SCIENCE

VOL. 97 FRIDAY, MAY 21, 1943 No. 2525

SCIENCE: A Weekly Journal devoted to the Advancement of Science, edited by J. MCKEEN CATTELL and published every Friday by

THE SCIENCE PRESS

Lancaster, Pennsylvania

Annual Subscription, $6.00 Single Copies, 15 Cts.

SCIENCE is the official organ of the American Association for the Advancement of Science. Information regarding membership in the Association may be secured from the office of the permanent secretary in the Smithsonian Institution Building, Washington, D. C.

THE CONSTITUTION OF PROTOPLASM[1]

By Dr. ALBERT CLAUDE

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, NEW YORK

AMONG the variety of elements which partake in the constitution of the cell, the nucleus is the largest single body and the one which has lent itself to the most successful investigation. The nucleus was seen as early as 1781 by Fontana, but it was not until the principles of the cell theory were established by Schwann, Remak and Virchow that its role in cell economy could take its full significance. With Flemming, Strasburger and van Beneden began a series of brilliant investigations on the nucleus, which culminated in the discovery of the phenomenon of mitosis and the demonstration of the unique role which the chromosomes assume in heredity. The success met with in the study of the nucleus was undoubtedly due to the circumstance that its structures were able to withstand the action of the fixatives which had come into use during the nineteenth century. This typical resistance of the nucleus to these agents and the nuclear affinity for basic dyes can in turn be traced to a substance present in abundance in all nuclei and segregated in the chromosomes during division, namely, thymonucleic acid.

The usual fixatives which had proved eminently suitable for the preservation of the nuclear framework destroyed the cytoplasmic structures, an effect due chiefly to the high concentration of acids and of alcohol which they contained. The artefacts so produced gave rise to erroneous views on the organization of protoplasm, such as the reticular and the froth theories. The outstanding advance in the study of cytoplasm came with the work of Altmann and his followers, who recognized the destructive action of acids and introduced bichromate as a fixative. This improve-

[1] Paper presented at the Gibson Island Conferences of the American Association for the Advancement of Science, Gibson Island, August 21, 1942.

ment in technique had the advantage of preserving most of the cytoplasmic inclusions and was responsible for the discovery of mitochondria. In the light of modern cytological studies, the cytoplasm appears to be essentially composite in nature, consisting of a continuous ground substance, the hyaloplasm, in which are found formed elements morphologically independent and varying widely in size and shape.[2] The morphology and distribution of mitochondria have extensively been studied and the conclusion is that these elements are constant constituents of cytoplasm. Secretory granules in animals and the plastids in plants are differentiated elements related to specialized functions of the cell and concerned with the elaboration of active substances as in the production of proteolytic enzymes in the pancreas, the production and storage of definite food materials such as starch, or the deposition of pigments. The Golgi body also appears to be a constant constituent of the cell, but its morphology, chemical composition and function are still obscure. The cytoplasmic elements just mentioned are large enough to be stained and studied by the usual cytological techniques. Their average diameter is appreciably greater than 0.2 μ, a value which represents approximately the limit in the power of resolution of the ordinary microscope. Visibility of minute objects within the cell can be considerably increased by means of intense lateral illumination as provided in the dark-field microscope. With the latter technique, it can be shown that the living cytoplasm, in addition to the "visible" inclusions, contains numerous highly refringent bodies of extremely small size, which may be at rest or in active Brownian movement. These ultramicroscopic bodies have escaped the attention of cytologists engaged in the study of fixed preparation, but have often been seen by students of living cells.[3] The chemical composition of the above cytoplasmic structures, their respective functions within the cell, their origin and the genetic relationship between the different classes of granules are problems which have not, and apparently can not be solved by purely microscopical techniques.

During the past few years, this laboratory has been engaged in the mechanical fractionation of normal and tumor cells by means of differential centrifugation, and purified fractions have been obtained from both nuclei and cytoplasm.[4,5,6] The first cell component to be isolated was a particulate substance of cytoplasmic origin which has been described in preceding papers under the provisional term, "small particles."[5] The present paper deals with a further study of this important cell constituent, together with a preliminary account of the isolation and analysis of zymogen granules from the liver and pancreas.[7] The position of the small particles in the organization of protoplasm and their possible relation to mitochondria and zymogen granules will be discussed.

Small particles: The method for the separation of the small particles has been described previously.[4,5] In this method, the cells are broken up and suspended in neutral water and the material is segregated and washed in a high-speed centrifuge. When the purified substance is concentrated in the centrifuge, it appears as a jelly-like pellet which is completely transparent. In this form, the material is not birefringent. Under transmitted light, the substance is somewhat amber in color, a property which is probably due to the large proportion of phospholipids which it contains. By reflected light, the color presented by the purified material may vary, depending on the tissue of origin. When the source of the particles is the liver, the color of the mass may be red or pink. It is usually light brown in chicken tumors and practically colorless in lymphoid tumors and in the pancreas. The unusually bright color exhibited by the liver fraction suggested that part of the purified material might have derived from the red corpuscles, since, in the liver, capillary blood may often form a large portion of the organ. For this reason, the work was repeated on livers which had been perfused prior to extraction. As regards color, yield and chemical composition (Table I), the results of this new series of experiments were identical with those already reported.[5] Therefore, it may be concluded that, in this case, the fraction under study had its origin in the hepatic cell.

When suspended in neutral water, the material forms opalescent preparations which, in the dark-field microscope, appear to be composed of extremely small bodies, highly refringent and in active Brownian movement. The size of the particles has been estimated to range approximately between 50 and 200 mμ in diameter, with no apparent segregation in definite size groups. The isolated particles have been shown to be complex formations in which a nucleoprotein of the ribose type occurs in association with a definite proportion of lipids, especially phospholipids.[4] The chemical composition of this cytoplasmic component is highly characteristic, as indicated by the consistent values obtained on chemical analysis and irrespective of the tissues from which it is prepared. Typical

[2] E. B. Wilson, "The Cell." The Macmillan Company, New York, 1925.

[3] R. Chambers, "General Cytology." The University of Chicago Press, Chicago, 1924.

[4] A. Claude, SCIENCE, 87: 467, 1938; *Proc. Soc. Exp. Biol. Med.*, 39: 398, 1938; SCIENCE, 90: 213, 1939; 91: 77, 1940.

[5] A. Claude, *Symposia on Quantitative Biology,* Cold Spring Harbor, 9: 263, 1941.

[6] A. Claude, *Trans. N. Y. Acad. Sciences,* Series II, 4: 79, 1942.

[7] Details of unpublished experiments, carried out with the collaboration of Dr. C. Auger, will appear in other journals.

values are close to 9 per cent. nitrogen and 1.5 per cent. phosphorus, except in embryos and pancreas, where the total phosphorus amounts to 2.1 per cent. The latter observation is of interest since embryonic tissues and pancreas have been found to be exceptionally rich in ribose nucleic acid. Tables I and II give the average values obtained on analysis of the small particles derived from rat and guinea pig liver (perfused) and from beef pancreas. Small particles of the type described above have been isolated from a great variety of tissues, and the value of 9 per cent. nitrogen appears to be representative for this class of cytoplasmic granules. A study of the available data indicates that the small particles are universal in distribution and that they represent a considerable portion of the cell (at least 10 to 15 per cent. by dry weight). The evidence suggests that the small particles are integral and, without doubt, important components of living protoplasm.

The position which the small particles occupy in the organization of the cell is of particular interest. This point has been under investigation in this laboratory for the past two years, not only with respect to the nature and role of the small particles, but also to the possible relation which may exist between them and other cytoplasmic structures. It was originally stated that the small particles might represent mitochondria or fragments of mitochondria.[4] This suggestion was based on apparent similarities in chemical constitution and on the estimate of the size of mitochondria, as found in the literature.[8] However, it can be shown that, as a rule, the width of mitochondria is appreciably greater than 0.2 μ, whereas the size range for the small particles, as found in our laboratory, appears to be roughly between 50 and 200 mμ in diameter; some particles are occasionally larger.[4] In the guinea pig liver, the red, small particles are definitely submicroscopic, although probably larger than our first estimate of 40 to 60 mμ in diameter.[5] In the spleen, pancreas and the liver of different species, the small particles have been found to be also submicroscopic. However, the sedimentation rate of the substance seems to be influenced by a number of factors, especially by the nature of the solvent and the pH of the solution and further study will be necessary before the actual size of the particles in different tissues can be ascertained. In rat leukemia, particles were found whose size was approximately that of the mitochondria, as seen in the living cells,[5] but a further study of rat leukemia extracts in the high-speed centrifuge showed that the protoplasm of the leukemic cells contained also, like that of other cells, a jelly-like substance composed of submicroscopic units.

[8] E. V. Cowdry, Carnegie Institution of Washington, *Contrib. Embryol.*, 8: 39, 1918.

The following observations on the intracellular segregation of cell constituents in high centrifugal fields indicate that, as a rule, the small particles do not derive from the visible elements of the cell but are undoubtedly part of the so-called ground substance. When hepatic or pancreatic cells are stained by the Altmann-Bensley technique, the zymogen granules and the mitochondria appear colored a vivid red against a diffuse background which contrasts by its slightly purple color. On the other hand, these various cell components can be forced to segregate within the cell by submitting a fragment of tissue to high-speed centrifugation. After 60 minutes at 18,000 × gravity, the different cell constituents are found segregated towards the centrifugal pole in the following order: the glycogen, the mitochondria and secretory granules, the "purple substance" and the Golgi body. The nucleus is at the level of mitrochondria and zymogen granules but above the glycogen. The upper surface of the "purple substance" appears as a straight line boundary. If the centrifugal force is sufficiently great, this boundary may be separated from the centripetal pole of the cell by an area which is clear and seemingly empty. This observation indicates that the apparently homogeneous ground substance contains a particulate, chromophilic component which dissociates itself from the true hyloplasm under moderately high centrifugal force. This "purple substance" which can thus be demonstrated in the cell by the combined techniques of staining and high-speed centrifugation constitutes probably the source of the small particles. This is indicated by the fact that the same color differentiation can be obtained *in vitro,* by staining the isolated fractions, namely, small particles and secretory granules, on the same slide and by the same technique. In this case, the substance of the small particles takes a purple color against the red color of the secretory granules. Staining the tissues with the Regaud technique leads to similar observations, where the sedimentable component of the ground substance (small particles) can be identified by a gray-blue color contrasting with the blue-black color of the secretory granules and mitochondria. Thus, the evidence, so far, indicates that the mass of the small particles does not derive from the grossly visible elements of the cell but constitutes a hitherto unrecognized particulate component of protoplasm, more or less evenly distributed in the fundamental substance and which impart to it, in well-preserved preparations, its staining properties. In order to differentiate the small particles from the other, already identified elements of the cell, it may be convenient in the future to refer to this new component under a descriptive name which would be specific. For this purpose the term *microsome* appears to be the most appropriate. The term microsome, meaning

small body, was applied originally by Hanstein (1880) to any granules, as seen in living protoplasm. The use of the word was progressively narrowed down, being retained as a general term to designate any small granules of undefined nature.[2] Under these conditions, it seems proper to suggest that the term microsome, already familiar to cytologists, should be restricted to designate the small particles exclusively.

Zymogen Granules: The technique for the separation of secretory granules from guinea pig liver has been described in another paper.[5] A new series of experiments on the perfused liver of guinea pigs and the liver of normal rats indicates that the results obtained with this method are highly reproducible, as shown by the very close values obtained on chemical analysis, even in two different species of animal (Table I). Extreme variations in individual experiments were less than 1 per cent. for the nitrogen, less than 4 per cent. for the phosphorus values. Twelve per cent. nitrogen and 1.25 per cent. phosphorus, or values very close to these figures, have been obtained consistently in recent experiments and it may be concluded that they constitute characteristic features of the liver secretory granules. These granules are readily separated from the other liver components, and neutral water can be used in their preparations. In the centrifuge, the liver granules form a loose sediment which is opaque and presents a buff color which resembles that of compressed yeast.

TABLE I

FRACTIONATION OF THE LIVER BY DIFFERENTIAL CENTRIFUGATION: CHEMICAL COMPOSITION OF SMALL PARTICLES AND OF SECRETORY GRANULES. (AVERAGE VALUES FROM 2 EXPERIMENTS)

Animal species	Fraction	N per cent.	P per cent.	C per cent.	H per cent.	S per cent.	Amount obtained (dry weight) per cent.
Guinea Pig	Small Particles	9.08	1.69	56.03	8.23	0.7	7.5
	Secretory Granules	12.08	1.26	54.55	8.09	0.82	4.6
Rat	Small Particles	9.14	1.62	55.44	8.26	0.68	10.0
	Secretory Granules	12.09	1.25	54.45	7.91	0.94	6.6

Separation and purification of zymogen granules from pancreas have presented much greater difficulties, due especially to the presence of a powerful lipase which rapidly attacks the lipid portion of the microsomes and that of the zymogen granules—an action which results in the destruction of their structure and which leads eventually to the denaturation of their proteins. An adequate technique was finally worked out, the details of which will be given in a later paper. This technique is based on a time centrifugation of 30 minutes at 2,000 × gravity. The pancreatic granules so obtained are rapidly destroyed in water, dissociating into a particulate component and an insoluble, highly colored substance. They are fairly well preserved in 0.8 per cent. NaCl solutions at pH 7.5. In the centrifuge, the zymogen granules form a loose and opaque sediment. The color of the material is characteristically yellow and often yellow-green. The chemical composition of the zymogen granules is strikingly similar to that of the liver secretory granules. This resemblance is especially apparent when comparing the results of analysis which are summarized in Tables I and II. In both cases, the value for nitrogen is equal to, or approaches 12 per cent. Moreover, it can be seen that in both liver and pancreas the small particles on the one hand, the secretory and zymogen granules on the other hand, have similar respective values. In both organs, the secretory or zymogen granules have a higher nitrogen and sulfur content, but a lower phosphorus, carbon and hydrogen content than the corresponding small particles. In their gross chemical composition, therefore, the secretory granules, whether from liver or pancreas, are fundamentally alike. The similarity of their elementary structure suggests that these granules represent differentiated members of a single class of cytoplasmic organs which are built on an identical framework, in spite of the specialized and exclusive functions which they may be called upon to perform in organs as dissimilar as the liver and the pancreas.

TABLE II

FRACTIONATION OF BEEF PANCREAS BY DIFFERENTIAL CENTRIFUGATION: CHEMICAL COMPOSITION OF SMALL PARTICLES AND ZYMOGEN GRANULES. (AVERAGE VALUES FROM 5 EXPERIMENTS)

Fraction	N	P	C	H	S
Small Particles	9.16	2.11	57.88	9.06	0.46
Zymogen Granules ...	11.94	1.88	50.39	7.82	0.69

Relation between Microsomes, Mitochondria and Secretory Granules: The studies reported above indicate that, on the basis of general physical properties and elementary chemical composition, there exist two definite classes of cytoplasmic elements, namely, the microsomes and the secretory granules. On the other hand, it has been shown previously that small particles and secretory granules are chemically related, both being complex formations composed of phospholipids and ribonucleoproteins associated in characteristic proportions.[4,5] These findings raise the important problem of the origin of the particulate components of cytoplasm and that of the possible developmental relationship which may exist between the microsomes and the other cytoplasmic structures. The secretory granules from guinea pig liver and from beef pancreas disintegrate spontaneously, when kept

in distilled water, leaving a residue composed of particles which form jelly-like pellets in the centrifuge and which, on analysis, were found to contain about 9 per cent. nitrogen. Thus, the secretory granules seem to contain a substance physically and chemically similar to the so-called "small particles." This observation may suggest that the secretory granules develop from the microsomes or that they have a common origin.

In 1934, Bensley and Hoerr isolated from the liver of guinea pigs a fraction referred to by them as mitochondria.[9] From its physical characteristics the fraction of Bensley and Hoerr seems to correspond to our liver secretory granules. However, inasmuch as the chemical analysis previously reported by these authors is different from our findings, it is impossible to know at the moment whether the two fractions are really identical. Secretory granules are abundant in the guinea pig liver, especially in the fasting animal, where they accumulate and seem to fill the cell completely, and it appears probable that up to the present, mitochondria have not been isolated in a pure or concentrated form, a large part of the so-called "mitochondria" fraction representing probably, to a large extent, mature secretory granules.

Nucleic Acids and Cell Structures: One point which may be discussed in the light of the new findings is that of the origin of the granular substance of cytoplasm. The following considerations suggest that the distribution of nucleic acids in the cell is intimately connected with this problem. Thymonucleic acid has been shown to be exclusively a nuclear constituent. More precisely, it constitutes the distinctive substance of chromosomes. Thanks to the Feulgen technique, it has been possible to show that thymonucleic acid is found nowhere except in the nucleus and that it is present equally in nuclei of animals and plants as well as in homologous formations of more primitive cells such as yeast and bacteria.

Ribose nucleic acid, a close relative of thymonucleic acid, has been known for some time to occur in animal cells. Caspersson and Schultz have shown recently that it is a constituent of nucleoli.[10] Work in this laboratory brought attention to the fact that ribonucleic acid in the cytoplasm is localized on particulate or granular structures. This was first demonstrated for the small particles[4] and more recently for the secretory[5] and zymogen granules. This observation assumes exceptional significance if we consider the fact that, so far, any organic structure which has been found to possess directly the property of self-duplication has also been shown to contain nucleic acid of the one or the other type. The outstanding example is the chromosome. The relation is even more striking when the self-perpetuating unit is not a complex structure but a simple substance such as the autocatalytic nucleoprotein of Stanley. The crystallized viruses of plants are nucleoproteins. Other viruses which have been successfully purified have proved to have a nucleoprotein as a major constituent. This is the case for the Shope papilloma virus.[11] The agent causing Chicken Tumor I has been shown to depend on the integrity of a nucleoprotein for its activity.[12] It appears unlikely that nucleic acid represents no more than an inert substrate whose main purpose is to hold the structure together, as it has often been suggested to be the case in the chromosome. In the light of modern research, it seems probable that nucleic acid plays a fundamental role, perhaps of an enzymatic nature, in the process which enables the structure to reproduce itself.

In the process of cell division, the duplication of chromosomes can be followed under the microscope. There is evidence that the centrioles and certain plastids in plants are reproduced by auto-division.[2] De Vries attempted to show that this was also the case for the tonoplasts. Outside of these isolated cases, there is no satisfactory explanation to account for the perpetuation of cytoplasmic structures, particularly for the increase in granular substance, which must necessarily take place at each mitotic division. Two mechanisms for the perpetuation of these structures are possible: either each element has the power to reproduce its own species or it is being produced by an outside agency which, itself, must be self-perpetuating. The findings that the small particles or microsomes and the secretory granules contain ribonucleic acid suggest that these cytoplasmic constituents, like the other nucleic acid-containing structures, may be endowed with the property of self-duplication. The latter assumption, which should be no more than a working hypothesis, offers a biochemical basis for the view that each vital element which contributes actively to the life of the cell has the power to reproduce its kind. Except for the plant plastids, the experimental proof that other differentiated cytoplasmic granules reproduce in this manner have been elusive and it has often been suggested that these granules are formed *de novo* in the ground substance. The existence of a reservoir of self-perpetuating microsomes from which the specific granules may develop would provide a satisfactory answer to this problem but much research will be needed before this point can be clarified. If our hy-

[9] R. R. Bensley and N. L. Hoerr, *Anat. Rec.*, 60: 449, 1934; R. R. Bensley, *Anat. Rec.*, 69: 34, 1937.

[10] T. Caspersson and J. Schultz, *Proc. Nat. Acad. Sciences*, 26: 507, 1940.

[11] J. W. Beard, A. R. Taylor, D. G. Sharp and D. Beard, *Surgery, Gynecology and Obstetrics*, 74: 509, 1942.

[12] A. Claude and A. Rothen, *Jour. Exp. Med.*, 71: 619, 1940.

pothesis regarding the mode or origin of the microsomes is correct, then these small particles would share with the cell itself, and within the cell, with the chromosomes, the centrioles, the plastids and possibly the tonoplasts, the most general law of living matter, that of genetic continuity. It must be emphasized that the above conception is concerned exclusively with the biochemical aspect of the origin and evolution of the granular substance of the cytoplasm. It does not deny the possibility that the cytoplasmic constituents may come, in the course of their evolution and activity, under the influence of the nucleus.

THE UTILIZATION OF AQUATIC FOOD RESOURCES

By Professor CHANCEY JUDAY
THE UNIVERSITY OF WISCONSIN

THE food and forage situations in Europe during the past three years have stimulated discussions regarding the availability of certain aquatic plants and animals not now generally used as sources of such material. The utilization of large aquatic plants as forage for animals and the use of both marine and fresh-water plankton as sources of human food have been mentioned. Little has been said, however, about the quantity of these materials found in fresh waters, and a brief consideration of this phase of the problem may be worth while.

Large aquatic plants have been used as forage for cattle in Yugoslavia for many years,[1] and it has recently been reported that they are now being used extensively for the same purpose in Sweden owing to the scarcity of fodder in that country. While these plants have a rather high mineral content (10 to 35 per cent. ash), they contain considerable quantities of nutritious materials; protein makes up 12 to 25 per cent. of the dry weight, fat 1 to 3 per cent. and the remainder of the organic matter consists of carbohydrates, of which crude fiber constitutes 16 to 21 per cent. Using the averages of these percentages of organic matter and assigning 4 calories to each gram of protein and of carbohydrate and 9 calories to each gram of fat, their energy value is about 1,450 calories per pound, dry weight. A mean of 18.5 per cent. of the dry plants consists of crude fiber and the greater part of this may be regarded as indigestible; deducting this part of the carbohydrate would leave an energy value of 1,100 calories per pound for the digestible organic matter in the plants.

Rather large crops of these plants are found in some of the Wisconsin lakes; in Mendota, for example, the annual yield has been estimated at 2,100 tons of air-dry material, or about one ton per acre of the shallow area in which they grow.[2] In Green Lake the crop was estimated at 1,600 pounds per acre, air-dry, in the shallow water zone and the total crop at 1,528 tons. In the soft-water lakes of northern Wisconsin, the yields of large aquatics are much smaller, ranging from 10 to 100 pounds per acre in the vegetated zones.

With respect to the use of plankton for human food, Clarke[3] has discussed this problem from a marine standpoint, referring especially to the plankton crustacea, while Hardy[4] and other authors have called attention to the possibility of using fresh water as well as marine plankton for food; both phytoplankton and zooplankton have been mentioned in some of the communications. It has been pointed out that the chief difficulty is to obtain enough plankton material to warrant the labor involved in collecting it. The smaller organisms which make up the great bulk of the plankton are especially difficult to capture. One author has suggested the use of the tons of plankton collected on the filter beds of cities that filter their water supplies, while others have considered various types of nets. The latter capture only the larger organisms, chiefly zooplankton forms, which usually constitute not more than 10 per cent. of the total plankton and frequently as little as 5 per cent.

Data obtained on Wisconsin lakes show that the dry organic matter of the plankton found in them ranges from a minimum of half a gram in the soft-water lakes to a maximum of 9 grams per cubic meter in some of the hard waters. This minimum in Crystal Lake represented a standing crop of 45 kilograms per hectare (41 pounds per acre), while the maximum in Lake Waubesa indicated a standing crop of 966 kilograms per hectare, or 862 pounds per acre; the live weight of this dry organic matter would be ten times as large, since 90 per cent. or more of the weight of the living organisms consists of water. The mean standing crop of plankton in Lake Waubesa over a period of two years was 242 kilograms per hectare, dry weight, or 216 pounds per acre, of which 49 per cent. consisted of protein, 5 per cent. fat and the

[1] Vilim Mršić, SCIENCE, 83: 391, 1936.
[2] H. W. Rickett, *Trans. Wis. Acad. Sci.*, 20: 501, 1921, and 21: 381, 1924.
[3] SCIENCE, 80: 602, 1939.
[4] *Nature*, 147: 695, 808, and 148: 115, 143, 314, 375, 1942.