The Structure and Function of the Nucleoli in Normal and Pathologic Cells[1]

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DURING the last three years and in spite of very trying working conditions, we carried on a comparative study of the cell elements of malignant lymphogranuloma (Sternberg cells of Hodgkin's disease) and of the reticulosarcomata. Abundant material for these studies was obtained by the process of organ puncture (puncture of lymphatic ganglia, bone-marrow, spleen, and liver) from patients at the St. Antoine Hospital in Paris: particularly at the Institute of Hematologic Investigations directed by Dr. A. Tzanck, and at Professor Loeper's clinics. The findings of this research will be published in full in another paper, but they led us to consider the problem of the structure and functioning of the nucleoli from the general viewpoint of cytology. In effect, the hyperplasia of nucleolar masses, their metachromasis, their deformations, surpassing sometimes in these diseases the nuclear monstrosities observed, claim the attention of the investigator and force him to attribute physiologic and physiopathologic importance to that part of the cell undergoing such changes.

Our present knowledge concerning nucleolar histochemistry and functioning does not allow us to establish conclusions of any great value. Such knowledge might be summarized as follows: (1) Cells with high potential capacity for development, reproduction, and further differentiation (such as the ovocytes of animals in general, or the primary cells of the blood of mammals: hemohistoblasts, hemocytoblasts, myeloblasts) possess multiple nucleoli (two to four, rarely five) which are spheroidal or ovoid in shape and unmistakably basophilic; (2) to all appearances there exists during the process of ovogenesis a close relationship between the chromosomes and the nucleoli (Brachet, Grassé and Lespéron, Gavaudan, Duryee and others); (3) all traces of the nucleoli are lost during the maturation of the ovum as well as during the maturation period and the functional differentiation of the primary cells of the blood; (4) the nucleoli seem to be linked with the growth of the chromosomes; perhaps at the beginning of the prophase, at the start of caryocinesis,

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the nucleoli may have some direct relation to certain special materials strictly localized in one definite point or area of one chromosome (Heitz and McClintock); (5) the nucleoli disappear during mitosis, later to reappear in two newly formed cells should these be homologous cells, from a functional viewpoint, as regards the mother cell; (6) metachromatic phenomena (such as loss of basophilia, change to violet, to a more or less intense lilac, to pink, when basic Romanowsky stain is used) are observed in the nucleoli of the pathologic cells of blastomata and in serious inflammatory disturbances of the hemopoietic organs or of the reticulo-endothelial system, such as malignant lymphogranuloma; (7) the nucleoli of the ovocytes (the only ones studied up to now) do not give a characteristic Feulgen reaction to thymonucleic acid; (8) however, in accordance with Brachet's observations of the ovocytes of amphibians, it is possible that the nucleoli may throw off at the time of maturity small quantities of newly formed thymonucleic acid, perhaps synthesized from other pre-existing substances; (9) by means of the Feulgen test and of microcinematography the ovocytes of the rat (Moricard) show a positive formation of thymonucleic acid (positive Feulgen) at the limits of the nucleolar gel end of the nuclear juice (nucleolar membrane), corresponding to the formation of the chromatic loops of the chromosomes; (10) the nucleoli contain glutathion (as proved by Joyet-Lavergne through the precipitation method of Binet-Weller with cadmium lactate) and it is therefore probable that they intervene in the oxygen reducing processes of the nucleus.

In vegetable cells (Zea), McClintock has proved the existence of a chromomere, a part of chromosome 6, that reconstructs or helps in the reconstruction of the nucleolus during the telophase. If we accept McClintock's designation, this chromomere may be known as a "nucleolar organizer." His investigations date back to the year 1934. Other studies of Gardiner, Geitler, Manton, Upcott, and McClintock himself (1935–1936) seem to confirm this phenomenon as also existing in animal cells and apparently establish a relationship between the nucleolus and the chromosomic system of the nucleus.

Darlington, in his book on cytology (1937), has summarized the facts gathered in the series of studies mentioned above and emphasizes the trophic function of the nucleoli as regards nuclear energesis during the periods preceding mitosis. However, we know almost nothing concerning nucleolar microchemistry or about those substances that make up the nucleolar mass during the various phases of

development and maturity of cells, whether these be normal (successive changes of the blood-forming cells in the hemopoietic organs, arising from multinucleated hemocytoblasts) or pathologic (blastomata's cells with great nucleolar hyperplasia).

The data which we have at hand are therefore rather limited. In his monograph on *Le Rôle physiologique et morphogénétique du noyau*, Brachet (1938) affirms that "en ce qui concerne la nature et les fonctions des nucléoles, on en est reduit aux hypotheses." Anything that may therefore contribute to the knowledge of the structure and the histochemistry of the nucleoli must be of general interest to cytologists.

From this point of view the study of the nucleoli of primary undifferentiated cells and of the pathologic cells of the blood would seem particularly interesting. In fact, this study would break away from the working material that has been almost exclusively utilized until now by cytologists, that is, the ovocyte (ovocytogenesis and development of the ovum in animals of different species), and would therefore enrich the field of cytologic investigation. At the same time it offers us abundant material that is easily available, thanks to the recently adopted technique of bone-marrow puncture of the sternum (myelogram) and of organs such as the lymphatic ganglia, the spleen, and the liver. It, at last, leads us back, apropos a question in general cytology, to the examination of problems that affect human physiology and physiopathology.

We are giving in the following paragraphs the results of our observations conducted by means of comparative studies of various preparations (bone-marrow smears) and microtomic sections (biopsies) of the bone-marrow, lymphatic ganglia, spleen, and liver, on which we carried out Feulgen's test, in addition to the study of the phenomena of metachromasis and the changes suffered by the nucleoli in mass and shape, especially those of the reticular cells—the hemohistoblasts, histocytes, and hemocytoblasts—from organs of numberless patients suffering from various diseased conditions.

I. THE FEULGEN TEST

Known for some time (Feulgen and Rossenbeck, 1914), Feulgen's test is in fact a specific application of the Schiff test for the determination of thymonucleic acid, producing a reddish violet coloration in the presence of aldehydes when these act on a basic fuchsin solution, decolorized beforehand by sylphurous anhydride. In the Feulgen test thymonucleic acid works as a reducing agent after partial

hydrolysis by hydrochloric acid. Thymonucleic acid which is relatively labile is broken up into its components, to wit: phosphoric acid, glucide, purine bases (adenine and guanine) and pyrimidine bases (cytosine, thymine). Glucide (carbohydrate) is really the reducing agent, in this specific instance, a desoxipentose; but in accordance with the studies of Levene and his school, it is even more specifically a d-ribodesose or thyminose. This substance, and it alone, is capable of recovering under the conditions of the Feulgen test the characteristic reddish violet coloration of Schiff's reagent previously decolorized. This test is exquisitely sensitive. In fact, it permits us to determine the presence of thymonucleic acid among the nuclear material, distinguishing it from pentose-nucleic acid, or zymonucleic acid, which is characterized by groups of nucleosids whose carbohydrate is d-ribose. The technique for this test, however, requires extreme care. Particularly, it is absolutely necessary always to employ control preparations, submerged without previous hydrolysis in Schiff's reagent. Under such conditions, the reaction should be negative. We shall not go into detail here regarding the technique utilized. Aside from slight modifications suggested to us by practice and the material used, we followed the exact procedures indicated by Lison in his *Histochimie animale* (1936), and by Moricard in his monograph, *Les Facteurs hormonaux et cytoplasmiques de la division cellulaire* (1940).

The results of our observations are as follows:

1. The nucleolar substance, clearly circumscribed within the nucleus of reticular cells, of hemohistoblasts, of histocytes, of hemocytoblasts, myeloblasts, and lymphoblasts, and of megaloblasts, never gives a positive Feulgen reaction. It must be concluded, therefore, that the nucleolar substance of these cells does not contain preformed thymonucleic acid.

2. Under such circumstances—let us make clear that we are referring to cells during their resting period from the point of view of reproduction—the nucleolus, or nucleoli, are surrounded by an edge, sometimes thick and dense, sometimes very finely granulated, made up of a substance that gives a clearly positive Feulgen reaction. This "perinucleolar crown," oftentimes made up of the juxtaposition of small masses colored an intense reddish violet by Feulgen, can be easily distinguished, thanks to this reaction, from the chromatin network of the nucleus. In nuclei of the lepto-hypochromatic type, characteristic of this group of cellular elements, this chromatin network presents a similar reddish-violet coloration, but somewhat

paler. On the other hand, in the proerythroblasts, the chromatin masses give an intense positive Feulgen reaction of a deep wine-red color, and the chromatin network always envelopes the nucleolus when this last exists and is visible (always Feulgen negative) in such cellular elements.

3. Preparations from this same material, obtained from bone-marrow punctures, lymphatic ganglia or spleen, when stained by the Romanowsky method (Giemsa or some other) show, as is well known, basophilic nucleoli of a rather palish blue color, but it is not possible, in the majority of cases, to observe the perinucleolar formation which the Feulgen test reveals.

4. The "visualization," or visibility of the nucleoli is, therefore, more clearly observed thanks to the Feulgen test which brings out a negative image of the nucleolar substance circumscribed by a crown of positive Feulgen substance, that is, a crown of chromatin rich in thymonucleic acid.

5. These same cells, when in mitosis, offer very interesting images by means of this same test. At the beginning of the prophase, the perinucleolar crown of positive Feulgen substance appears more dense on certain occasions; however, more frequently it acquires a diffuse aspect the while one can note a true dissolution or disintegration of the nucleolus, which loses its characteristic of being a circumscribed mass within the nucleus. Its presence is hardly recognizable, appearing blurred or dissolved in the nuclear juice among the forming chromosomes that are stained a deep reddish violet by the Feulgen test.

6. Pathologic cells of like mesenchymal origin from innumerable cases of reticulosarcomata, reticuloendotheliomata, histocytomata, sarcomata, melanic sarcomata, and Hodgkin's disease (Sternberg cells) studied under the same technique, show, besides nucleolar metachromatic hyperplasia (Giemsa) always negative to the Feulgen test, a perinuclear corolla or crown of positive Feulgen substance, sometimes very dense, often distinctly separated from the lax chromatin network of the heavy nuclei characteristic of these cellular elements and which at times attain monstrous proportions.

7. Certain well-defined differences that coincide with differences in nuclear mass and structure can be noted in both the nucleoli and the positive Feulgen perinucleolar crowns distinguishing from each other the various types of pathologic cellular elements mentioned above. Thus, it is possible to record specific distinctions between: (a) blastomatosic cells of the reticulosarcoma; (b) of the reticuloendothelioma, *strictiori sensu* (Oberling); (c) of the histocytoma or histocytosarcoma; (d) of Hodgkin's lymphogranuloma (Sternberg cells). The first (a) show almost always a degree of nucleolar hyperplasia that is really impressive; the nucleoli—of a huge size at times—are surrounded by positive Feulgen perinucleolar crowns that are very dense, thick, and compact and which send out radiations of the positive Feulgen substance from the nucleolar periphery towards the periphery of the nucleus, but rarely reaching the nuclear membrane. In other instances, the positive Feulgen perinucleolar crown is broken up into small scattered masses. On the other hand, the cellular elements of the second type (b), that is, of the reticuloendotheliomata, strictly speaking, offer greater uniformity in nucleolar images, exhibiting almost always a single and less voluminous nucleolus which never reaches the exaggerated hyperplasia characteristic of the nucleoli of the reticulosarcomata. The nucleolar mass, fairly regular in shape, metachromatic in the majority of instances—even though at times it retains to a certain degree the normal basophilia—and always Feulgen-negative, is surrounded by a more finely granulated corolla of positive Feulgen perinucleolar substance which, on occasions, seems to be separated from the nuclear chromatin network by a colorless, annular space. The third type (c) frequently discloses numerous nucleoli (four or five, rarely more), usually small and also surrounded by a delicate border of positive Feulgen substance. Although the images are less uniform in this type than in the preceding one, as regards the shape and dimension of the nucleoli, these never reach the degree of hyperplasia observed in (a). One is dealing here always with negative Feulgen nucleoli, metachromatic to the Romanowsky basic stains although they sometimes display a slight basophilia. Sternberg's cells, (type d) are quite different. The nucleoli here show the most frequent and radical alterations in shape. While hyperplasia is characteristic of the nucleoli of the blastomic cells of the reticulosarcomata, deformation (misshapen condition) capable of reaching monstrous proportions is typical of the nucleoli of Sternberg's cells in malignant lymphogranuloma. Let it be clear that one always finds gross nucleolar masses, huge at times, but their variegated, multilobulated appearance is more frequent and more pronounced than the increase in bulk of the nucleolar mass. On the whole, nucleolar hyperplasia is more spectacular in the cells of the reticulosarcomata; the deformation is more characteristic of the nucleoli of Sternberg's cells. Furthermore, in these last, the perinucleolar

crowns of positive Feulgen substance are less accentuated, frequently missing, and the nucleolar mass sometimes appears diffused and not well differentiated from the nuclear juice. Nonetheless, Feulgen's reaction is negative for this nucleolar substance.

8. A secondary light-green coloration (*verde-luz*: acid green: sodium salt of tri-sulpho-dimenthyl-dibenzyl-diamido-triphenyl-carbinol acid) of the preparations, previously stained by the Feulgen method (complementary coloration) makes the reddish violet of the positive Feulgen substances stand out intensely. The perinucleolar edges and crowns appear strongly silhouetted because the nucleolar masses acquire a slight, delicate green color, sometimes rather accentuated, not only in the pathologic cells with metachromatic nucleoli but also in normal cells of the hemopoietic series. It is thus proved that the substances that constitute the nucleolar structure are not made up exclusively of basophilic material.

9. A goodly number of blastomic pathologic cells, particularly those of the reticulosarcomata, appear more or less profusely vacuolized, as is well known. The vacuoles, rather large on occasions, are not limited to the cytoplasm but also seem to perforate through the whole thickness of the nucleus. Such nuclear vacuoles are never surrounded by a crown or an edging of positive Feulgen substance. It is easy to distinguish in these cells, together with the vacuoles, the Feulgen negative nucleolus or nucleoli which are always surrounded by a more or less thickened perinucleolar crown. In our way of thinking, these images proved that the crown of material rich in positive Feulgen thymonucleic acid could not be attributed to an inert accumulation or concentration caused by a merely mechanical or physical phenomenon taking place around a nuclear lesion, or around a mass of substance of physiochemical makeup different from that of the nucleus, as the nucleolus undoubtedly is. On the contrary, one deals here with a process of perinucleolar neoformation of positive Feulgen substance in whose production the nucleolus has a part through the diffusion of material indispensable in the synthesizing of thymonucleic acid.

10. The thick caryosomes of certain cellular elements (very frequently found, for example, in the nucleus of the proerythroblasts) give a very intense positive Feulgen reaction. We, therefore, have to consider them as being very rich in performed thymonucleic acid, while the Feulgen-negative nucleoli may be regarded as reservoirs for materials (pentosenucleic acid or zymonucleic acid?) necessary in the production of thymonucleic acid.

11. During the process of maturation and functional differentiation, the primary blood cells (hemocytoblasts, myeloblasts) lose their nucleoli through the phenomena of dissolution, of disintegration, of endonuclear lysis (nucleolysis), similar in all respects to the process of nucleololysis observed in chromosome formation during mitosis. In the granulocytic series of leucocytes, for example, the transition forms (premyelocytes) may occasionally show a nucleolus or remains of nucleoli, which are never circumscribed or surrounded by Feulgen positive edges or perinucleolar crowns. The cytoplasmic granulations (azurephils) appear during the transition period—myeloblast to myelocyte—sometimes already accompanied by specific acidophilic or neutrophilic granulations. The completely differentiated myelocytes (eosinophilic myelocytes and neutrophilic myelocytes), whose nuclear chromatin gives a positive Feulgen reaction, do not possess now any trace of the nucleoli. The premyelocyte phase, with its various cytologic characteristics, whose significance and diagnostic interest we have discussed elsewhere (*Sur les caractères cytologiques du promyelocyte et sur le hiatus leucaemicus*, *Le Sang*, Paris, 1939), represents the transformation period of the myeloblast into myelocyte, when the nucleolus is lost. Differentiated myelocytes and mature granulocytes (eosinophils and neutrophils) never possess the faintest trace of nucleolar substance. We are led to conclude, therefore, that nuclear cinesia, occurring during reproduction (mitosis) of these cells, as well as the specific activities—glandular in nature—of cellular energesis and metabolism that give such cells a definite and non-revertible character, condition the existence of the nucleoli and that the latter, at the moment of disappearance by the process of nucleololysis, take part in the metabolism of the nucleus (enriching it in thymonucleic acid) by means of a physiochemical mechanism of molecular interchange.

II. METACHROMASIS

Numerous cellular elements, particularly those of mesenchymal origin, (reticular cells, macrophages, histocytes, polyblasts, and hemocytoblasts) show a nucleolar alteration during diverse pathologic conditions, independent of the modifications they may suffer in size and shape. In fact, whatever may be the degree of hyperplasia and deformation of the nucleoli, these do not show in such instances that selective affinity towards methylene blue (basophilia) which, in the preparations stained by Romanowsky's method, gives to the nucleoli of normal cells their characteristic blue tint (pink

when stained by Pappenheim's pyronine-methyl green). Under the above conditions, the nucleoli of the pathologic cells show a different coloration: lilac, purple, violet blue, more or less intense reddish violet, with a shading of different tones in the same nucleolar mass (Romanowsky). We have studied these phenomena of nucleolar metachromasis in hundreds of blastomic cells (reticulosarcomata, reticuloendotheliomata, histocytomata, melanosarcomata), of Sternberg cells in malignant lymphogranuloma, of pathologic histocytes from fungoid mycoses, of pathologic reticular cells from lymphatic ganglia in Nicolas-Favre disease, and in zona (herpes zoster). In this last disease (gangliar puncture), the presence of rather small metachromatic nucleoli of a deep violet color (Giemsa), frequently sharply outlined by a delicate perinucleolar crown, is observed in a large number of cells of the histocyte-cianophil type (histocyte-plasmocyte). However, these same cells at times exhibit more voluminous metachromatic nucleolar masses, more or less modified (nucleololysis), surrounded by a very definite colorless halo, or zone. This same material may show healthy reticular cells, normal to all appearances, with small, basophilic nucleoli, almost always very pale (hypocromasia). In Nicolas-Favre's disease (poradenitis inguinale), the gangliar puncture reveals slightly marked lesions of the nucleoli during the intermediary period in the evolution of this morbid process. The nucleoli of the numerous macrophages of reticular origin appear sufficiently hypertrophied, sometimes very voluminous, somewhat misshapen (like a three-leaved clover, or else elongated, lobulated or in the form of a horseshoe, etc.), more or less plainly basophilic. The mass of histocytes accumulated or proliferated in the ganglion do not show, as a rule, anything but small basophilic nucleoli. In such inflammatory processes due to an ultravirus, the cells of mesenchymal origin of the ganglionar lesion reveal, therefore, a collection of evolutional nucleolar changes, relatively not too pronounced, representing successive phases of nucleolar degeneration that later reaches a higher degree of hyperplasia, deformation, metachromasis and complete disappearance of the perinucleolar crown of positive Feulgen substance.

Such changes reach a maximum stage, with really extravagent characteristics, in the Sternberg cells of Hodgkin's disease. Here, the hyperplasia of the nucleoli and especially their deformation (causing utterly bizarre shapes) and their metachromasis are extremely pronounced, even though one can observe all of the intermediate phase beginning with the basophilic nucleoli of the reticular cells. It is

well known that lymphogranulomatous tissue offers a large variety of cellular types in the different zones, or areas, of the same ganglion (or other organ). Microscopic examination, particularly of the smears made from punctures of lymphogranulomatous ganglia, sometimes reveals accumulations of reticular cells, to all intents healthy ones, with nucleoli of a reduced size, basophilic, and surrounded by a fine chromatin corolla or perinucleolar crown of positive Feulgen substance. At the same time, the examination also reveals elements of the same nature with hypertrophied metachromatic nucleoli that mark the transition towards the Sternberg cells (properly speaking), which display monstrous nuclei in cariolysis and metachromatic nucleoli, diffused and enormous, without any defined edge or perinucleolar crown.

Lastly, the blastomic ganglionar cells which have been studied by us with special reference to the reticulosarcomata and to the metastasis of sarcomata of varied origins, among which are two cases of melanic sarcoma, show the highest degree of nucleolar hyperplasia. We are referring here to a global hyperplasia of the nucleolar mass, since there are frequently found multiple nucleoli (up to twelve or more in number), scattered throughout the nucleus. This hyperplasia of the nucleolar mass, with gross metachromatic nucleoli, reaches its maximum in certain blastomic cells of the reticulosarcomata.

The phenomenon of metachromasis has been known for a long time. However, a satisfactory explanation has not yet been found for it, in spite of the work of Michaelis, Hansen, Ostwald, and others, leading up to the most recent researches of Lison (1935). As Langeron points out, it is possible that the stain responsible in the Romanowsky reaction (Giemsa, May-Grünwald-Giemsa, Tribondeau, Wright, Pappenheim's Panchromo, and others) for the production of a marked metachromasis in the presence of certain substances, whether these be cytoplasmic (granulations of the mastocytes), nuclear or nucleolar, might be methylene violet. This stain acts when mixed with methylene blue, from which it is derived through alkalinization, and always exists in old Giemsa solutions, as has been demonstrated since 1906 by the observations of Pappenheim, MacNeal, and many others. If the presence of methylene violet were indispensable (necessary and sufficient) to determine the phenomena of metachromasis, we would be in fact dealing with an "allochromasia" in the sense of the word as used by Michaelis, that is to say, a selective affinity for a particular coloring matter,

within the combination of basic dyes, on the part of certain substances of the cellular structure. At any rate, we are always dealing with basic dyes whose metachromatic (or allochromatic) staining shade or tint corresponds to the color of their free base. Thus, for example, thionin (chlorhidrate of amido-diphenyl-thiazine), a bluish violet stain, bestows that color to the greater part of the basophilic material tissues, or cells; on the other hand, it stains red the fundamental substance of cartilage, mucine, the cytoplasmic granulations of mastocytes (Cajal's fattened cells), the metachromatin or volutin of the protista and the nucleoli of the pathologic cells already mentioned.

Now, this metachromatic veering or turn in color is not an accidental staining phenomenon. One deals here with a true microchemical, or histochemical reaction that may be reproduced experimentally in test tubes with solutions of chemically pure substances (Lison). A pure solution of chondroitin-sulphuric acid, which is a characteristic of cartilage, immediately turns a solution of metachromatic stain (thionin, aged methylene blue). In the same way, the mucoitin-sulphuric acid of mucine produces identical metachromasis that corresponds to that of the mucous substances of the tissues in the presence of stains of like kind. In order to establish the phenomenon of metachromasis, the following are necessary: (a) a basic stain capable of veering or turning in color; (b) a substance that is capable of producing such a change when it comes into contact with the stain. We know a group of substances gifted with this property. Levene and his collaborators went deep into this matter and proved that these substances constitute the prosthetic group of conjugated proteins known by the names of "glucoproteids" or "mucoproteids." They are in fact sulphuric esters of polysaccharides (polyoses) of high molecular weight. Different substances with a molecular make-up analogous to that of chondroitin-sulphuric and mocoitin-sulphuric acids and belonging to the group above mentioned are found scattered throughout the flora and the fauna. All of them produce a metachromatic change in the presence of the stains already mentioned.

Under various pathologic conditions, similar substances are formed by virtue of complicated processes of tissue or cell degeneration that involve deep modification in the molecular constitution of the proteins. Amyloid degeneration is probably an example of such processes; amyloid substances belong to the group of the chondroproteids. We are not yet in a position to identify the involutional or degenerative phenomena of the nucleoli—as described in the preceding pages—with the processes involved in the transformation of cellular nucleolar protides and glucides into sulphuric esters of the polysaccharides. We do not yet know the chemical make-up of the substances that produce nucleolar metachromasis. Nevertheless, it is extremely probable that we are dealing with substances of this same type, belonging to the group of the glucoproteids that react, as has been said, in like manner, producing on certain basic stains the same chromatic change. These substances of involution, signs of a degenerative breakdown in the normal microchemical structure of the nucleoli (metachromatic nucleoli), are, no doubt, derived through a process of sulpho-esterification of the polyoses (d-ribose of pentose-nucleic acid?) from the constituent material of the nucleoli in their normal state. If we should apply to these nucleoli Caspersson's technique, based on the absorptive power of the nitrogenous bases of nucleic acids in ultraviolet light and in their previous digestion by lanthanum-trypsine, the results might at some future date contribute to the clarification of these problems.

III. NUMBER, MASS, SHAPE, AND CONSISTENCY OF THE NUCLEOLI

We have seen that the primary cells of the blood (above all, in the hemocytoblastic phase) can and usually do contain several nucleoli within their nucleus. These are pale hyperchromic basophilic nucleoli. Their number is almost always below a possible maximum of five small definitely individualized nucleolar masses. It is only in the pathologic cells, particularly in blastomic elements (including the rare condition described by the name of "hemocytoblastoma") that a larger number of nucleoli is found (the maximum observed by us was fifteen nucleolar masses).

The total mass of the nucleoli, considered as a whole, is much more interesting than their number. Even in terms of surface, as revealed by the cellular images, the total mass of the nucleoli gives us valuable clues to establish the phase of phylogenetive evolution of the cells, the period of functional differentiation in the normal series (polyblast-hemohistoblast-hemocytoblast-myeloblast . . .), or their pathologic degeneration. The Mexican hematologist, González Guzmán, has been carefully studying this problem since 1936 and it is to him that we owe important suggestions for the procedure which may fix the evolutionary phase and the physiopathologic condition of the cell in accordance with the comparative sizes of the

total nucleolar mass and that of the nucleus. The data gathered from these researches, confirmed by the majority of investigators, show that the cellular elements attain a maximum size in their nucleolar mass during the period of functional differentiation; that this diminishes rapidly thereafter; and that this maximum limit, which we could call "physiologic limit of nucleolar magnitude," is quite overstepped only under pathologic conditions in the cellular elements of serious morbid processes, especially in the blastomata (reticulosarcomata, etc., of the hemopoietic organs). The hyperplasia of the nucleolar mass runs an even course, in this instance, with alterations in shape. However, hyperplasia is the most significant finding in blastomatosic cells of the reticulosarcomata, histocytomata, etc. Sometimes spheroidal or ovoid nucleoli, 12 to 16 micra in diameter, are found inside nuclei having a diameter of 22 to 25 micra. Sometimes these are multiple nucleoli (six, eight, ten nucleolar masses), of a rather regular round shape, whose total aggregate is usually considerable, oftentimes enormous, in comparison to the nuclear mass. The reverse is usually true in Sternberg cells of malignant lymphogranuloma (Hodgkin's disease), the deformation or alteration in shape of the nucleoli—corresponding to the changes in appearance, at times monstrous, of the nucleus—predominates over the hyperplasia. One can then see lobulated nucleoli, elongated or curved, in the shape of a horseshoe, or in the form of knots, etc.

As regards the consistency of the nucleoli, we shall state here only some of the findings arrived at through personal observation. The images examined in preparations of pathologic material (ganglionar puncture, melanic sarcoma) lead us to conclude that under such circumstances the nucleoli present at times a rigid, semisolid consistency with regard to the gel of the nuclear mass. In effect, such nucleoli separate easily from the main mass of the nucleus by simple traction. Sometimes they appear adhering to the edge of the nuclear membrane, on the outside of the nucleus, with their boundaries clearly defined. In such cases, the nucleoli are always made up of metachromatic substances. This "expulsion" of the compact and rigid nucleoli, produced by pressure or traction applied to the cellular material, has been noticed by us only on rare occasions and exclusively on sarcomatose cells.

Our contribution to the study of the structure and functioning of the nucleoli permit us, in spite of its modest limits, to arrive at a few conclusions. In accordance with almost all previous observation, carried out almost exclusively on ovocytes of different animal

species, we have seen that the nucleoli of the cells of hemopoietic organs (undifferentiated primary cells and pathologic cells) to all appearances do not contain preformed thymonucleic acid. On the other hand, we are able to confirm and supplement Brachet's observation (on the ovocytes of amphibians) and Moricard's (on the ovocytes of rats) as regards the intervention of the nucleoli in the formation of the perinucleolar crowns of positive Feulgen substance, rich in thymonucleic acid. The nucleoli probably contain reserve material for the synthesis of thymonucleic acid. This synthesis seemingly takes place in the virtual space between the nucleolus and the nucleus. The positive Feulgen perinucleolar crown, particularly characteristic of certain phases of cellular activity, should be considered as an expression or indication of the work of molecular interchange going on between nucleolus and nucleus. When chromosomes are being formed in the nucleus (prophase), rich in thymonucleic acid, a real dissolution or breakdown of the nucleolus is observed, the latter pouring all its substance into the nucleus during the process of nucleololysis. Coinciding with this, the chromatin of the chromatic loops seems to grow rich in thymonucleic acid when judged by the intensity of the Feulgen reaction. It is probable that among the materials that make up the nucleolar substance pentose-nucleic (zymonucleic) acid, which gives a negative Feulgen reaction, or a similar substance, may play an important role as reserve material for the synthesis of thymonucleic acid after the disintegration of the molecules. It is also conceivable that pentose-nucleic acid, or the nucleotids of similar molecular groups, may be broken up beforehand in their constituent elements due to the action of the nucleases, nucleotidases, and other ferments existing in the nuclear juice, so as to be later taken up and synthezised into thymonucleic acid when coming in contact with the chromosomes. The studies of Dalcq, Dalcq and Simon, Brachet, Caspersson and Schultz show that the synthesis of thymonucleic acid has characteristics in common with the biochemical reproduction of the molecules of virus-proteins, the best example of which is, perhaps, that of the virus of "tobacco mosaic," which contain a group of nucleic acid (Bauden and Père, 1937). The consumption of nucleic acid (thymonucleic) by the nucleus (nuclear chromatin, chromosomes) would have a source in the nucleotids and nucleosids of other acids characteristic of nucleolar substance. In certain pathologic conditions that we have examined above, this nucleolar substance changes its molecular structure when affected by a degenerative process due to profound

(toxic) modifications of its cellular metabolism. The hydrolysis of the nucleic acids produces molecular groups that, in the presence of sulphuric acid, give rise to the formation of sulphuric esters of polysaccharides and display the phenomenon of metachromasis.

It must be avowed that it is still necessary to fill with hypotheses the gaps of such an extremely difficult microchemical study, of observations that require abundant proof. In spite of it all, there is sufficient ground for affirming that the nucleolus exercises a trophic function during certain periods of the life of the cell and conditions nuclear activity in a certain way, depending on the availability of neoformed thymonucleic acid. In pathologic cells, the nucleolar substance is transformed too soon into metachromatic substance, an indication of deep modifications (metaplasia) in cellular activity.

T. B. (Trans.)

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