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CLINICAL AND EXPERIMENTAL

INTRACELLULAR STRUCTURES IN MONOCYTES IN CASES OF MALIGNANT DISEASE*

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THE occurrence of bodies of spiral form within the large mononuclear leucocytes in cases of malignant disease was noted in 1916[1] but without arriving at any definite conclusion as to their nature. It was considered possible that their presence might afford a means of diagnosis.

Since that time, a search for bodies of this kind has been made in every subsequent available case, whether hospital or private (in England), and it was found that when they were present, the case was very often one of malignant disease. It was, therefore, concluded that their presence could not be accidental, or a coincidence, but that they must represent genuine structures and have a positive significance. The problem remained as to whether they were possibly mitochondrial in nature, or perhaps belonged to the category of the inclusion bodies described in the case of virus diseases by various observers.

Technical Note.—Ordinary air-dried blood films are used. They are stained either by Pappenheim's panoptic method or by Leishman's stain. The latter is time saving and satisfactory. No other methods of staining fulfill the requirements. A strong light is essential for the examination. The endothelioid monocytes are specially studied. When the filamentous forms are not very distinct they are traceable by searching first for their nodal points.

Forms Noted.—Coccoid and bacillary (batonnet) forms are well known to occur in the mononuclear leucocytes of normal blood and of exudates. They

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vary in size from the ordinary azure granule to the granules and rods known as chondriomes and mitochondria found in various tissue cells. It is not these that form the subject of this communication, but linear forms; namely, (a) irregularly wavy or filamentous lines which may assume a definite spirillar form. These correspond to the chondriocontes of cytology (Fig. 1). (b) Curved or angular lines marked by nodal thickenings. Some of these are definitely flexed at the nodal thickenings and may be called "geniculate bodies." (c) Branching lines or "mycelioid" bodies. There is sometimes a round thickening at one end as shown in Cell 6 of the figure. (d) Other forms include (1) "beaded" forms, which appear as rows of fine dots lying in a linear vacuole. These correspond to

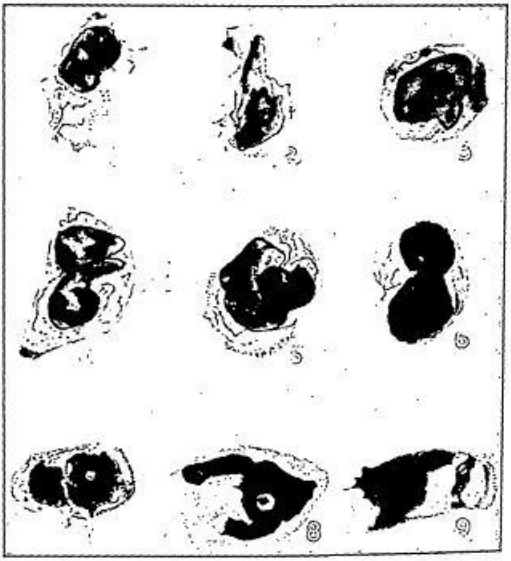

Fig. 1.—Nine cells are shown, each from a different case of malignant disease. Eight show cytoplasmic structures, and one shows an intranuclear body. Magnification: Slightly larger than that apparent with the ordinary oil immersion lens and medium high ocular. Cells 1 and 3 are hyaline leucocytes. The rest are monocytes. *Coccal forms:* Cells 1, 2, 4, 5, and 7 show the ordinary fine azure granules. Cell 1 also shows a single granule in a vacuole and a diploid body in a vacuole. *Undulant and spiral forms* are shown in cells 3, 4, 5, and 7. *Noded or beaded lines* in cells 1, 2, 4, and 5. *Spirillar form* with globular end in cell 2. *Mycelioid form* in cell 6. *Protozoal form* in cell 9. Cell 7 was considered possibly a sarcoma cell.

chondriomites of general cytology, (2) intranuclear bodies in vacuoles, showing a very fine granulation (Cell 8 of figure), (3) larger bodies occasionally occur which might easily be mistaken for protozoa.

Possible Errors of Interpretation.—Spiral bodies may be simulated by edges of cell substance wrinkled by spreading the film, so that an optical effect is produced. Nodal points may be passed by as being simply azure granules, the filaments being overlooked. Azure granules may appear to be connected simply

through an optical effect. Granules in intracytoplasmic clefts may appear as chondriosomes. Only forms which appear definitely red or purple red are accepted as genuine intracellular structures.

TABLE I

[illegible] WITH WHICH INTRACELLULAR BODIES WERE NOTED IN THE MONONUCLEAR LEUCOCYTES IN PATIENTS WITH MALIGNANT DISEASE

[illegible]

TYPE OF CASES	NO. CASES EXAMINED	% POSITIVE	[illegible]	[illegible]	[illegible]	[illegible]	TOTAL
Alimentary tract	77	100	84	20	9	22	[illegible]
Epithelioma	13	92	73	17	5	5	128
Cervix and uterus	25	96	[illegible]	[illegible]	9	17	116
Breast	29	100	55	26	7	10	[illegible]
Other sites	27	93	76	13	8	28	[illegible]
Sarcoma	22	87	70	22	10	11	113
Total untreated patients	223	95	71	21	7	21	120
Patients under radium treatment	21	73	51	23	9	7	91
Patients definitely nonmalignant	15	67	5.6	0.2	[illegible]	2.4	8

Comment.—The third column shows that intracellular structures of the types described occur in a high percentage of cases of carcinoma or sarcoma. Cases are counted as negative if these bodies are not seen within the conditions selected, namely during the scrutiny of between 100 and 200 leucocytes. The last column indicates how soon the bodies will be seen. Thus, in the alimentary cases, 132 out of every 1,000 mononuclear leucocytes showed them; in other words, about every eighth mononuclear.

This serves to show that this form of study can come within the scope of routine work.

As regards the first column, cases in the alimentary tract include all tumors primary in the stomach or onward, that is, not epitheliomas. The term "epithelioma" covers cases which were external or buccal or in the upper alimentary tract as far as the cardiac orifice. The negative cases in this group were mostly esophageal. Sarcomas appear to provide most of the negative results, one case was a myxosarcoma and one a fibrosarcoma; there was one case of fibromyoma turning malignant. Perhaps it is not surprising that these should yield negative results.

The cases described as "definitely nonmalignant" fall into four groups: (1) definitely healthy persons, (2) students who were apparently in good health, (3) patients admitted to hospital without organic disease as far as known (hernia, fracture, hemorrhoids), (4) various medical and surgical cases considered to be certainly nonmalignant. Some of these gave positive results. Of these, details were available in six, two being cases of suppuration, one of tuberculosis with pyopneumothorax, one of simple goiter, one of Hodgkin's disease and one a chronic mastitis (therefore possibly precancerous).

A considerable number of "suspect" cases could not be included in the table, as it was felt that pathologic confirmation was desirable before the case was eligible, considering how effectively other diseases sometimes mimic cancer

Patients under Radium Treatment.—A definite fall in the proportion of patients who show these bodies is noticed, and in the others a longer search was required before they could be discovered.

No note is here made of the relation between positive findings and *stage of disease.* It has been found that even histologically, early cases may show the inclusions. But some of the "negative" results were furnished by clinically early cases.

DISCUSSION

Filamentous and other cytoplasmic structures are considered by cytologists to be integral components of any cell. But they are not specially referred to in textbooks on hematology as being discernible in ordinary blood films. The exact counterparts of those under discussion are, however, figured in, for instance, E. B. Wilson's classical work on *The Cell.* Similar bodies are described and discussed in the literature on virus diseases, and the question as to their nature and significance may be discussed on the same lines.

A. They may be intrinsic in the cell: (i) normal products of cell metabolism, or of growth, or of cell division. Here belong mitochondria, filamentous chondriocontes, beaded chondriosomes, lepidosomes, metachromatic, secretory, or other granules, Golgi apparatus;[9] (ii) nuclear in origin; chromidia, spirem threads, chromosomes; (iii) degenerative products.

One has to decide between (i), (ii), and (iii) before deciding that a given cell really contains the particular bodies in question. Granules are not noted. Hence, the difficulty of deciding whether a granule is a degenerative product or a secretory product is evaded. The position of *filamentous mitochondria* in the cell economy is also not agreed upon by cytologists. For the present purpose, it is, however, convenient to refer to these bodies as mitochondrial, though they do not give the classical staining reactions.

Definite mitochondria can be seen quite frequently in neutrophile leucocytes, especially in pyogenic infections, as well as in malignant disease, presumably owing to the depletion of the granules, which renders them visible. But the similar bodies in the monocytes do not give the same staining reaction with Leishman stain.

They may be classified as chondriosomes in that the latter are regarded as phospholipoid in nature[9] (pp. 47 and 223), and Giemsa will stain such material the same way as it does these bodies.

ii. Nuclear origin: Chromidial extrusions are commonly seen in circulating blood cells, and they have been noted especially in cases of malignant disease. They are also numerous in health under a purin rich diet.[3] But these extrusions never attain the length of the bodies in question; they are of drumstick or spiculated form. Perhaps the occurrence of long ex-nuclear lines likewise denotes increased nuclear activity, material passing out into the cytoplasm.

The noded lines may be of nuclear origin also, if one regards the cytoplasm as permeated by canaliculi down which granules of nuclear matter proceed outward into the circumcellular fluid. The internodal lines would then be taken as optical effects produced by the canaliculi. The mycelioid forms might be

explained in a similar manner, the extranuclear material running into the canalicular network without separating into droplets or granules.

Such an explanation would justify taking the presence of these bodies in general as an index of chromophilia of tissue, and this would be in harmony with the generally favored view as to the nature of malignant disease.

The nodal lines bear a resemblance to linin threads. But this is likely to be accidental, for there is no reason why they should enter the cytoplasm en masse, especially considering that circulating leucocytes do not undergo mitosis. Portions of chromatin have, therefore, no opportunity to become detached or to fail to reenter daughter nuclei. In any event iron hematoxylin has not shown their presence.

B. They may be extrinsic to the cell. (1) Nonspecific to malignant disease: (a) artefacts, such as contamination with extraneous objects, pseudospirochetes, physical staining effects; (b) phagocytosed organisms. (2) Specific to the disease (a) in etiologic relationship, (b) not so.

Re (i) (b). In favor: (1) The Staining reaction. On the one hand, ordinary pathogenic bacteria do not stain red with Leishman stain, whether free or within neutrophile leucocytes. On the other, experiments made by me have shown that protozoa and mycomycetes from insect intestine stain the same color as to these mitochondrial bodies after phagocytosis by human leucocytes, using opsonic technic. In the case of the leucocytes in malignant disease both neutrophiles and monocytes may show the bodies, yet those in the neutrophiles stain like ordinary bacteria, whereas the others take on the red color. It is, therefore, feasible that one is dealing with phagocytosis in each case, but the type of organism is different or its reaction in the cell is different. (2) Carcinomatous tissue frequently contains bacteria. Ulcerated tumors are necessarily infected. But even other tumors prove to contain various organisms. It would appear that neoplastic tissue attracts various organisms which may be circulating (as they do even in health), so that they gradually accumulate in the tissue.[5] A number of recent publications discuss the bacterial content of tumors and suggest it as a cause of increased virulence of the neoplasm, especially in the cervix cases.[6, 7] In fact, bacteria might even be necessary to the growth of the neoplasm, so that the therapeutic attack should commence upon them.

Now the flora of these infected tumors is multiple. They, therefore, may provide both types, those taken up by the neutrophiles, and those by the macrophages, with the corresponding difference in staining effect. (3) Histologic study shows that there is at least every opportunity for both orders of cells (invariably present in and among the tumor cells) to take up these organisms and return to the blood. Fragments of mycelium, and noded or geniculate bodies have been identified in specimens from cases in which the blood has shown the inclusions.

Against: (1) even though ingested by monocytes, even the higher bacteria should be stainable with special bacterial stains, yet they are not. (2) There must be enormous numbers of these organisms circulating in the cancer case, seeing that some are present in every cubic millimeter.

Re ii. (Are they specific?) (a) In etiologic relationship? In favor: (1)

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They are found in the tumor cells also; (2) some authorities on virus diseases incline to the idea that the inclusions in those diseases are specific; (3) absence of ordinary staining reaction for known bacteria (at one time the mycelium of actinomycosis could not be demonstrated in the tissue-lesion); (4) the nodes in the geniculate bodies may be similar in function to the arthrospores of streptococci, etc., or of streptothrix.

Against: (1) The evidence that they may be simply phagocyted organisms which certainly do not cause cancer; (2) tumor cells are able to phagocyte such organisms (but the presence of such bodies is taken as itself evidence that tumor cells can ingest organisms); (3) most workers do not regard inclusion bodies in virus diseases as causal (on the other hand, this is merely an expression of opinion, either way); (4) they have not the individuality, e.g., of malarial plasmodia.

(b) Not in etiologic relationship? That is, are these bodies not the actual virus, but vectors of virus? In favor: (1) There is nothing intrinsically impossible in ex-nuclear material being a bearer of ferment or zymogen, seeing that intracellular ferments must inhere in some concrete cell constituent anyway. Therefore, such material can equally harbor a virus. (2) There is the analogy of the chromosomes, which bear highly complex potentialities.

Whatever be the origin and nature of these bodies, there remains one practical fact, namely, that the subject of malignant disease has his blood "loaded" with the material in question, even at an early stage in the case. Therefore, if the answer to question B ii (a) is positive, it would mean that the monocytes carry virus about the body, and that metastases might arise even if tumor cells did not become detached. The whole body would be diseased almost from the outset, and it would not be a matter simply of an initial local lesion. But if the answer to question A ii be positive, the findings in the blood films would be interpreted in the sense of a nonspecific chromoplastic activity, and one would understand why such conditions as extensive abscess formation sometimes also furnish similar bodies in the blood cells.

On that last view, the findings may simply reflect the outpouring of cytotoxic and cytotaxic products by the neoplasm itself, an event which need not be continuous, and need not occur in all stages. The new tissue may be "closed" by fibrosis, or "open" (only a lymphocyte barrier). In the former case the mononuclear leucocytes reveal nothing, whilst in the other case they contain these bodies. It has been found in the course of this study that the blood is actually not always equally rich in the mitochondrial bodies even at all times in the day. The nucleo-irritative products seem, therefore, to vary in amount, apart from the defensive tissue reaction. Perhaps this defensive reaction explains the disappearance of the inclusions after successful radium therapy.

It may be noted that in a few cases the benign or malignant nature of a palpable tumor could be decided correctly by means of this search for cell inclusions.

SUMMARY

A description is given of certain filamentous, spirillar, and geniculate structures seen in (especially) the monocytes in a high percentage of cases of malignant disease, and in very few cases which were clinically nonmalignant.

They are demonstrable only with Giemsa staining or by Leishman's stain. They may disappear from the blood after successful radium therapy. Their possible nature is discussed.

During the past year, liberal facilities for bringing this study to maturity were furnished by Dr. E. W. Archibald, Director of the Department of Surgery of McGill University, so that material became accessible from the Royal Victoria Hospital, the Montreal General Hospital, and other hospitals in Montreal. Thankful acknowledgment is here extended to the physicians and surgeons who placed their cases at disposal.

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THE TOXEMIAS OF PREGNANCY*

II. The Nitrogen Metabolism

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In a previous communication[1] the senior author has discussed some evidences of the hepatic factor in the group of widely diverse conditions generally called the "toxemias of pregnancy." These evidences were among the results of an elaborate chemical and laboratory study of fifty pregnant women who exhibited some one of these toxic conditions.

The basis of selection, the method of study, and the general composition of the group have already been considered in the paper noted above.

Data derived from the study of seventy-seven women studied repeatedly both throughout a normal pregnancy and for a number of weeks thereafter,[2] have served as a control; these will be drawn upon, as needed, for the present discussion.

This present paper is concerned with the protein metabolism, as evidenced chiefly in the levels of blood and urine nitrogen.

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