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Demonstration of a Cytoplasmic Structure in Plasma Cells

By H. Braunsteiner M.D., K. Fellinger M.D., and F. Pakesch M.D.

THE MOST DIFFICULT PROBLEM in electron microscopy is caused by the rather limited penetrating power of the electron beam. Only slices of a thickness of approximately 0.1 μ can give a sufficiently transparent picture. The first method used consisted in spreading tissue culture cells over a "formwar" film and transferring them directly to electron microscope screens.[1, 2] By this method parts of the cytoplasm of cultured cells could be visualized, but the nucleus and a large perinuclear zone always remained far too dense. However, with a slight modification of this method, certain hematologic studies concerning normal and pathologic leukocytes and thrombocytes could be undertaken.[3-6] Decisive progress has recently been made by developing new methods of embedding and by technical improvements in conventional microtomes.[7, 8] As a result, it is now possible in some laboratories to obtain sections of suitable thinness of almost any type of cells and tissues.

An unexpected result of these studies was the discovery of a characteristic structure in the cytoplasm of liver cells. Filamentous or canalicular elements may be observed in the ground substance. They are about .05 to .08 μ thick and up to several μ long. They lie close to each other and nearly parallel to and surrounding the cell nucleus and the cell particulates in concentric whorls. This structure was described independently by Dalton et al.[9] and Bernhard et al.[10] Both groups related them to the basophilic, ribonucleoprotein (RNP)-containing substance of the cytoplasm. In search for other cells containing rich amounts of RNP, a similar structure was found to exist in the exocrine cells of the pancreas, the cells of the salivary gland and the pepsin-producing cells of the stomach.[10, 11] It has also been located in this laboratory in the follicular cells of the thyroid, and a certain dependency of this structure on the secretory activity of this gland has been demonstrated.[12]

Caspersson and his school[13] have proved the close relation of RNP and protein synthesis. Therefore, Bernhard and co-workers identify the structure described above as the morphologic representation of RNP. We are not entirely convinced by this theory, because certain RNP containing cells do not show this structure.

To clarify this problem further, the present paper reports a series of observations on plasma cells of the bone marrow.

Methods

Small amounts of bone marrow were withdrawn by sternal puncture and immediately fixed for 24 hours in a 2 per cent solution of OsO_4 in buffered Thyrode at a pH of 7.5. Thereafter the pieces were rinsed for 1 hour in flowing tap water. They were transferred for dehydration successively into 50 per cent, 70 per cent, 96 per cent, and, twice,

From the Department of Medicine, School of Medicine, University of Vienna, Vienna, Austria.

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100 per cent ethanol and kept for 1 hour in each. After remaining in benzol for 2 hours, the pieces were embedded in "esterwax" at 53 C. for 24 hours. They were then kept at 5 C. for 20 hours and at −20 C. for 5 hours.

The microtome used was a Rocking Cambridge microtome. The sectioning was performed at a temperature of 5 C. A smooth advance for very fine sectioning was obtained only by the slight extension of the metallic block according to the change of temperature from −20 C. to 5 C. The sections from the knife were floated on a liquid surface in order

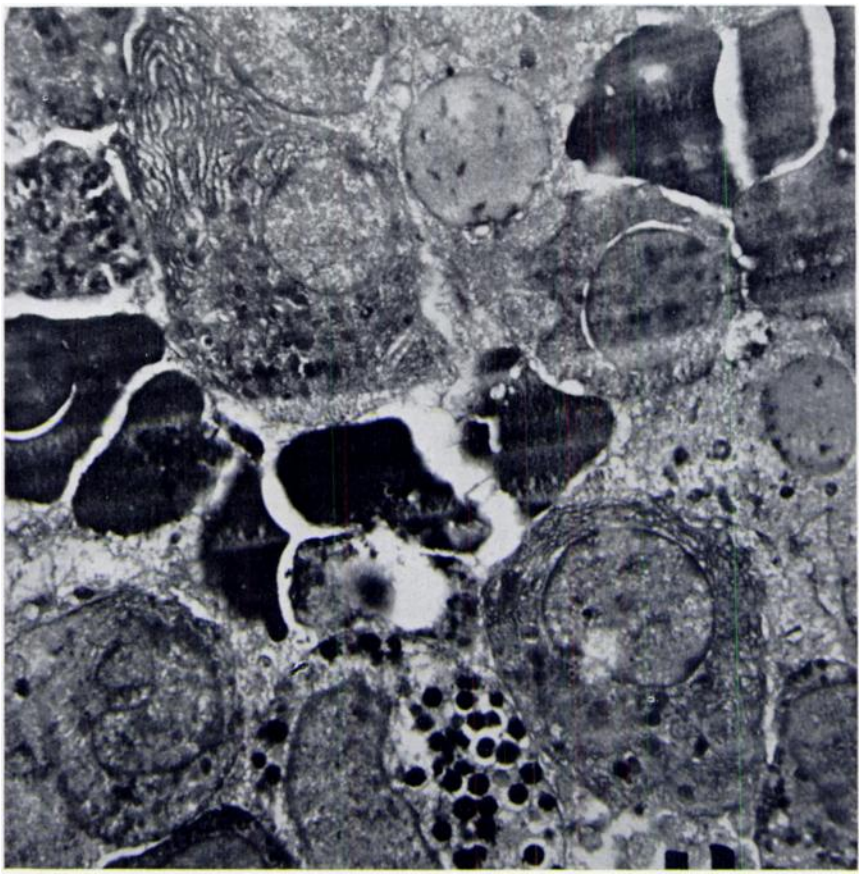

FIG. 1.—Two normal plasma cells in a bone marrow section. The cytoplasm of these cells consists in large part of concentric filaments surrounding the nucleus. 4000 ×.

to facilitate spreading. From there they were lifted on to electron microscope screens of fine copper mesh coated with a film made from a 0.1 per cent "formwar" solution. Then they were placed on filter paper, gently immersed in Xylol in order to remove the "esterwax" and allowed to dry.

The sectioned material was examined in a Philips (model 1951) electron microscope. The micrographs were taken at an original magnification of 5000 × or 10,000 ×. The high tension was generally 60 kv.

OBSERVATIONS

We examined normal plasma cells and plasma cells of one case of β- and two cases of γ-myeloma. The results were fundamentally the same.

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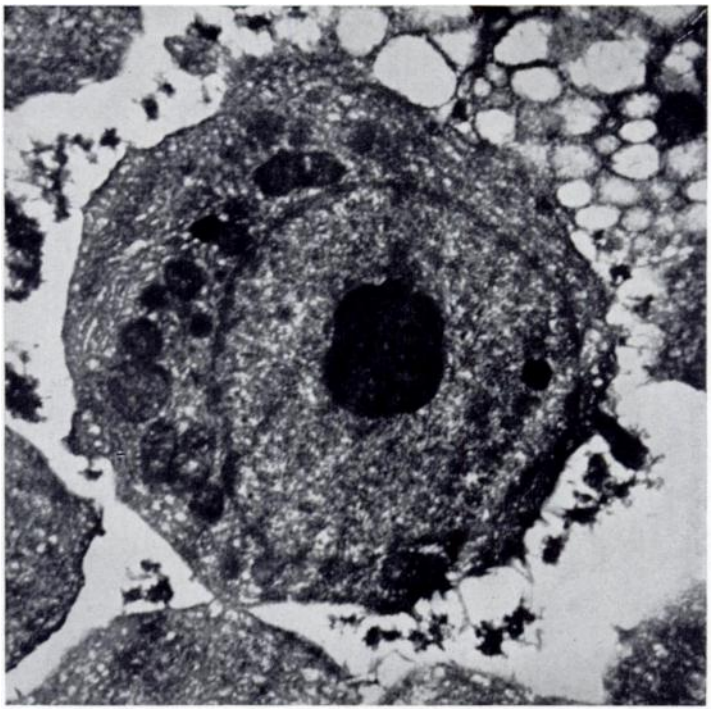

FIG. 2.—Plasma cell of a β-myeloma. Large nucleolus with a dense, convoluted structure. In the cytoplasm numerous mitochondria can be observed. 8000 ×.

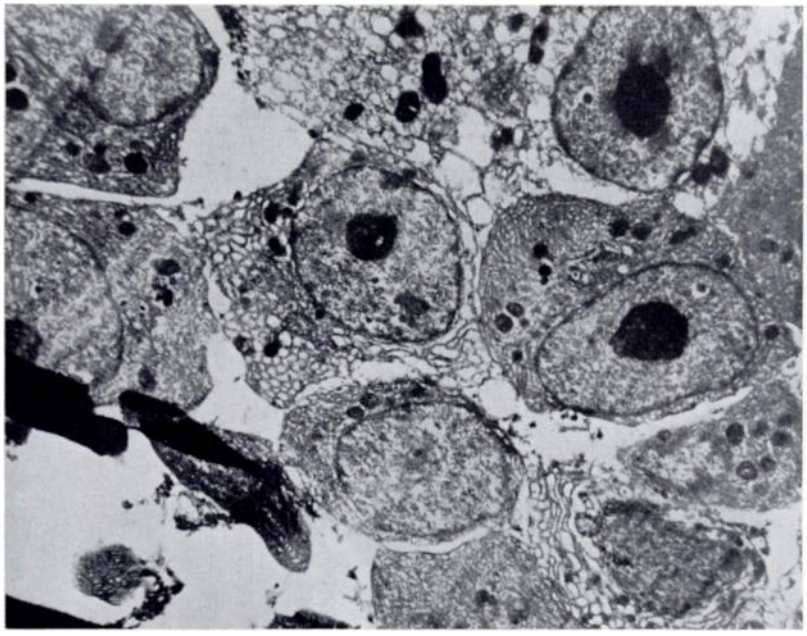

FIG. 3.—Bone marrow section of a β-myeloma. All transitory states from the closely-knit filaments to vacuoles can be seen. 4000 ×.

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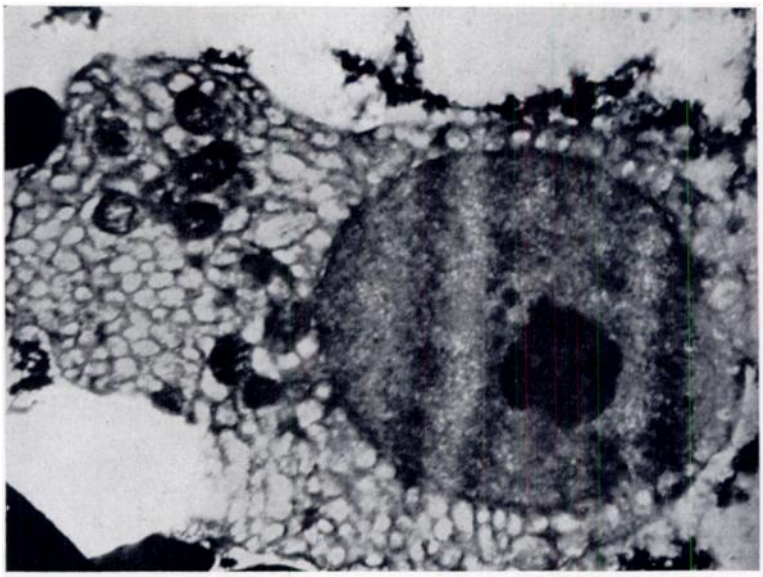

FIG. 4.—Vacuolated plasma cell of a β-myeloma. Many of these types of cell show inclusion bodies in the vacuoles. 8000 ×.

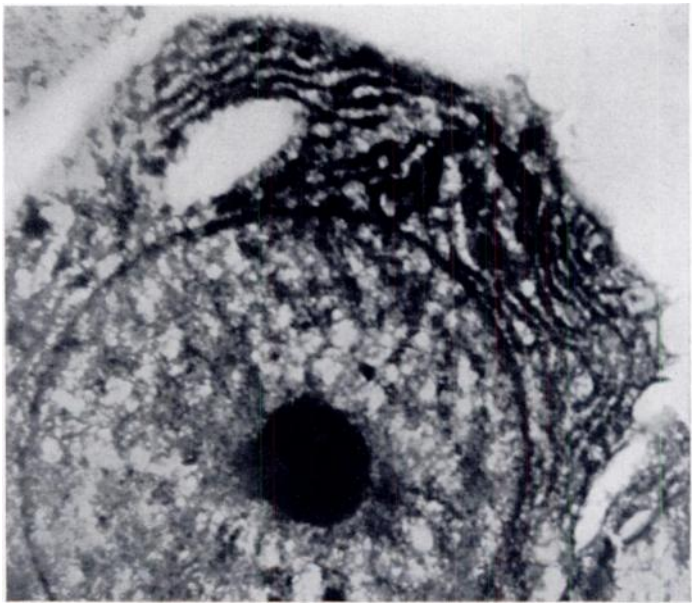

FIG. 5.—Plasma cell of a γ-myeloma. 10,000 ×. Note concentric filamentous structure crowding the nucleus.

The Nucleus

The nucleus showed a distinctly visible membrane. One or several nucleoli were clearly seen, often very large in pathologic cells. These nucleoli generally showed a convoluted structure, as previously observed in the nucleoli of liver

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cells.[10, 14] The significance of this structure still remains obscure but, according to the central position of the nucleolus in intracellular protein metabolism, it may be of considerable importance.

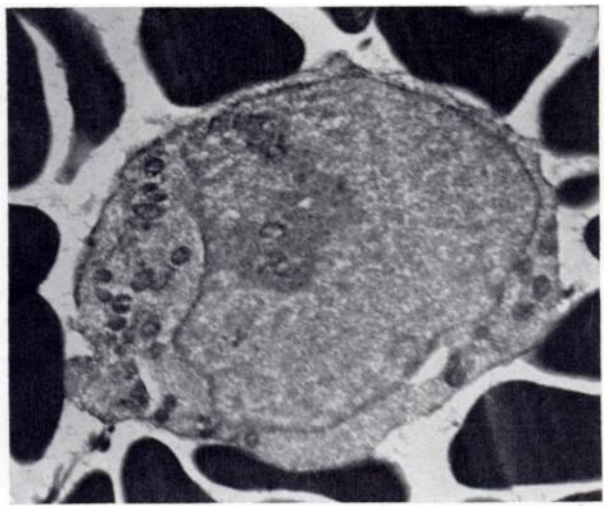

FIG. 6.—Normal myeloblast of the bone marrow. Large nucleolus. Numerous mitochondria in the cytoplasm. The cytoplasm is entirely homogeneous. 6000 ×.

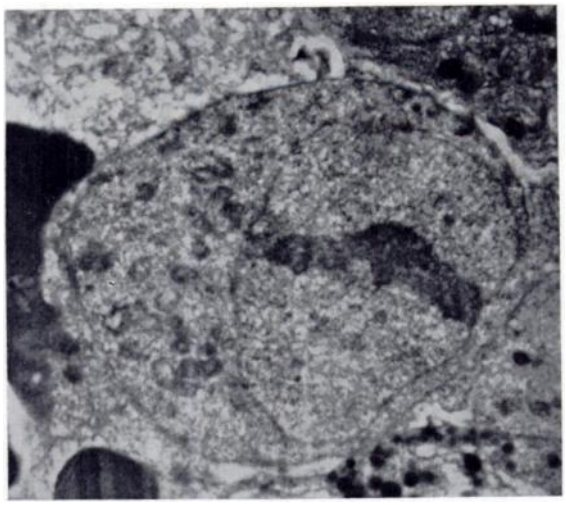

FIG. 7.—Paramyeloblast of the bone marrow of an acute leukemia. Large nucleolus with convoluted structure, horizontally sectioned. No filamentous structure of the cytoplasm. Numerous mitochondria. 8000 ×.

The Cytoplasm

The cytoplasm consisted almost entirely of fine filamentous elements lying next to one another around the nucleus. In other cells these filaments were

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partially dislocated and the empty space so formed was vacuolated. In a terminal state, the entire cytoplasm might be vacuolized. Sometimes inclusion bodies were present in the vacuoles.

A considerable number of mitochondria were often observed in the cytoplasm.

Discussion

In comparing the filamentous structure of the cytoplasm in plasma cells with those previously described in liver cells, pancreatic exocrine cells, cells of the salivary gland, pepsin producing cells of the stomach, and follicular cells of the thyroid, the morphologic identity becomes obvious.[9-12]

All these cells are rich in RNP and have in common the ability of differentiated production and secretion of protein. Therefore, this identity of structure may be

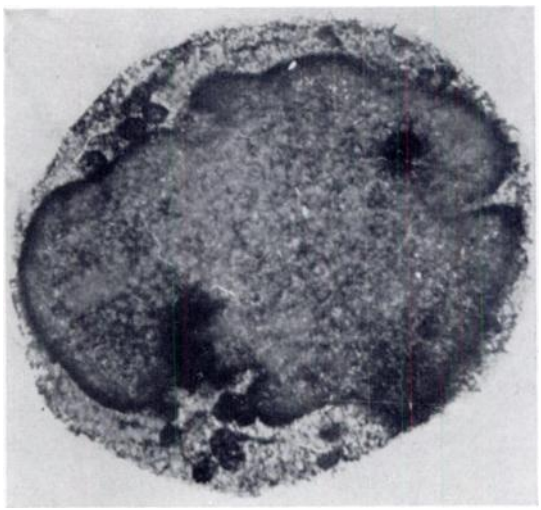

Fig. 8.—Cell of a lymphosarcoma. No filamentous structure of the cytoplasm. Numerous mitochondria.

due to both of these properties. We have, therefore, examined RNP-containing cells without any known protein secreting activity. This was performed under the same conditions.

The following types of cells have been observed: paramyeloblasts in two cases of acute leukemia, cells of a lymphosarcoma, cells of an experimental myelosarcoma of the rat, normal lymphocytes and lymphocytes in several cases of lymphatic leukemia, and finally normal myeloblasts and proerythroblasts. In none of these cases did the cells show a similar structure, although they contained a considerable amount of RNP as was demonstrated by specific staining.

Deducing from these observations two explanations remain

1) Only when very high contents of RNP are present, does the filamentous structure in the cytoplasm become visible.

2) The filamentous structure is a RNP containing apparatus of the cytoplasm

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for the production of differentiated protein and for its extracellular secretion. The latter theory is supported by the authors.*

SUMMARY

In normal and pathologic plasma cells of the bone marrow, a specific filamentous structure of the cytoplasm has been revealed by electron microscopy. The significance of this structure is briefly discussed.

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* Very recently, Pallade has shown convincing micrographs which suggest the presence of the basophilic substance either in granular or in tubular form. The tubular form would correspond to the filamentous structure described in this paper.[15, 16]

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