large granules of various origin have enough in common, chemically and biochemically, to be treated as a group of related elements. Mitochondria obtained from the undifferentiated cells of a lymphosarcoma on analysis were found to have an elementary composition not very different from that of the large granules from liver.[6]

The large granule fraction is made up of elements which are characterized by the presence of a limiting membrane, apparently semi-permeable, and highly sensitive to variation in the salt concentration of the medium.[8] Thus they behave like osmometers and can be observed to swell or shrink when the salt concentration is lowered or increased. The existence of a membrane has been demonstrated by electron microscopy.[16] When concentrated in the centrifuge, the large granules give a mass which is opaque, yellow-brown in the case of liver, of a lemon color in the case of pancreas.[8] Chemically,

TABLE II

Chemical Composition of Melanin Granules (Ash Free)

MELANIN GRANULES FROM	N	P	C	H	S	Fe	Cu	ASH	LIPIDS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Amphiuma Liver	11.80	0.09	50.00	4.66	2.16	0.24	0.02	11.6	2.1
Mouse Melanoma	11.60	0.42	53.00	7.00	1.05	0.02	—	—	—

the major constituents of the large granules are proteins, phospholipids to the extent of 25 per cent, ribose nucleic acid, ribose nucleotides, riboflavin, and probably many other compounds not yet identified.[8, 9] The most characteristic feature, however, is that they contain a number of enzymes or enzyme systems which are not found in other cytoplasmic fractions.[17] This fact, therefore, makes the large granules functionally distinct from the other elements of the cell.

The investigations dealing with the distribution of enzymatic activities, and now to be discussed, were carried out in collaboration with Drs. Rollin D. Hotchkiss, George H. Hogeboom, and W. C. Schneider.

Providing that the separation of the cell elements has been effective, a fact to be ascertained by suitable microscopical methods, the observations cannot take their full significance and indeed can be misleading, unless the chemical and biochemical tests are performed on a strictly quantitative basis and the values pertaining to each individual fraction are estimated in terms of the total capacity of the unfractionated cell, and that of the other cell portions. When Hotchkiss inquired by preliminary tests on the catalase activity of the microsome fraction, he noticed an impressive outburst and we could have concluded that the microsomes were characterized by notable catalase activity. When quantitative measurements were made, however, with the precautions indicated, it was found that the microsomes had a negligible portion, no more than one-half per cent, of the total activity possessed by the original liver extract.[17] This point needs to be emphasized because, in contrast to our own observations, other workers have occasionally reported that the microsomes were endowed with characteristic succinoxidase and cytochrome oxidase activity, and this has been adduced to ascribe to these submicroscopic elements special and important metabolic functions.[18, 19, 20]

In the fractionation procedure now considered, a method was adhered to whereby strict account was made of all the fractions and their respective washings, by volume and by dry weight. In this manner the amount of substance in each of the fractions, and their respective enzymatic activities, could be expressed in specific

values and also in per cent of the total substance, or the total activity, contained in the original cytoplasmic extract.[9] This procedure has been summarized diagramatically in Fig. 8.

The first enzyme investigated was d-amino-acid oxidase. The substrates used in these studies were the *d, l,* and *dl* forms of ala-

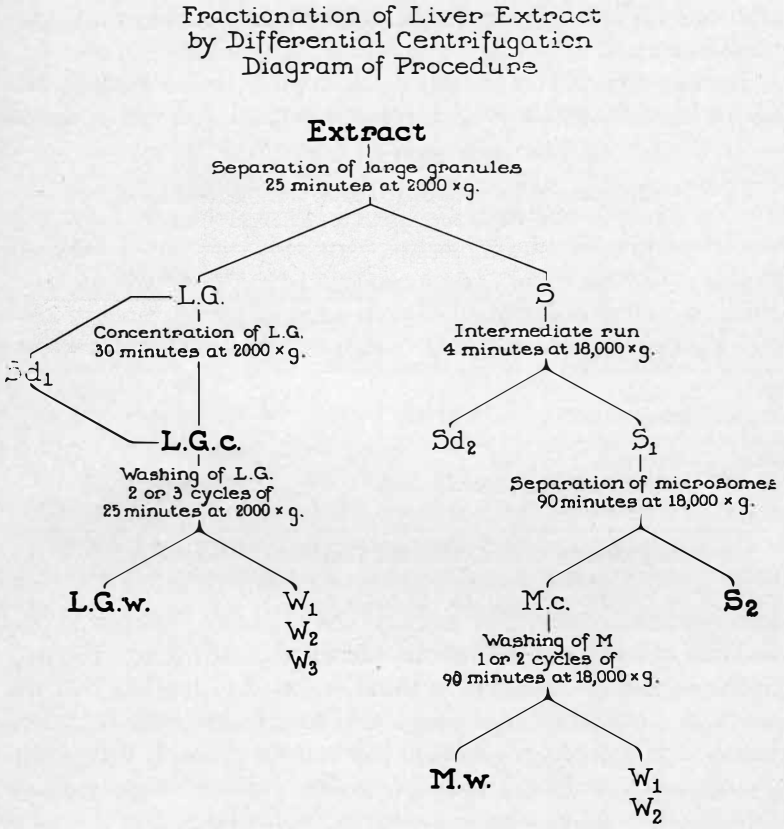

PLATE 5

FIG. 8. Fractionation of mammalian liver by means of differential centrifugation at various speeds. The diagram illustrates the procedure adhered to in fractionating liver extract into three main portions, namely, a) a *large granule* fraction (L.G.), consisting of mitochondria and secretory granules; b) a *microsome* fraction (M), composed of submicroscopic elements; and c) a *supernate* fraction (S_2), left after the two preceding fractions have been removed.

nine, leucine, and phenyl-alanine. The determination of enzyme activity was made by measurements of oxygen uptake in the manometric apparatus of Warburg, and confirmed by tests demonstrating the formation of ammonia. The results were equally clear cut, using guinea pig and rat liver, in that they demonstrated that d-amino acid oxidase activity was localized in the large granule fraction, while completely absent in the other portions of the cytoplasmic extract.[8, 17]

Further experiments in which the large granules were repeatedly washed showed a progressive and parallel decrease in amino

TABLE III

*Distribution of Cytochrome Oxidase and Succinoxidase in Cytoplasmic Extract of Rat Liver**

LIVER FRACTION		PER CENT RECOVERY	
		CYTOCHROME OXIDASE	SUCCINOXIDASE
E	Extract	(100)	(100.0)
L.G.c.	Large Granules (concentrated)	71	74.0
L.G.w.	Large Granules (washed)	70	52.0
M.w.	Microsomes (washed)	< 4	6.5
S_2	Supernate (after removal of L.G. and M.)	0	0

* According to Hogeboom, Claude, and Hotchkiss, *J. Biol. Chem.*, 1946, *165*, 615.

acid oxidase activity. The activity was restored, however, upon addition of co-factors in the form of heated yeast extract. The particular interest of these observations is that they indicate that the essential components of d-amino acid oxidase are both contained in the large granules but that one component, probably the protein moiety, is retained while the other is slowly diffusible, presumably through the semi-permeable membrane of the large granules. It is probable, however, that this *in vitro* effect is the result of some injury and that such escape does not occur normally in the intact living cell.[21]

Observations of a similar nature were made with respect to certain components of the cytochrome-linked enzyme system, namely, cytochrome oxidase, succinoxidase, and cytochrome c.[17, 22, 23, 24]

As shown in Table III, nearly 75 per cent of both succinoxidase and cytochrome oxidase activities were recovered with the large granule fraction and considerable concentration was achieved through washing.[22] Independent measurements by Schneider, following the same method of fractionation, are in close agreement with these findings.[23] These results have been supported by recent experiments concerned with the distribution of cytochrome c in the cell.[24] The tests were made through actual isolation of cytochrome c from the various cell fractions, and photometric determination of the amount present. A considerable proportion of the cytochrome c of the liver cell was found in association with the large granules. Tests of succinoxidase activity showed further that the cytochrome c present was biologically active, providing that the isolation of the granules had been conducted in isotonic saline.

Together, the preceding observations offer conclusive evidence to support the view that most, if not all, of cytochrome oxidase, succinoxidase and cytochrome c, three of the most important members of the respiratory system, are segregated in mitochondria, or in granules of the same type. These results seem to be in agreement with the early findings of Warburg who had observed that the large granules of guinea pig liver were responsible for most of the oxygen uptake of the cell-free extract.[3] These observations indicate that the power of respiration is not to be found in a diffused state in the cytoplasm, and that mitochondria may possibly be considered as the real power plants of the cell.

FRACTIONATION OF MITOCHONDRIA

So far, we have been concerned with the fractionation of the cell itself, and the separation of its most conspicuous elements. In the course of experimentation with isolated mitochondria certain observations were made which suggested that these elements might lend themselves to fractionation, thereby offering an opportunity to investigate further their morphology and their constitution.

As already noted, mitochondria are profoundly affected by changes in the salt concentration of the medium.[8] When mitochondria, previously washed in saline, are placed in distilled water, they swell enormously, in the manner of red cells under the same conditions, and finally disintegrate.

It has been found that the substance of the mitochondria, now dispersed, can be fractionated into three distinct portions, as follows: 1) particulate components of submicroscopic size which are found to carry practically all the ribose nucleic acid originally de-

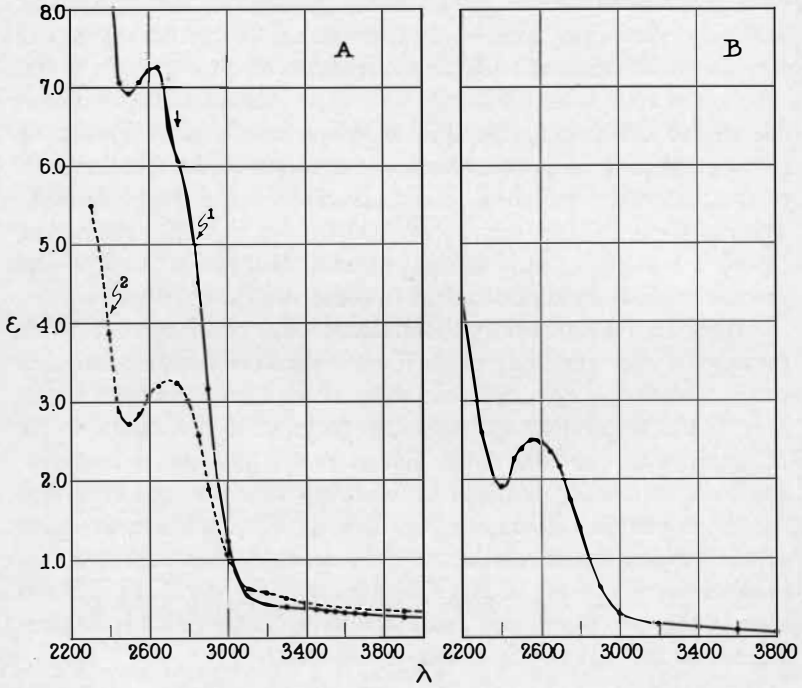

PLATE 6

FIG. 9. *Fractionation of Large Granules (predominantly mitochondria) in distilled water.* The figure illustrates the ultraviolet absorbing power of three main components separated from the large granules by centrifugation and dialysis. Curve 1, section A, corresponds to the water soluble components, which include nucleotides and proteins. Curve 2, section A, represents the protein portion, after the nucleotides have been removed by dialysis. The curve in section B corresponds to the ribose nucleic acid fraction, found to be associated with the microsome-like components of the large granules.

tected in mitochondria; 2) a protein fraction possibly comprising a number of enzymes; and 3) a nucleotide fraction; from qualitative tests this latter fraction is known to contain mono- and di-phospho-nucleotides, and riboflavins.

The particulate component liberated by the effect of water can be removed from the suspension by high-speed centrifugation, leaving proteins and nucleotides in the supernate. The proteins can then be separated from the nucleotides by means of dialysis. Some of the features of this fractionation are illustrated in Fig. 9, the different fractions being expressed by their power to absorb ultra violet light. Experimental details concerning this fractionation procedure will be found in a preceding paper.[9] Fig. 9 shows in part the respective ultra violet absorption spectra of the nucleotides (A_1), proteins (A_2), and ribose nucleic acid (B), of mitochondria.

The observations just discussed permit to conclude that mitochondria are small elements limited by a semi-permeable membrane, and which contain as major constituents: a) small particulate elements, about 0.1μ in diameter and in which the ribose nucleic acid of the mitochondrion appears to be concentrated; b) proteins; c) ribonucleotides; and, d) riboflavins. We already know that some of these constituents are represented by active enzyme systems and co-factors, such as d-amino acid oxidase, cytochrome oxidase, succinoxidase, and cytochrome c.[8, 17, 21, 22, 24]

MICROSOMES

In the course of this talk, certain constituents of cells have been repeatedly referred to by name, the microsomes, but have not been described. Although we have learned very little yet about their possible function, these elements cannot be ignored since they represent as much as 15 to 20 per cent of the cell mass.

The microsome fraction constitutes that part of the tissue extract which is brought down by high-speed centrifugation, about 20,000 x g for one hour, after the mitochondria and the nuclei have been removed.[5, 8, 9]

The microsomes are small elements, approximately 100 to 200 mμ in diameter, and therefore beyond the resolving power of the light microscope.* They are complex structures consisting mostly of phospholipids and nucleoproteins of the ribose type. Lipids, two thirds of which are phospholipids, account for as much as 40 per

* The recent suggestion of Monné (26) that microsomes and chromidia may be identical is obviously erroneous since microsomes are submicroscopic elements whereas chromidia (undefined inclusions currently considered as artifacts) were described on the basis of light microscope observations.

cent of their mass. Most of the nucleic acid of the cytoplasm, except for the relatively small amounts detected in mitochondria, is found to occur in association with these elements.[8, 9, 25]

Like mitochondria, microsomes are very sensitive to changes in the H-ion concentration of the medium, and react very similarly.[9] Fig. 10 illustrates the behavior of the microsomes when placed in media of various H-ion concentrations; they are most stable at pH 7.0 to 7.4; they agglutinate in acid, and disperse in alkaline media. At pH 3.5, the microsome substance disintegrates, with the release of ribose nucleic acid in solution. These properties of microsomes and mitochondria may explain to some extent the features of cell fixation and the granular appearance of cytoplasm, with redistribution of basophilic substance, and ribose nucleic acid, in certain histological preparations.

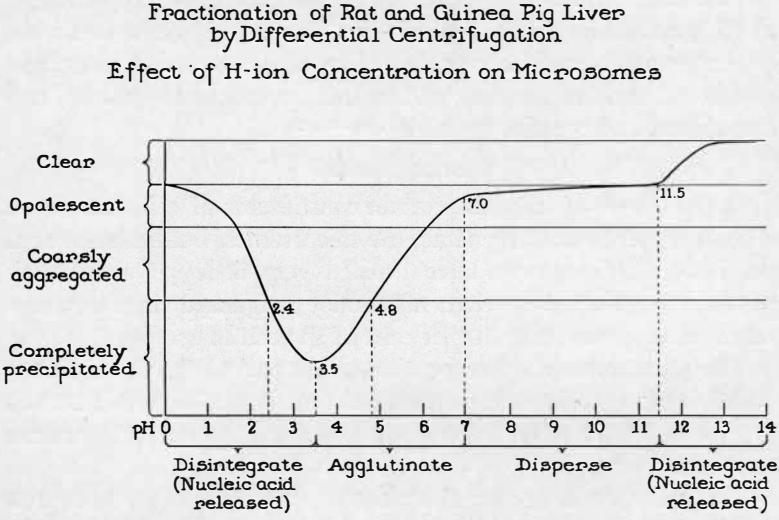

PLATE 7

FIG. 10. *Effect of H-ion concentration on the Microsomes from Rat and Guinea Pig Liver.* The curve illustrates the degree of dispersion, or aggregation, at various pH's, of a suspension of purified microsomes; the values on the ordinate are arbitrary.

At, or above, pH 12, even at ice-box temperature, the microsomes disintegrate, proteins are denatured, and nucleic acid is released. If the suspension is then brought to pH 4.8, the proteins no longer soluble at that point can be removed by simple filtration through

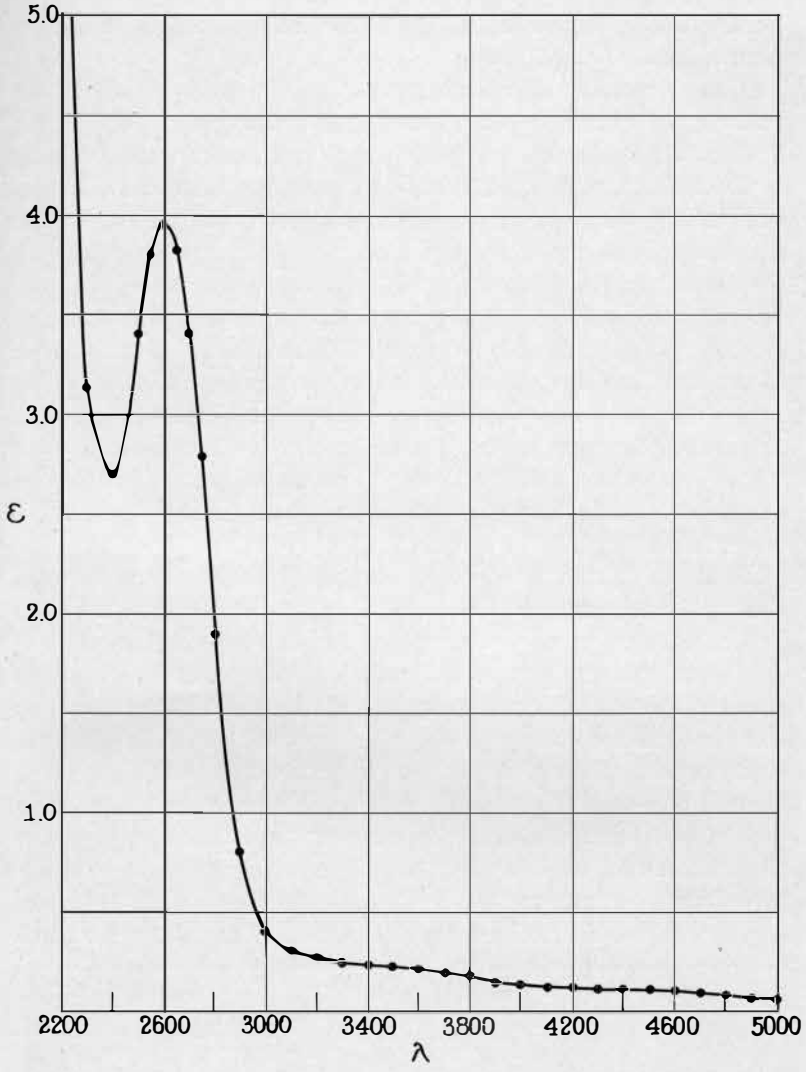

PLATE 8

FIG. 11. *Isolation of Ribose Nucleic Acid from Microsomes.* The figure illustrates the ultraviolet light absorbing power of a neutralized pH 4.7 filtrate, each cubic centimeter of which contained material originally derived from 10 mg purified microsome preparation.

paper, leaving in the filtrate the ribose nucleic acid in practically pure solution (Fig. 11).

The microsomes have been encountered in high proportion, 15 or more per cent of the cell mass, in every tissue so far investigated. Like mitochrondria, they appear to be constant constituents of protoplasm. Because of their abundance and their universal distribution, it is reasonable to assume that microsomes play a fundamental role in the economy of the cell. In order to distinguish them from the other components encountered in tissue extracts, they have been referred to under the name, microsomes, a non-committal term which simply means small bodies.[8, 27] This choice was rather wise if we consider that, since the microsomes were isolated in the laboratory, some ten years ago, no sure clue has been found to reveal their function although several attempts have been made, on theoretical grounds, and especially because of their high content in nucleic acid, to have them play some important role in the cell, either as plasmagenes, or agents of protein synthesis.

Since the microsomes were first encountered in tissue extracts, the question arose whether these elements were artifacts produced by the breaking up of the cell, or existed in the living cell as morphological entities. Because microsomes are too small to be resolved by light microscopy it was necessary to resort to indirect means to investigate this point.

Fragments of liver, in the present case amphiuma liver, were submitted to centrifugation at 20,000 x g for one to two hours, then fixed, sectioned and stained.[8] It can be seen from the micrographs in Figs. 12 and 13 that the effect of this centrifugation was to segregate the various cell components within the cells, just as they had segregated in bulk in the tube of the centrifuge, and in the same

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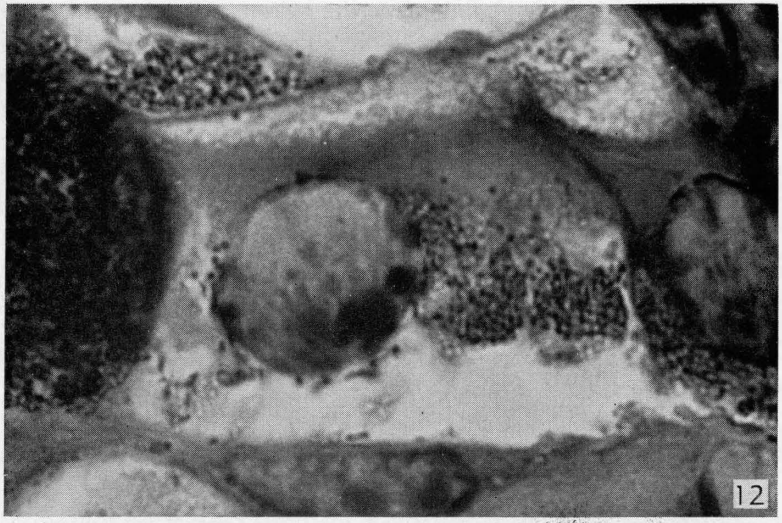
PLATE 9 →

Amphiuma Liver. Intracellular segregation of the morphological constituents of the hepatic cells by 60 minutes centrifugation at 18,000 x g. At the centrifugal pole is found the glycogen (unstained); above it, the nucleus and mitochondria; above these, the microsomes (basophilic components of the ground substance); and, at the centripetal pole, fat globules, or Golgi bodies.

FIG. 12. Fixation: Osmic Acid-Potassium Dichromate (Altmann); Stain: Anilin Acid Fuchsin and Methyl Green (Bensley).

FIG. 13. Fixation: Formalin-Potassium Dichromate (Regaud); Stain: Iron Hematoxylin. Light microscope photographs taken at (1000 x), and enlarged to (2000 x).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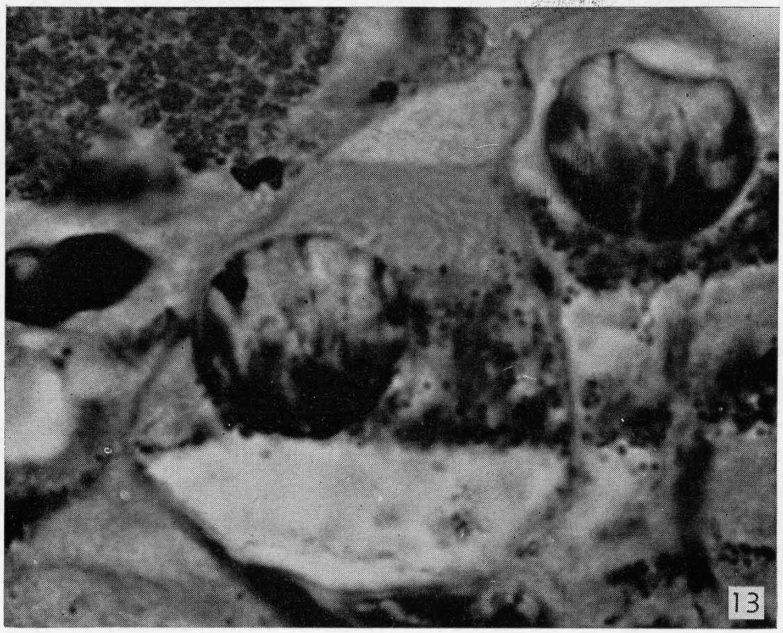
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order. The glycogen appears to be the heaviest component in the cell since it accumulated at the centrifugal pole. Side by side, on and above the glycogen layer, are found the nucleus and the mitochondria. Above these, and in a position where, because of their smaller size, the microsomes might have been expected to segregate, there is a rather wide, strongly basophilic zone separated from the next and last layer by a relatively straight boundary. That the material of this region is probably particulate in nature, although of structureless appearance in the light microscope, is suggested by the fact that it has been displaced by centrifugal force just as the other formed elements of the cell, but at a different level.

From this, and other similar observations, it has been concluded that the microsomes constitute the chromophilic component of the cell ground substance.[8] The strong affinity of the microsome substance for basic dyes, both in the cell and *in vitro*, is in agreement with their high content in ribose nucleic acid.

ELECTRON MICROSCOPY

During the first part of this presentation, we have been discussing, on several occasions, cellular elements that could not be seen because they were too small; yet, they were isolated, measured, and analyzed. For a while, the centrifuge had taken over from the microscope and was exploring regions left unknown, morphologically speaking, because of lack of resolving power.

Today, with the electron microscope at our disposal, limitation in resolving power is no longer a problem since we have been brought in a single step, theoretically at least, to the dimensions of molecules and atoms. But we have already been made to remember that resolving power alone constitutes only a part of the science of microscopy. Past developments in cytology have repeatedly demonstrated the importance of auxiliary techniques, such as staining and fixation, without which some of the cell structures might have remained undiscovered. It is already clear that progress in the study of cells and tissues by electron microscopy will likewise depend to a large extent on the success in devising techniques adapted to the needs of the new instrument. The difficulties facing us, however, are not precisely those which had been predicted.

The first problem in line, and the one which must be solved before further progress can be made, is that of the thickness of the specimen. With the instruments now in use, conditions are such that a preparation of organic material, to allow for the desired differential penetration of electrons, should not be more than 0.1 μ in thickness, and preferably less. Therefore, part of the results which I am about to discuss will be dealing, to a large extent, with this technical problem.

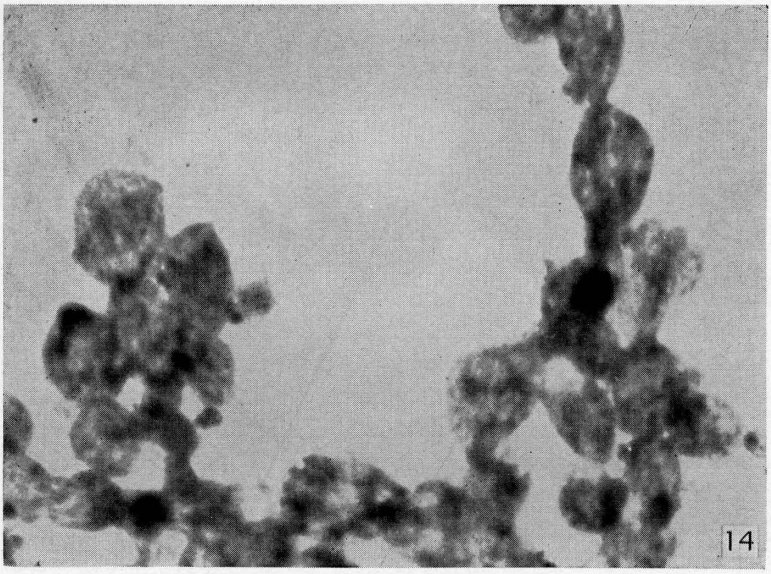

PLATE 10

FIG. 14. Mitochondria isolated from rat lymphosarcoma cells by differential centrifugation. The mitochondria were fixed in a 4 per cent formaldehyde solution buffered at pH 7.0, and the suspension was kept 48 hours at 4° C. Electron micrograph taken at (2550 x), and enlarged to (10,000 x). The mitochondria appear agglutinated. Certain aspects of the mitochondria suggest the presence of a limiting membrane (left); small, internal bodies, about 0.1 μ in diameter, are apparent (right).

During the next few minutes I should like to consider briefly some of the different techniques that have been tried in the study of cells and tissues by electron microscopy. It will be apparent

that each of these methods has its own merits and also its own limitations.

ISOLATED CELL ELEMENTS

In the preceding work, we have reviewed a method whereby certain cell structures can be isolated by means of differential centrifugation. It is clear that success in the electron microscopy of cell components isolated in this manner will depend a great deal on how well their morphology will be preserved during isolation, and preparation of the mount, and also on whether the cell elements themselves are sufficiently thin.

The first material to be examined in this respect were isolated mitochondria. As you may recall, these elements are approximately 0.5 to 1.0 μ in diameter and therefore normally too thick to permit differential penetration of electrons. Through fixation and washing, however, mitochondria may lose some of their substance so that morphological details can be revealed.

The electron micrograph shown in Fig. 14 is of particular interest because it shows two of the features brought to your attention this evening. In the present case, mitochondria were fixed in formalin buffered at pH 7.0 and the suspension allowed to stand for two days at ice-box temperature, giving the opportunity for some of the substance to leak out, thus making the element more transparent.[16] In the picture, the presence of a membrane is clearly apparent. In some of the mitochondria can also be detected a number of small granules, approximately 0.1 μ in diameter. These may correspond to the small elements of approximately that size, and charged with ribose nucleic acid, which we had isolated previously from mitochondria, by means of differential centrifugation at high speed.

CELLS IN TISSUE CULTURE

The next method, developed in collaboration with Porter and Fullam, takes advantage of the fact that cells in tissue culture

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PLATE 11 —→

FIG. 15. Electron micrograph of a chick embryo fibroblast-like cell, grown in tissue culture. The extended cytoplasm is thin enough to allow for the differential penetration of electrons. Mitochondria are clearly outlined and a delicate, cytoplasmic network is revealed. The center of the cell, especially the nucleus, remained too thick. Composite picture, (1600 x).

b
b
a

have a tendency to flatten out to extreme thinness, especially at the periphery, so that areas can often be found which are suitable for electron microscopy.[28, 29] Essentially, the method consists in growing cells on a plastic film and, after proper fixation, transferring the cells together with the film to which they adhere to the supporting screen of the electron microscope.

The composite picture shown in Fig. 15 represents the first record of a complete cell in the electron microscope: a fibroblast, or possibly a myoblast, grown in tissue culture from the gut of a chick embryo. Mitochondria are clearly outlined and their appearance confirms the conclusion that these bodies constitute elements which are sharply differentiated from the surrounding cytoplasm. The ground substance of cells in tissue culture, as in this and other specimens, is found to contain numerous small particles or vesicles,[28, 30] often embedded in a fibrous network, and which are thought to correspond to the microsomes, previously described, and first recognized through their isolation by centrifugation at high speed;[5, 8] such small elements are especially apparent in Fig. 24. With a better understanding of the technical problems involved, especially those concerned with fixation, there is no doubt that the tissue culture method will give an excellent opportunity to study the fine structure of protoplasm, at least as it appears in the thinnest portions of the cells. Unfortunately, the central part of extended cells in tissue culture remains too thick to permit the differential penetration of electrons so that the region of the nucleus is rarely available for direct examination. So far, chromosomes have not been seen *in situ* in the electron microscope, although mitotic divisions are of frequent occurrence in tissue culture, as shown under the light microscope.

Of greater interest, perhaps, is the contribution that the tissue culture method can make to the study of viruses since it offers an opportunity to observe at close range the conditions of their proliferation within the cell. The high resolving power of the electron microscope will permit to investigate what kind of relationship viruses establish during growth with the normal cellular constituents of the host. Because of our growing knowledge of the enzymatic constitution of mitochondria, for example, it would be of

interest to find out whether certain viruses require the medium of mitochondria for their growth.

Observations already made on the distribution of the causative agents in chicken tumor cells have given valuable information on their mode of proliferation, not unlike that of certain microorganisms: depending on the type of tumor examined, the chicken tumor viruses are found in cells disposed in typical colonies (Fig. 22), or in short arrays and doublets (Fig. 23), arrangements which recall the growth patterns characteristic of staphylococci and streptococci, respectively.[30] This electron microscope study of chicken tumor viruses strongly supports the view that these agents are self-duplicating entities, and that the intracellular environment provided by the receptive cell serves merely as an appropriate medium for their growth.

TISSUE SECTIONING

In preparing specimens for electron microscopy, it would seem that the method of sectioning, so widely successful as an adjunct to light microscopy, would be the technique of choice because of its applicability to all cells and tissues, irrespective of their particular constitution and origin. Early and repeated efforts have been made to adapt current methods, or to find new ways of sectioning, but these have been far from satisfactory. At the moment no practical method exists by which sections sufficiently thin, and satisfactory in quality, can be prepared with the desired regularity.

Among the various methods of sectioning that have been proposed the high-speed microtome, for a while, had given hope of achievement and, occasionally, interesting sections have been obtained. With experience, however, it is becoming more and more apparent that in preparing thin sections for electron microscopy it is not so much the instrument which is the limiting factor, but our methods of fixation and embedding: the high resolving power of the electron microscope painfully reveals the defects of histological techniques which, for the light microscope, had been accepted as satisfactory.

Fig. 16 shows a light microscope photograph of a section of

guinea pig liver. Fig. 17 represents the electron micrograph of another section derived from the same block, 0.3 to 0.5μ thick, and made by means of a high-speed microtome with the cutting blade moving at 50,000 revolutions per minute.[31] For this experiment, the liver of a guinea pig under ether anesthesia was prepared by perfusion with Tyrode's solution, fixed by perfusion with a 2 per cent osmic acid solution, and embedded in a eutectic mixture of camphor and naphthalene, in place of the usual paraffin. The advantage of this embedding material is that it sublimes so that a section, once made and properly transferred on the supporting screen of the electron microscope, can be examined directly without the need to be subjected to the disrupting action of solvents.[31]

The purpose in showing these two sections together is to illustrate, on the same material, the difference in the resolving power of the two microscopes. It can be seen that the electron micrograph reveals, especially in the ground substance, a number of details not apparent in the light micrograph. It is possible that the numerous small elements, about 0.1μ in diameter, which appear in the ground substance of these liver cells correspond to the microsomes, previously detected, but indirectly, by centrifugation and staining.[8]

In preparing sections for electron microscopy a number of steps must be taken into consideration: a) the construction of a suitable microtome; b) the sharpness of the cutting edge of the blade, and the cutting angle; c) the fixation of the cells; d) embedding; and, e) the handling of the section. Although of a different nature, each of these steps appears to be equally important.

In recent years, and especially with the construction of expensive

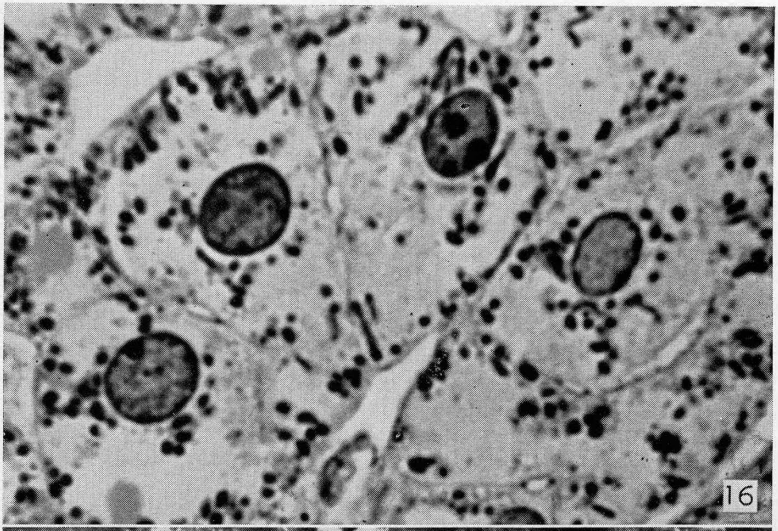
PLATE 12 ——→

Sections of Guinea Pig Liver. The liver was fixed by perfusion with a 2 per cent solution of osmium tetroxide, and the tissue was embedded in camphor-naphthalene.

FIG. 16. Light microscope picture of a 1 μ section. Micrograph taken at (1000 x), and enlarged to (2000 x).

FIG. 17. Electron micrograph of a 0.5 μ section; unstained. Micrograph taken at (1650 x), and enlarged to (4000 x).

The pictures illustrate the difference in the resolving power of the two instruments. This is especially noticeable in the ground substance which, in Figure 16, appears structureless; the greater resolving power of the electron microscope reveals in the same cell region innumerable small particles, about 0.1 μ in diameter (Figure 17). These particles may correspond to the microsomes, previously described. Compare with the microsome layer of Figure 12.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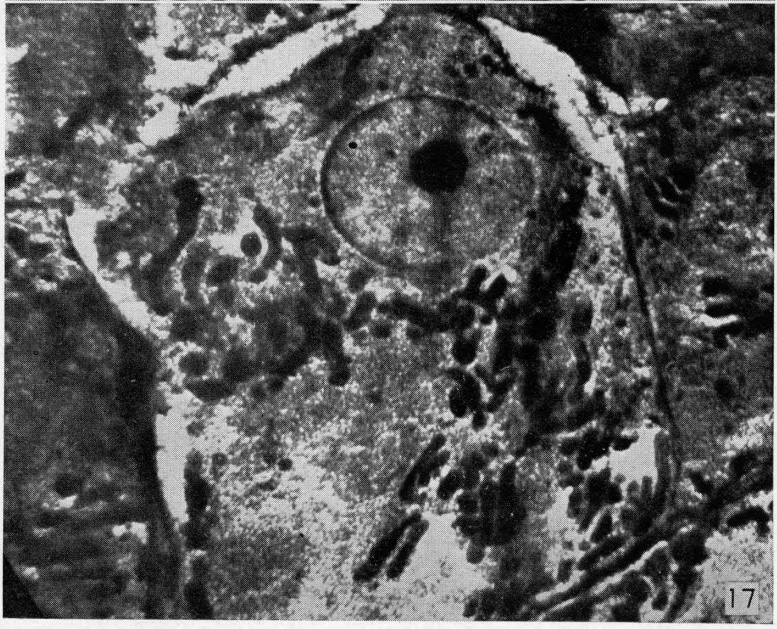
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high-speed microtomes, the idea has been prevalent that the problem was one of engineering and that, if the right microtome could be constructed, the solution of the problem would be at hand. In my opinion, this is not the case: technically there is no special difficulty in building an instrument which will provide a motion by

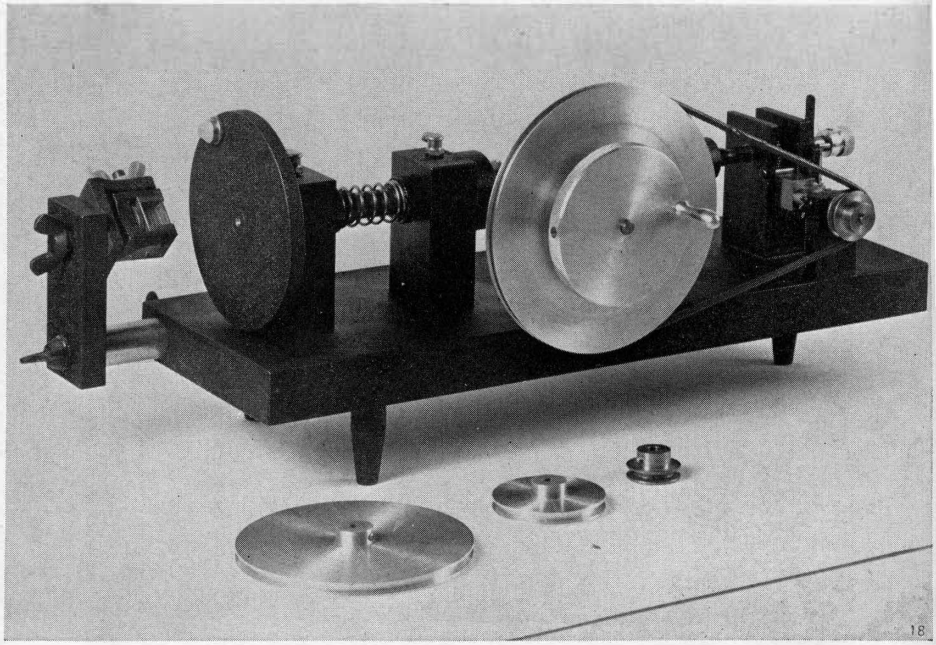


PLATE 13

FIG. 18. Continuous motion microtome for thin sectioning. Continuous motion is provided through a reduction gear, a differential screw, and a free turn-table. The instrument can be adjusted so that, per each revolution, the turn-table supporting the specimen will move forward by increments which may vary from 1 to 0.1 μ. Serial sections are collected on the water surface of a well, placed in front of the blade.

increments of, let us say, one tenth of a micron. We have had such a device for a long time in the micrometric screw of our microscopes. The difficulties reside, in fact, in the proper preparation, fixation and embedding, of the cells, and also in the handling of the section, once it has been cut. Since the problem of sectioning

is on the mind of most electron microscopists at the moment, I hope that I will be permitted to include here a progress report on the subject.

The microtome shown in Fig. 18 was built in the shop of The Rockefeller Institute by Mr. J. Blum, who is responsible for the accurate construction of the instrument. The original purpose, in designing this microtome, was to obtain an instrument which would provide a continuous motion, instead of the back and forth movement characteristic of the current microtomes where alternate changes in position, and even in the thickness of the oil film, may involve differences greater than the actual thickness of the desired section. In this instrument the continuous forward motion, with increments which can be adjusted for thicknesses varying from 1 to 0.1μ, or less, is insured by a differential screw, and a free turntable supporting the specimen. To this device, and in front of the blade, is added a well, kept filled with water during sectioning, so that each section, or ribbon of sections, can expand directly on the water, helped in this by the surface tension at the meniscus.

The instrument just described is capable of doing the work expected from it, but it may be of theoretical value only, for a while longer, if the sections produced are not worth looking at. In the near future, therefore, the task ahead is to find better ways for the preparation and the preservation of the specimen. In the meantime, I should like to describe another possible approach to this difficult problem of morphology: a recent attempt to apply, to the study of cells, the method of replicas.

REPLICAS OF CELLS AND BIOLOGICAL MATERIAL

As is known, the absorption and scattering of electrons is not affected by molecular differences, as is the case with light of various wave lengths, but is determined by differences in atomic densities. With biological material, therefore, where the major elements, carbon, nitrogen, and oxygen, have nearly the same atomic weight, the absorption and scattering observed in the electron microscope is a function of variations in the local concentration of substance and can be said to be a measure, but in inverse proportion, of the distribution of water originally present in the fresh material.

The following experiments take advantage of this fact, and were based on the assumption that differences in the distribution and the concentration of substance, as found in cells and tissues, are reflected, after drying, at the surface of the specimen. The results indicate that surface replica of dried cells and tissues can picture, with surprising accuracy, morphological details of their internal constitution. This method has been found to be readily applicable to blood cells and bacteria.[32, 33]

The technique is simple. It consists in immersing a dried smear of blood cells or bacteria, previously fixed, in a 0.5 to 0.7 per cent solution of Formvar in ethylene dichloride, draining rapidly, and allowing the plastic film to dry with the slide kept in a horizontal position. The preparation is then placed in water, an area selected under the dissecting microscope and the film, or replica, peeled off gently by means of fine, watchmaker's forceps. For electron microscope study the free film disc is lifted from the water on a screen and the mount dried on blotting paper. The plastic mold of cells prepared in this manner can be so faithful that it may be difficult to distinguish, under the light microscope, between the replica and the original preparation. Fig. 19 shows a light microscope photograph of a replica of chicken blood cells: the nuclei of the nucleated erythrocytes, and the mitochondria and granules in a white cell, are clearly apparent. Fig. 20 represents an electron micrograph of a replica of chicken blood cells: on the left the image of an intact erythrocyte, and on the right the "ghost" of a red cell, with remnants of the nucleus. Fig. 21 shows an electron micrograph of the replica of an elongated form of *B. coli,* as the organisms often appear in aging cultures. This replica reveals in the bacterium a series of distinct bodies apparently arranged along a spiral path;

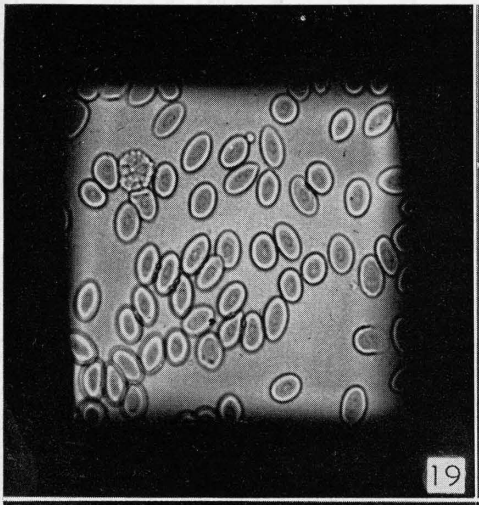
PLATE 14 →

Replicas of Blood Cells and Bacteria.

Fig. 19. Formvar replica of a smear of chicken blood. The film is resting on the wires of the supporting screen. Light microscope photograph, taken at (250 x), and enlarged to (450 x).

FIG. 20. Replica of a chicken erythrocyte (left), and a "ghost" (right). Electron micrograph taken at (2200 x), and enlarged to (4400 x).

FIG. 21. Replica of an elongated form of B. Coli. Electron micrograph taken at (2600 x), and enlarged to (4000 x).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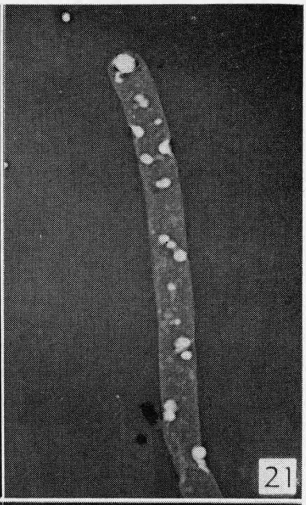
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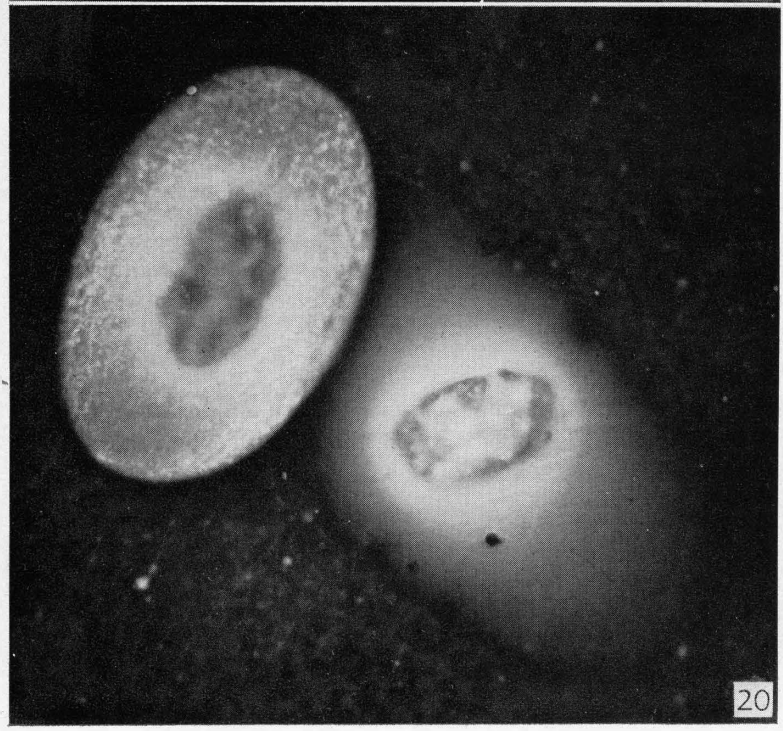
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the fact that these granules are stainable with methyl green indicates that they contain chromatin and that they correspond, probably, to nuclei.

The method of replicas may prove useful in a number of problems involving the electron microscope study of blood cells, for example, in investigating the penetration and growth in cells of malarial parasites. It may prove of value also in other biological problems, where the other methods of preparation, such as tissue culture or sectioning, are not applicable.*

MICROSOMES, PLASMAGENES, AND VIRUSES

During the past few years a number of authors, especially Darlington[34] and Haddow[35] have elaborated on the deductions of modern genetics and the concept of plasmagene has been proposed to account for some of the new findings. According to this concept, the chromosomes might not constitute the sole reservoir of genetic characters but some of the constituents of cytoplasm might be endowed, at least temporarily, with a sort of genetic continuity. Because of their abundance in the cytoplasm, their small size, and especially because of their high content in nucleic acid, the microsomes have been mentioned as the possible physical expression of the plasmagenes. Following the same line of reasoning, the theory has unfolded further, to link plasmagenes and microsomes with the appearance and the growth in cells of certain viruses, and especially the tumor-producing viruses such as those of chicken tumors and of the mammary adeno-carcinoma of the mouse. Thus, after a long period of philosophical tranquillity we are faced once more, through the refinements of modern cytology and modern chemistry, with the ancient problem of spontaneous generation. I admit that

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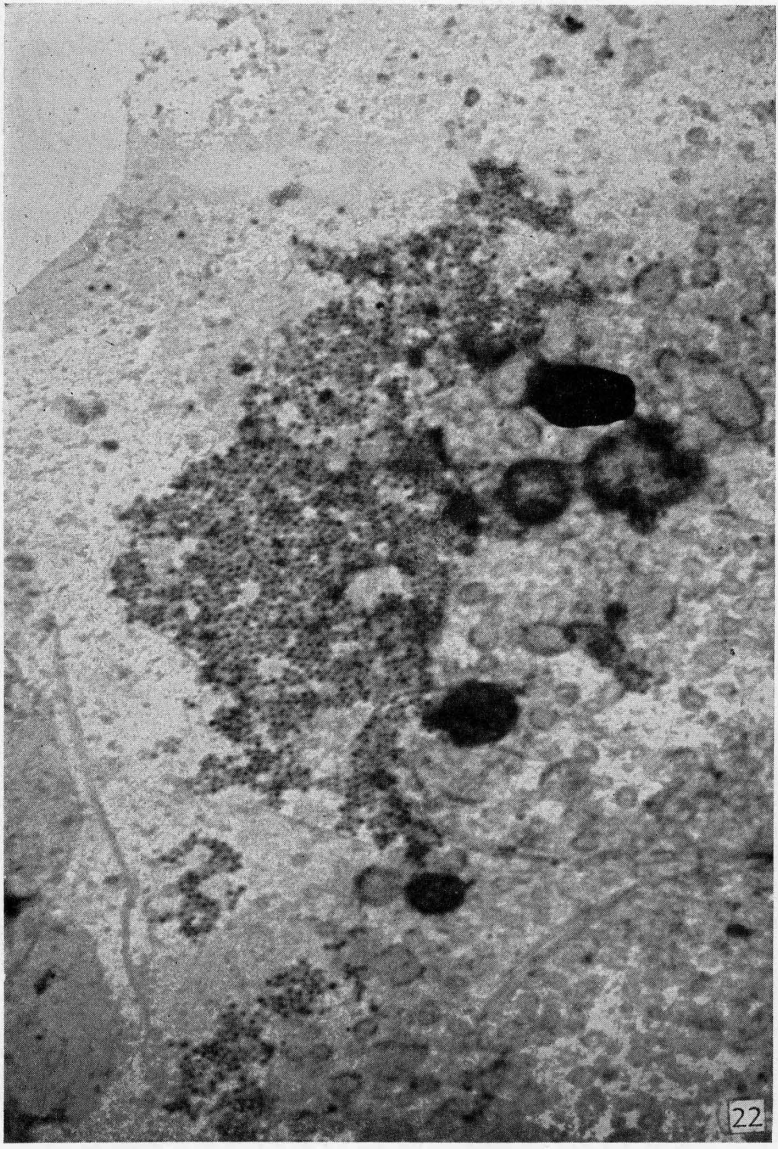
PLATE 15 →

FIG. 22. Electron microscopy of chicken tumor cells. Portion of a Chicken Tumor 10 cell, grown in tissue culture. The picture shows "colonies" of the causative agent; the virus particles are of relatively uniform size, approximately 65 mμ in diameter. Electron micrograph taken at (1960 x), and enlarged to (10,800 x).

* Since the presentation of the present paper, the method of replicas has been applied with some success to the study of salivary gland chromosomes.[39]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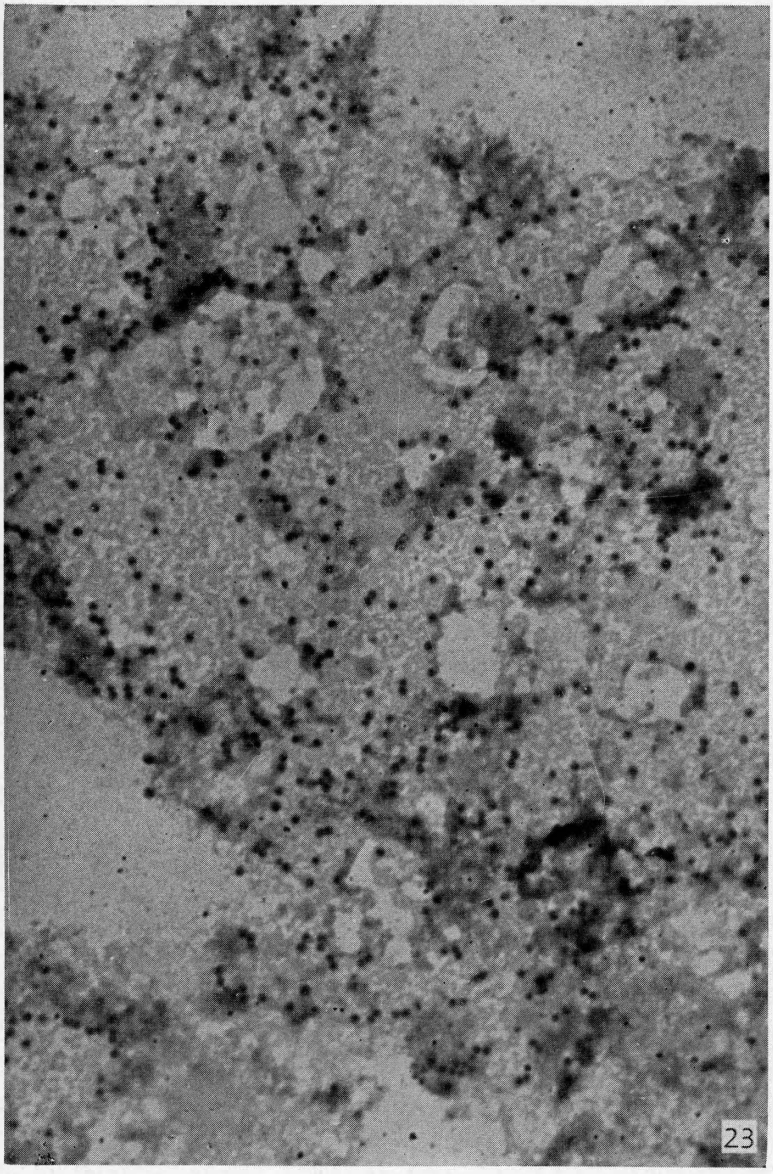
the question cannot be dismissed lightly: our knowledge has increased tremendously since the times of Schwann and Pasteur, and we find ourselves working at the molecular level where life and simple chemical reactions cannot readily be distinguished.

The study of normal and tumor cells by electron microscopy offers an opportunity to examine the possible relation between tumor viruses and the normal constituents of the cell; some of the results concerning two tumors, namely, Chicken Tumor I and Chicken Tumor 10, have already been discussed. Fig. 22 is an electron micrograph of the thinly extended rim of a Chicken Tumor 10 cell, grown in tissue culture. The characteristic feature in the preparation is the presence of a number of patches, never seen in normal cells, and composed of small, juxtaposed elements of uniform size, i.e., 60 to 70 mμ in diameter. Elements of approximately the same size have been observed in cultured cells of Chicken Tumor I (Rous sarcoma). Fig. 23 represents the electron micrograph of a preparation derived from a culture of Chicken Tumor I cells. In this particular case the mass of the cell body was removed at the time of fixation, leaving exposed numerous discrete elements of the type just mentioned, and remnants of the cell membrane. It will be noticed that, instead of appearing in patches, the tumor elements in this preparation are disposed singly, in short strings, or frequently in pairs, an arrangement characteristic for Chicken Tumor I. In the case of both tumors, the elements found in tumor cells have the size predicted for the tumor agents, from previous filtration and centrifugation experiments and it has been concluded that they constitute the causative agents of the tumors.[30] Fig. 24 is the electron micrograph of a sector of a Chicken Tumor I culture, where cells were actively growing. At the time of fixation a tumor cell had just divided and the two daughter cells were still united by a protoplasmic bridge, apparent across the upper part of Fig. 24.

PLATE 16 →

FIG. 23. Electron microscopy of Chicken Tumor I cells, grown in tissue culture. The scattered bodies shown in the micrograph are considered to represent the causative agents of the tumor. They appear in short arrays, and more frequently in pairs, indicating a growth habit different from that of the causative agent of Chicken Tumor 10 (see Figure 22). Electron micrograph taken at (2280 x), and enlarged to (10,850 x).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Electron micrography of the neighboring fields showed the two young cells filled with numerous tumor particles, here also disposed in short chains and doublets, and some of them were caught and carried along in the protoplasmic bridge (Fig. 24). The underlying cell, part of which occupies most of Fig. 24, appears to have remained practically normal, since no typical tumor agents were detected in it. In this apparently normal cell are seen a number of mitochondria of various shapes and, in addition, numerous small granules or vesicles, scattered throughout the cytoplasm, and which presumably represent the microsomes of the cell. Other tests have shown that microsomes swell considerably in water, if previously fixed with osmic acid, and their appearance as vesicles in the present micrographs is probably due to this fact. Inspection of the various chicken tumor micrographs reveals profound differences between microsomes and the tumor viruses, not only in size and shape, but also in texture. The fact that they appear to react differently to the action of fixatives would suggest the existence of appreciable differences in their chemical constitution. On the basis of these observations, and because of their growth patterns in doublets, chains, and typical colonies, it is possible to conclude that the chicken tumor agents appear with the earmark of self-reproducing entities, probably exogenous, and with no visible counterpart in homologous, normal cells. At the moment, at least, a relation between the tumor viruses and the normally occurring microsomes is not apparent.

In a virus disease of this sort, the most conspicuous feature of malignancy, i.e., the tendency of the cells to divide, may be consid-

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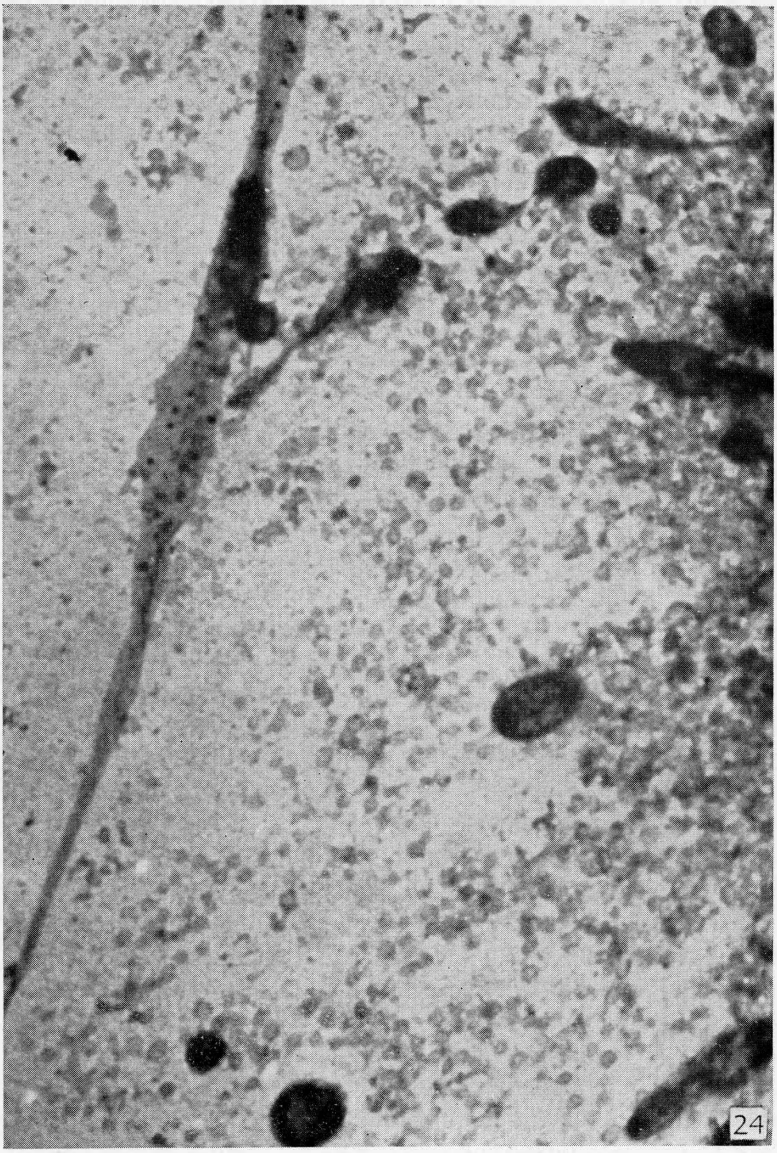
PLATE 17 ⟶

FIG. 24. Electron microscopy of Chicken Tumor I cells grown in tissue culture. The electron micrograph shows a portion of an apparently normal cell containing a number of mitochondria and numerous, scattered bodies of smaller size. The latter bodies which, in the picture, are about 120 to 220 mμ in diameter, and vesicular in appearance, probably correspond to the normal microsomes, described in the text. The picture is crossed by a protoplasmic bridge uniting two Chicken Tumor I cells which had just separated, following division. Virus particles, apparently carried along passively during division, can be recognized as small, dense bodies in the protoplasmic bridge. Differences presented by the virus particles and the normal microsomes are evident. Electron micrograph taken at (1960 x), and enlarged to (8,800 x).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ered as a defense mechanism, rather than a degenerative process, or a viciation of the normal metabolism of the cells.

It is conceivable that a cell line could rid itself of the virus providing that, during a certain period, the rate of division of the cell is greater than that of the virus. In such favorable cases, the virus particles in the successive daughter cells would become progressively less numerous until, finally, a new cell generation would be free of the virus and, therefore, behave again as normal cells. In this respect, the formation of a tumor might be considered as the expression of a losing race, between the affected cells and the virus.

RESPIRATION, NUCLEIC ACID, AND PROTEIN SYNTHESIS

In the biochemical work reviewed tonight a number of respiratory enzymes were shown to be localized in the large granules, probably exclusively, and I have said that mitochondria could be visualized as microscopic power plants where the energy of molecular oxygen is ultimately transferred and utilized. Since it is probable that some of the synthetic activities of the cell take place near the source of energy, it may be assumed that the large granules represent also important manufacturing centers.

In a series of papers Caspersson[36, 37] and Brachet[19, 20, 38] have developed the view that ribose nucleic acid is related in some way to protein synthesis. This opinion was based, first, on the observation that ribose nucleic acid is always present in the cytoplasm of metabolically-active cells and, second, on the fact that ribose nucleic acid is found in greatest abundance in actively growing cells such as embryonic and tumor cells, where synthesis of proteins is presumably needed, and in cells primarily engaged in the production and excretion of proteins, as in the pancreas. It may be recalled in this connection that most of the ribose nucleic acid of the cell ground substance occurs in association with particulate elements of submicroscopic size, i.e., the microsomes.

If the ribose nucleic acid of the microsomes is to play a part in protein synthesis, it must be without the immediate participation of the cytochrome-linked respiratory system since cytochrome oxidase, succinoxidase, and cytochrome c are absent. On the other

hand, the rôle attributed to ribose nucleic acid as a factor in protein synthesis is not the only hypothesis that could be held to account for the abundance of this substance in embryonic and tumor cells since its presence coincides with other outstanding functions of these cells. It may be noted that, in general, the cells which have been found to have a high ribose nucleic acid content, such as embryonic and tumor cells, have been shown by Warburg and his followers to possess to a high degree the power of anaerobic glycolysis. It might be suggested, therefore, that the presence of ribose nucleic acid in cells is related, beside its possible role in protein synthesis, to their capacity for anaerobic respiration. This view would seem to be supported by the concurrence of large amounts of ribose nucleic acid, and of active fermentative processes, in yeasts and in certain bacteria. Ribose nucleic acid might be involved either in some phase of the anaerobic mechanism, or act as an intermediate in the energy transfer for various synthetic reactions.

From these considerations one might venture the conclusions, partly facts and partly hypothesis, that, whereas most the metabolic activity of the cell is found in the cytoplasm, the supply of energy may be segregated in various cytoplasmic entities: the aerobic respiration in the mitochondria, as already demonstrated, the anaerobic processes in the ground substance. This might explain the intense basophilia of cells in young embryos, in fast growing tissues, and in tumors, especially in areas where the circulation and the fresh supply of oxygen may be inadequate, or defective. Demonstration that a relation exists between the power of anaerobic glycolysis and ribose nucleic acid distribution would permit us to consider further the possibility that the nucleus, where the cytochrome-linked respiratory system is apparently lacking, derives its energy at least in part, from loci where ribose nucleic acid is present, especially the nucleolus, and certain chromosomal regions.

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