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A Note on the Histology of a Case of Myelomatosis (Multiple Myeloma) with Bence-Jones Protein in the Urine (Myelopathic Albumosuria)

BY

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A Note on the Histology of a Case of Myelomatosis (Multiple Myeloma) with Bence-Jones Protein in the Urine (Myelopathic Albumosuria).

By F. Parkes Weber and J. C. G. Ledingham.

In a paper on multiple myeloma (myelomatosis) which one of us read before the Royal Medical and Chirurgical Society in 1903[1] records of about forty cases were collected in which the presence of Bence-Jones protein in the urine ("myelopathic albumosuria" of Bradshaw) had been observed. Histological examination of the tumour-like growth in the bone-marrow had already been made in a good number of myeloma cases, and since then several other cases have been examined. In giving histological descriptions, however, it is probable that cases characterized clinically by the presence of myelopathic albumosuria have not been sufficiently distinguished from the other myeloma cases. In some cases, indeed, in which careful histological examinations have been made practically nothing was known of the symptoms during life, especially as to whether there was or was not "myelopathic albumosuria" present at any time. In the case now under consideration there is fortunately no uncertainty in this respect. The condition of the urine was recognized several years ago.

The patient was a woman, aged 65, who was long under the care of Dr. H. Savory in the Bedford County Hospital. Afterwards she was again in the same hospital under the care of Mr. Gifford Nash, and died there on January 25, 1909. Dr. Savory and Dr. Gowland Hopkins, of Cambridge, carried out most careful investigations with regard to the influence of diet on the protein excretion, and we doubt whether the metabolism in any other case of this disease has ever been studied so thoroughly. One of us (F. Parkes Weber) was kindly allowed to make the post-mortem examination at Bedford in conjunction with Dr. Gowland Hopkins, and it is by the courtesy of Dr. Savory and Dr. Hopkins that we have been permitted to publish this note on the histological features. They will shortly publish a detailed account of the case, and in regard to the clinical features it is sufficient to add that, as they tell us, the protein excreted in the urine was of typical character and averaged 12 grm. per diem.

[1] F. P. Weber, *Med.-Chir. Trans.*, Lond., 1903, lxxxvi, pp. 395-467.

Necropsy.

The body was that of a medium-sized, rather emaciated female with considerable kyphosis of the lower dorsal region and apparently abnormal projection of the right great trochanter. The head and skull were not examined. We shall for convenience first describe the macroscopic changes noted in the bones and bone-marrow and then proceed to the viscera and the histological examination of the growth.

The bones examined included the sternum, ribs, clavicles and part of the vertebral column, part of the right humerus and part of the right femur. The sternum, ribs, clavicles, humerus and femur were all found transformed into mere shells of very hard, brittle, compact bone filled with a dark red opaque jelly-like substance which replaced the normal bone-marrow. Not only were the medullary canals of the long bones greatly enlarged at the expense of their osseous walls, but much of the cancellous tissue of the bones (*e.g.*, of the head of the humerus) was found to have been replaced by the dark red opaque jelly-like substance in question. If it is permissible to speak of this altered bone-marrow as bone-marrow at all, evidently the total amount of bone-marrow in the body must have been immensely in excess of the normal amount. There were no local bulgings on the ribs or sternum, nor were any fractures detected such as have been noted at necropsies in some cases, but, on the contrary, it was seen that a former fracture of the surgical neck of the right humerus (which had occurred a few months before the patient's death) was completely healed by good bony union of the fragments. The projection of the right great trochanter was found to be due to the fact that the neck of the femur, though shortened, was nearly at right angles to the shaft, as if it were the site of an old fracture. The portion of the vertebral column examined was the lower dorsal region—that is to say, the site of the greatest kyphotic bend. The bodies of the vertebræ consisted of hard bony shells containing only a remnant of the normal cancellous tissue, much of the cancellous bone having been replaced by a red opaque jelly-like substance exactly similar to that already found in the other bones. This jelly-like substance, or altered bone-marrow, was seen on microscopic examination to consist of a diffuse tumour-like growth, with fat-cells and normal bone-marrow constituents interspersed amongst the collections of the cells proper to the growth. Histologically the growth was a "plasmacytoma," "plasmoma," or "myeloma plasmacellulare," but we shall return to the histological

features of the growth after describing the results of macroscopic and microscopic examination of the viscera.

The heart (weight 9½ oz.) showed nothing abnormal. The aorta, as much of it as was examined, showed no excessive atheroma, excepting some calcification quite at its commencement, close to the aortic valves. There were a few old pleuritic adhesions. There was hypostatic congestion of the bases of the lungs, and the hardened condition of the left lower lobe suggested the presence of actual pneumonia. Microscopic examination of this part of the lung proved that there was pneumonia and that the pulmonary alveoli were

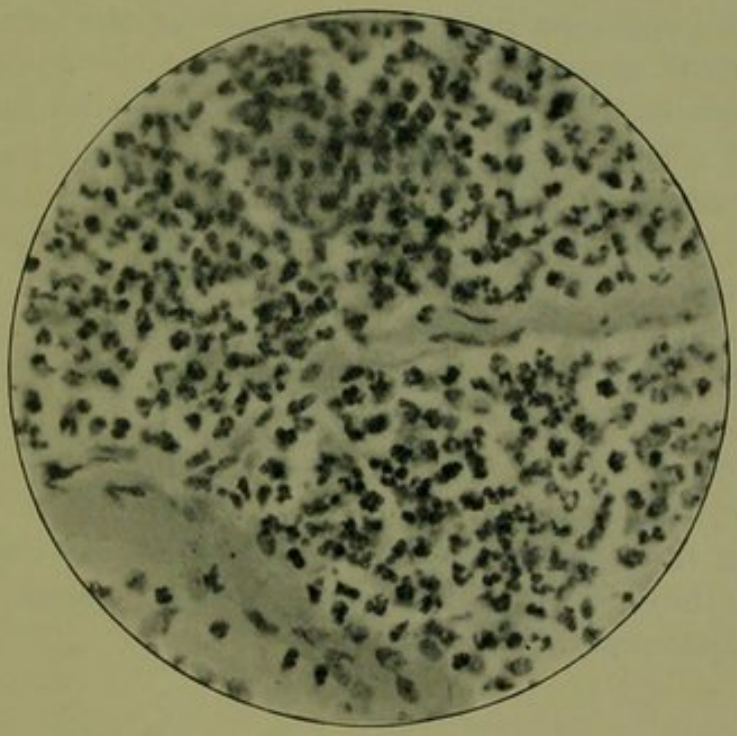

Fig. 1.

Microphotograph showing the pneumonia. (Magnification, 500.)

typically infiltrated with pus-cells (fig. 1). Examination of blood-films from the blood in the heart gave the following differential count (J. C. G. Ledingham) of white cells: small lymphocytes 1·2 per cent.; large lymphocytes 19 per cent.; large mononuclears and transitionals 0·6 per cent.; polymorphonuclears 77·3 per cent.; eosinophiles 0; myelocytes 1·8 per cent. A good many of the polymorphonuclears appeared immature, approaching the myelocyte type. No plasma-cells could be distinguished. No nucleated red cells were seen. No changes were observed in the red cells.

Macroscopic and microscopic examination of the liver (weight 51 oz.) and pancreas showed no disease. There was no cholelithiasis. The spleen (weight 3 oz.) was small and seemed rather shrivelled. On cutting into it there appeared to be an increase of the fibrous trabeculæ; microscopic examination showed a certain amount of fibrosis and thickening of the walls of the arteries. The suprarenal glands were not examined.

The kidneys weighed together 12 oz. One was slightly bigger than the other, but there was no macroscopic evidence of disease. Microscopic examination showed nothing abnormal beyond scattered spots of a chronic interstitial fibrotic change of very slight degree. The urinary bladder appeared healthy. The body of the uterus contained a mass of fibroids, several of them calcified, and the uterine canal was elongated to about 5 in. The ovaries were shrivelled, and the right one contained a cherry-sized cyst. There was a right parovarian cyst filled with clear straw-coloured fluid. The stomach and intestines, as far as they were examined (small intestine examined microscopically), appeared normal. Ordinary hæmatoxylin sections of the lowest dorsal segments of the spinal cord were pronounced by Dr. Gordon Holmes, who kindly examined them for us, to show nothing abnormal, except swelling of the dorsal root-fibres in the root-entry zone, suggesting slight compression of the dorsal roots; in one section there was evidence of slight compression of one lateral surface of the cord.

There was no evidence of metastasis of the bone-marrow growth anywhere in the body outside the bones. No enlargement of any lymphatic glands was observed.

Examination of the Bone-marrow Tumour-like Growth (J. C. G. Ledingham).

Pieces of the marrow from the femur, humerus and sternum were fixed in Orth's fluid and embedded in paraffin. The stains employed were Laurent's methylene-blue-eosin mixture, Unna-Pappenheim's methyl-green-pyronin, Twort's Licht-Grün-neutral-red mixture and hæmatoxylin-orange-rubin.

The microscopical features of the marrow were essentially the same in all three situations, but the following description will apply mainly to the humerus marrow, in which the peculiar cell-infiltrates constituting pathological change occurred in their most characteristic form. Examination of the sections even with the naked eye shows irregular densely

stained areas standing out prominently among a looser and less cellular tissue. Microscopically (figs. 2-4) the fat-cells of the marrow are still intact, and in some places appear to have elbowed out the other cellular elements. Fat-spaces are less numerous, however, in the densely stained areas; these present a somewhat tesselated appearance, consisting of cells of uniform type arranged in columns or rows around the still remaining fat-spaces; frequently between two adjacent fat-spaces the intervening infiltrating cells appear to be laterally compressed and of smaller dimensions. Otherwise these cells, though conforming to the same type, show extraordinary variations in size, nuclear arrangement, and condition of cytoplasm.

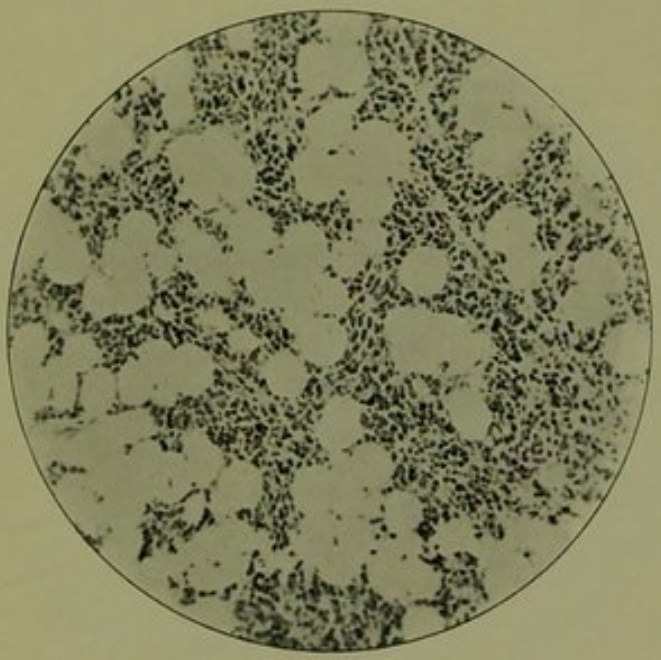

FIG. 2.

Microphotograph showing the bone-marrow growth. (Magnification, 100.)

With the Unna-Pappenheim stain (Plate I) the cells present all the morphological characters of plasma-cells. The nucleus is wheel-shaped, with the chromatin granules arranged mostly at the periphery. In the centre of the nucleus is usually seen a clear space which lodges the nucleolus, a round, sharply defined body, which stains a bright red in contrast to the green nuclear chromatin. The cytoplasm is often voluminous, with intensely basophil reaction. In many cases the cytoplasm is highly vacuolated, the intervening granular substance staining deeply with the pyronin. Round the nucleus, which, as a rule, is placed eccentrically, is the clear unstained "halo" characteristic of plasma-cells.

The nucleus may be single or double, and some large forms have been noted with three nuclei. Mitotic figures are only rarely met with in these cells, and when present do not conform to the usual type. In Plate III are drawings of three cells with mitotic figures. It will be noticed that during mitosis the basophilia of the cytoplasm is greatly reduced and the chromatin is arranged in the centre of the cell as a twisted highly pyknotic band.

Some of the cells (Plate II) show a peculiar partition of the nuclear chromatin into three or four spindle-shaped, densely stained masses, which may represent an early phase in the mitosis. In the case

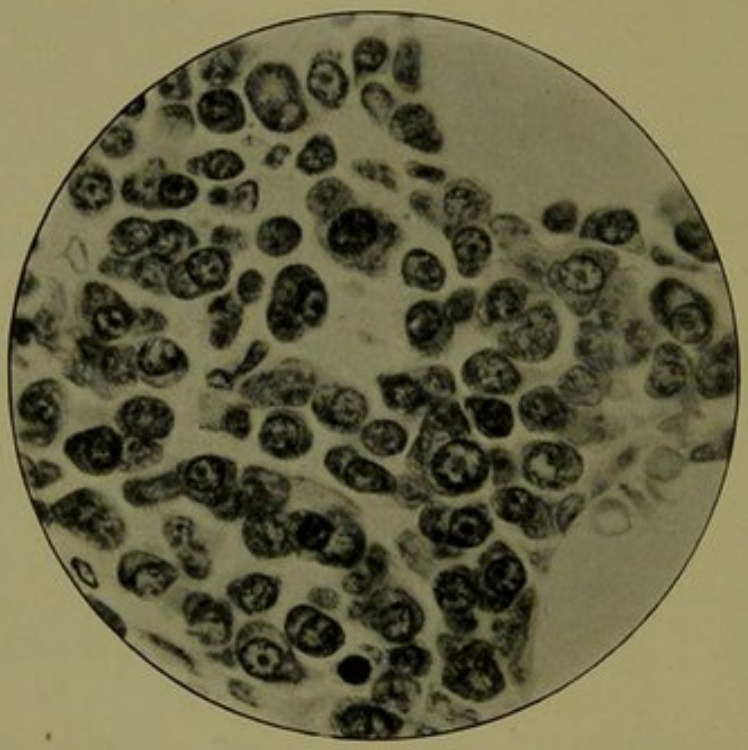

FIG. 3.

Microphotograph showing the bone-marrow growth. (Magnification, 750.)

reported by J. H. Wright (*Johns Hopkins Hospital Reports*, 1900, ix, p. 359[1]) no mitotic forms were seen, but a few of the cells contained elongated, hour-glass or dumb-bell nuclei. These evidently correspond to the mitotic forms in our case. Wright also noted cells with two

[1] In Rudolf Hoffmann's case, as well as in Wright's, the myelomatous growth was found to be histologically a "plasmacytoma," or "plasmoma," as in the present case. *Vide* Hoffmann, "Ueber das Myelom, mit besonderer Berücksichtigung des malignen Plasmoms," *Beiträge zur path. Anat. und. zur allg. Path.*, Jena, 1903, xxxv., p. 317. Since then other examples of "myeloma plasmacellulare" have been described (*see* Addendum).

nuclei connected by a thin band of chromatin, suggesting amitosis (direct division; that is to say, division other than by karyokinesis). Evidence of direct division was not found in our case.

It is remarkable that so few mitotic forms occur. As a matter of fact, degeneration of these plasma-cells is more in evidence than active proliferation. The nucleus in numerous cases is seen to be undergoing lysis (Plate I), and frequently one or two spots of chromatin are all that remain of the nucleus. The cytoplasmic residue still retains, however, its marked affinity for pyronin. Lying alongside perfect cells are seen numerous cytoplasmic cell-residues staining deeply with pyronin and containing no trace of a nucleus.

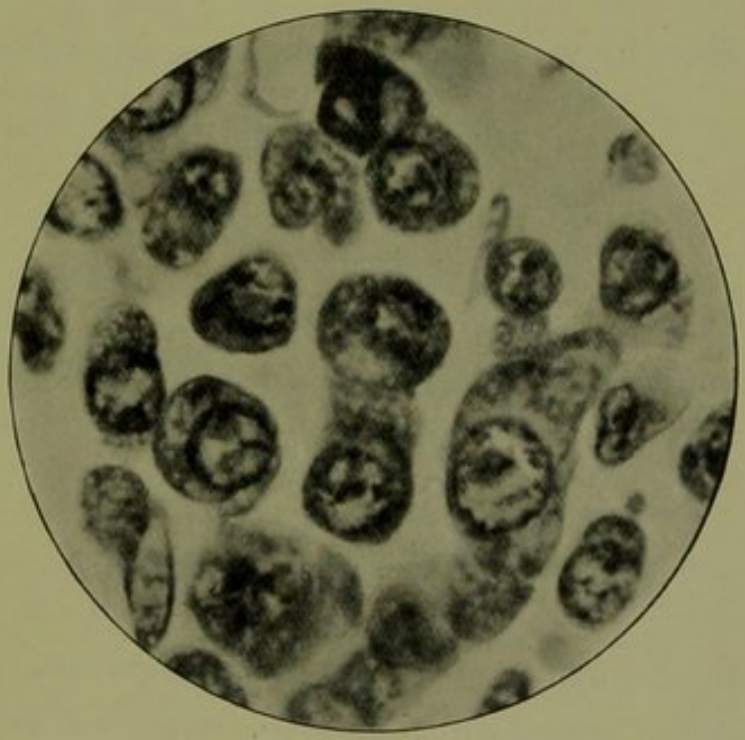

FIG. 4.

Microphotograph showing the bone-marrow growth. (Magnification, 1,500.)

With Twort's stain the various elements of the plasma-cell are thus differentiated. The nuclear chromatin and the granular portion of the cytoplasm stain a brick-red. The nucleolus is stained a light red. The perinuclear "halo," the intranuclear space, and the substance contained in the cytoplasmic vacuoles take up the green or acid stain (Plate III). This green staining of the nucleoplasm is an interesting feature. It has been noted by one of us (J. C. G. Ledingham) in the nucleus of the *Entamœba histolytica*. The perinuclear "halo" of the

EXPLANATION OF PLATE I.

SECTION OF PART OF THE BONE-MARROW GROWTH.

(Magnification, 650.)

Methyl-green-pyronin stain (Unna-Pappenheim method). The plasmacyte infiltrate surrounds the fat-vesicles. The vacuolated cytoplasm and the nucleoli (stained red) of the plasma-cells are well shown.

A—Plasma-cell in process of karyolysis.

B—Fat-vesicle.

C—Cytoplasmic remnant of plasma-cell after complete karyolysis.

D—Large kind of plasma-cell with double nucleus and well-marked peri-nuclear "halo."

E—Erythroblast with lobed nucleus.

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plasma-cells remains after complete karolysis as a green irregular space (Plate III). With Laurent's stain (Plate II) the perinuclear "halo" is very well marked, but remains unstained. The nucleolus is stained a bright pink in contrast to the dark blue nuclear chromatin.

Outside the dense infiltrates all varieties of the ordinary marrow elements are present, together with plasma-cells, which find their way into all parts of the marrow. The adventitial sheaths of the vessels are invariably surrounded by rows of plasma-cells. Eosinophil myelocytes are very numerous. Small erythroblastic foci are seen here and there. The giant-cells of the marrow are not increased in number. No micro-organisms of any kind were detected, and staining by Schmorl's method revealed no spirochætes.

The question now arises, Does the histological evidence afford any clue to the source of the Bence-Jones albumosuria? In Weber's case (1903), which was investigated chemically by Hutchison and MacLeod, no body giving exactly the same reactions as those of Bence-Jones protein was found in the bones, blood, or organs. From the vertebræ and ends of the femur, however, a protein was obtained giving very similar reactions, differing somewhat in the temperature at which it coagulates, and in not being redissolved on boiling. Moreover, no protein like that detected in the myelomatous marrow could be isolated with the same methods from normal marrow.

Conclusion.

From the histological evidence in the present case we are inclined to offer the suggestion that the cytoplasmic residua of karyolyzed plasma-cells may be the source of this peculiar protein. It must be remembered that the total quantity of the (myelomatous) bone-marrow is, as we have already pointed out, greatly in excess of the normal. Moreover, the position (in the bone-marrow) of the tumour-like growth shows that metabolic or degenerative products of the plasma-cells, of which it consists, must readily gain access to the circulating blood-stream; and from the blood it has been shown experimentally that Bence-Jones protein is readily (like hæmoglobin or glucose, when present) excreted through the renal filter with the urine, or at least, according to von Decastello, if the renal tissue is in any way damaged.[1] In regard to the relationship of myelopathic albumosuria to the bone-marrow disease, we will merely

[1] On this point *see* A. von Decastello, *Verhandl. d. Kongresses für inn. Med.*, Wiesb., 1908, xxv., p. 620.

EXPLANATION OF PLATE II.

SECTION OF PART OF THE BONE-MARROW GROWTH.

(Magnification, 900.)

Laurent's eosin-methylene-blue mixture stain. Shows plasma-cells of the growth, especially very large forms of the plasma-cells, between fat-vesicles.

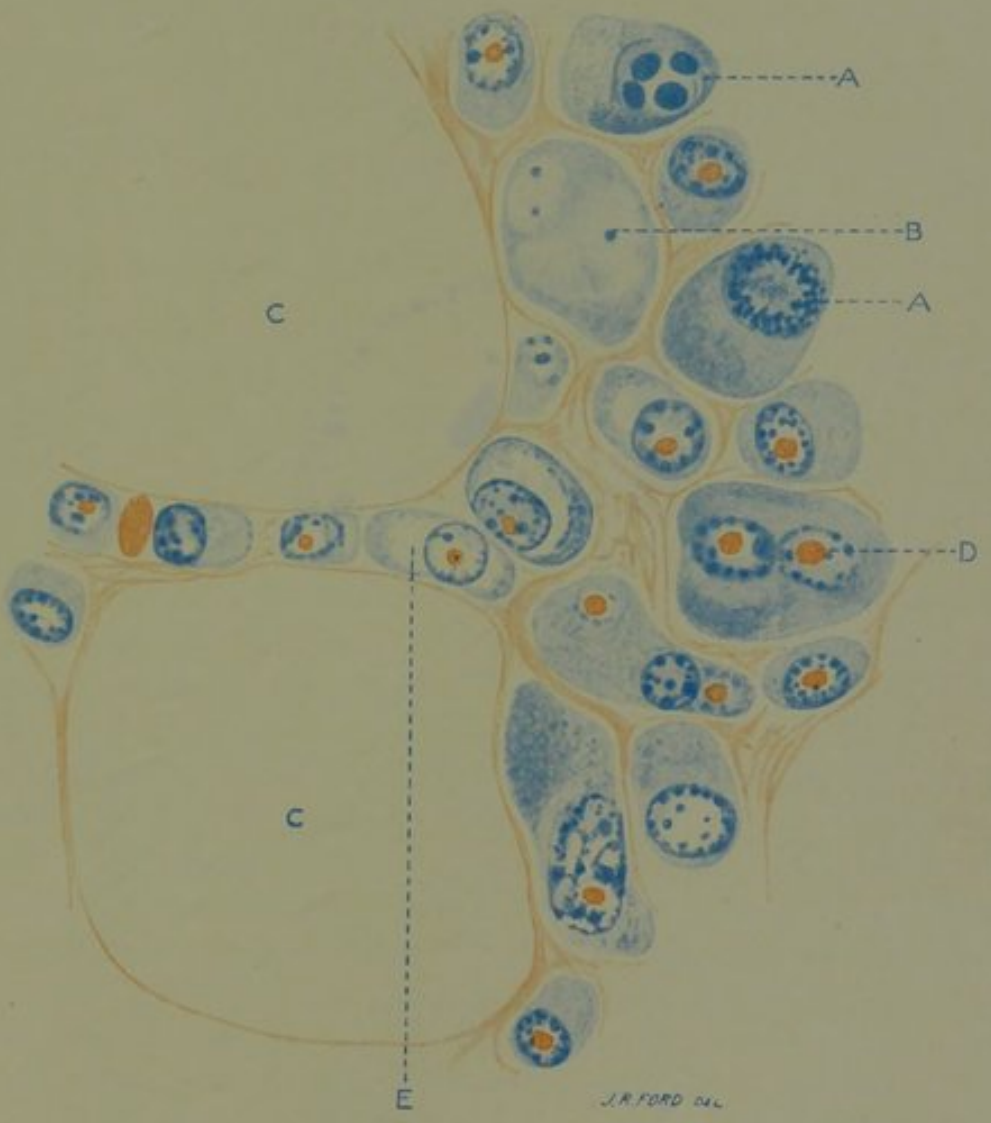
A—Form of plasma-cell, showing peculiar position of the nuclear chromatin (? prophase of karyokinesis).

B—Plasma-cell, showing extreme karyolysis.

C—Fat-vesicle.

D—Nucleolus, stained pink, lying in clear intranuclear (perinucleolar) space.

E—Perinuclear "halo."



PARKES WEBER & LEDINGHAM: Histology of a Case of Myelomatosis. Plate II.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remark that, for practical purposes, whenever a copious and persistent excretion of Bence-Jones protein in the urine has been observed, the patient has been sooner or later found to be affected with diffuse primary tumour-formation of the bone-marrow. Only one or two apparently genuine exceptions to this rule have as yet been recorded.[1]

One of us (F. Parkes Weber) in the paper of 1903 (already alluded to) ventured to draw an analogy between the bone disease, "myelomatosis," on the one hand, and the skin disease, "mycosis fungoides," on the other. The justification of such an analogy becomes evident now that it is known that in at least some cases of myeloma the growth consists of plasma-cells, whilst in mycosis fungoides the sarcoma-like tumours of the skin have been found to be "plasmomata."

Addendum—Consideration of the Term Myeloma.

There is no occasion to take up unnecessary space here by referring individually to all the recorded cases of myeloma. A review of all those described previously to 1903 will be found in the papers already referred to by F. P. Weber and R. Hoffmann. The histology of more recent cases has been recorded or discussed by MacCallum, Lubarsch, Jellinek, Menne, Abrikossoff, Saltykow, Sternberg, Permin, Aschoff, Scheele and Herzheimer, Ribbert, Simmonds, v. Verebely, Hueter, Charles and Sanguinetti, degli Occhi, H. A. Christian, Umber, Tschistovitsch and Kolesnikowa, A. Herz, and Benda (*see* references at the end of the paper).

The main points round which discussion has centred have been the differentiation of the various forms of bone-marrow growth, the presence or absence of metastases, and the morphology of the cells constituting the bone-marrow growth. A myeloma has been defined as a primary diffuse or multiple tumour of the bone-marrow, consisting of elements peculiar to that tissue and giving rise to no metastatic growths. Cases which do not conform to this description, either because of peculiar histological structure or because of the occurrence of metastases, would be excluded from the group. These criteria were employed by Menne in his arrangement of the cases recorded up to 1906.

It might certainly be advisable at present to exclude those rare cases of alveolar sarcoma and endothelioma which have been recorded, but it does not seem possible to exclude, on the ground of the occurrence of metastases, those cases in which the tumour elements are at any rate

[1] *See* von Decastello, loc. cit.

EXPLANATION OF PLATE III.

SOME ISOLATED CELLS OF THE BONE-MARROW GROWTH.

(Magnification, 900.)

In the two upper figures Twort's Licht-Grün-neutral-red mixture has been used, and marked differential staining has been obtained.

A—Cytoplasm, stained light brown, studded with vacuoles, the contents of some of which have taken on the green (acid) dye.

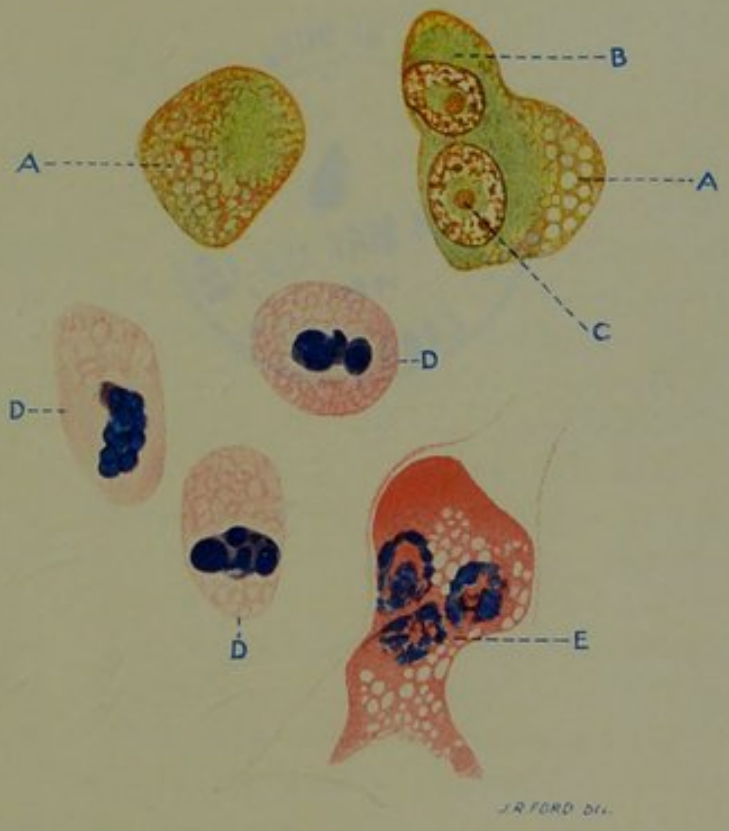
B—Perinuclear "halo," also stained green.

C—Nucleolus, stained light brown, surrounded by the intranuclear (perinucleolar) space, the contents of which have likewise taken up the green stain. The nuclear chromatin is stained reddish-brown. In the upper left figure the nucleus has undergone complete karyolysis; but the perinuclear "halo" (green) remains.

The four lower cells have been stained with methyl-green-pyronin (Unna-Pappenheim).

D—Peculiar mitotic form with twisted pyknotic nucleus. The cytoplasm (coloured pink) shows a reduced affinity for the pyronin (that is to say, diminished basophilia) during the process of mitosis.

E—Large unusual form of tumour-cell with triple nucleus undergoing pyknosis.



PARKES WEBER & LEDINGHAM: Histology of a Case of Myelomatosis. Plate III.

not foreign to the normal bone-marrow. If one regards the disease as a systemic affection of the hæmatopoietic apparatus the objection to the inclusion of those very few cases associated with metastases falls to the ground. With our modern views as to the occurrence of bone-marrow elements in organs like the spleen and liver under certain conditions (myeloid transformation), the occasional presence in these organs of so-called metastatic nodules can readily be explained.

It would seem to us of far more value to classify the cases of myeloma according to the presence or absence of Bence-Jones protein in the urine, but we cannot discuss this question here. A very convenient, but probably artificial, classification can be made on the basis of the morphology of the tumour-cells. Five types would thus be distinguished, viz:—

(1) *Lymphocytic* (Benda's case ?).[1]

(2) *Pre-myelocytic*, or *Myeloblastic*.—The cells in these cases lack the definite granulation of the myelocytes, but in most respects resemble the non-granular "pre-myelocytes" or "myeloblasts." To this type apparently belong the cases of Tschistovitsch and Kolesnikowa, Hueter, MacCallum (1905), Abrikossoff, Saltykow, Menne, and Permin.

(3) *Myelocytic*.—To this type several cases have been found to conform, but even in these the granulation of the myelocyte-like tumour-cells is generally not completely developed. Besides F. P. Weber's case (1903) the cases of Sternberg (1903), Charles and Sanguinetti (1907), A. Herz (1908) and Umber (1908) apparently belong to this group.

(4) *Erythroblastic*.—Ribbert's case of this supposed character remains isolated.

(5) *Plasmacytic*.—Since the cases (already referred to) of Wright (1900) and Hoffmann (1903), several myeloma cases have been described in which the growth has been found to consist of plasma-cells or of cells closely resembling plasma-cells (Aschoff, v. Verebely, H. A. Christian, degli Occhi).

The plasma-cell growth has been variously described as malignant "plasmoma," "plasmacytoma" or "myeloma plasmacellulare." Our present case constitutes about the seventh belonging to this category. Unlike our case and that of Wright, Hoffmann's case presented a so-called metastatic nodule in the liver consisting of plasma-cells.

[1] In F. P. Weber's case of 1896 (*Trans. Path. Soc. Lond.*, 1897, xlviii, p. 169) no special staining for plasma-cells was undertaken till 1903. Then, however, Dr. J. M. H. MacLeod found that the cells of the bone-marrow growth showed a greater resemblance to lymphocytes than to the typical plasma-cells of the granulomata.

Wright observed that plasma-cells occur in the normal bone-marrow, and we now know that they may occur in all organs. Myeloid transformations may sometimes, as already stated, occur in the spleen, lymphatic glands and liver. Hence, as Lubarsch remarks, the occurrence of "metastatic" nodules consisting of myelocyte-like cells or plasma-cells would readily fit in with the conception of myeloma as a systemic affection.

At present, however, the time is not ripe for the adoption of very dogmatic views as to the exact place of myeloma in the classification of diffuse affections involving the hæmatopoietic apparatus.

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