THE TYPE OF PHAGOCYTIC CELL AND ITS RELATIVE PROPORTIONS IN HUMAN BONE MARROW AND SPLEEN, AS IDENTIFIED BY THE SUPRAVITAL TECHNIQUE, WITH SPECIAL REFERENCE TO PERNICIOUS ANEMIA.

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In a recent publication, Peabody and Broun (1) have reemphasized, in the light of the increasing appreciation of the rôle of phagocytosis, the relatively large number of phagocytic cells to be found in the bone marrow in pernicious anemia. Sabin, Doan, and Cunningham (2), in a survey of the phagocytic cells of the spleen, bone marrow, connective tissues, and serous exudates, by means of a supravital technique, have separated these mononuclear cells, the so called reticulo-endothelial apparatus of Aschoff, into *two* distinct strains, monocytes and clasmatocytes. The basis of the separation is fourfold, derivational, developmental, cytological, and functional. The clasmatocyte, probably of endothelial origin, and now of recognized importance in the physiological destruction of old red blood cells, is of particular interest in its possible relationship to another function attributed, at least tentatively, to endothelium (3); *viz.*, that of being the ultimate source of the red blood cells. It was with the purpose of determining the type of phagocytic cell, which is found in increased numbers in the bone marrow in pernicious anemia, that these supravital studies were made.

All cases reported were followed clinically on the Thorndike and Fourth Medical Services of the Boston City Hospital, and the diagnoses were personally confirmed. To the cooperation of the Pathological Department of the Boston City Hospital we are indebted for our postmortem material, all of which was secured within 5 hours of death, several cases within 2 hours. During the past year we have studied preparations of living cells from four cases of pernicious anemia and from six patients dying of other causes. Two cases have had biopsy exam-

inations of the bone marrow. The preparations of bone marrow and spleen were made in their own serum if a sufficient amount was present, or with the addition of warm physiological salt solution when necessary. Films of vital neutral red-Janus green were used, according to the technique and criteria described elsewhere (3). Several different preparations were always studied and from 500 to 1000 cells counted in arriving at the relative percentages of the different types.

TABLE I.

Case	1	2	3	4	5	6	7	8	9	10
Diagnosis	Pernicious anemia.				Aplastic anemia.	Coronary infarct.	Acute myeloblastic leucemia.	Nephritis.	Cirrhosis.	Lobar pneumonia.
	Bone marrow.									
				Biopsy.						
PMN. active	0.5	12.0	7.0	1.0	1.0	12.0	2.0	5.0	15.0	19.2
Myeloblasts	3.0	0.0	0.0	0.5	0.0	0.0	38.0	0.0	0.0	4.0
Myelocytes A.	0.0	1.0	0.6	1.3	2.0	0.0	35.0	1.0	2.0	4.0
" B.	48.0	19.0	29.3	18.0	9.0	10.0	9.0	10.0	10.0	10.0
" C.	30.0	50.0	44.0	25.0	34.0	70.0	6.0	60.0	40.0	50.5
PME. " C.	6.0	7.0	4.6	3.2	14.0	4.0	2.0	1.0	2.0	0.6
PMB. " C.	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.5	0.0
Lymphocytes	0.0	0.0	0.6	5.5	15.0	0.0	1.0	2.0	4.5	0.0
Monocytes	0.0	0.0	0.0	0.4	2.0	0.0	0.0	1.0	2.0	0.0
Clasmatocytes	8.0	10.0	10.0	6.0	2.0	3.0	3.0	2.0	3.5	0.3
Megacaryocytes	0.5	0.0	0.3	0.2	0.0	1.0	0.0	1.0	0.5	0.0
Normoblasts	—	—	—	22.0	21.0	—	4.0	15.0	16.0	8.4
Erythroblasts	—	—	—	14.0	0.0	—	—	2.0	4.0	3.0
Megaloblasts	4.5	0.0	3.3	2.1	0.0	0.0	0.0	0.0	0.0	0.0
	Spleen.									
Clasmatocytes	0.5	1.0	0.5	—	5	4.0	8.0	6.0	5.0	4.0

Table I summarizes the differential cell counts on the series of cases studied. It will be noted that the first four cases show a noticeably higher percentage of clasmatocytes in the bone marrow

than do the last six. The first three, all cases of fatal pernicious anemia, have strikingly similar numbers of phagocytic cells, 8 to 10 per cent. Qualitatively, these cells all showed extreme phagocytosis, many containing more than twenty ingested red blood cells, apparently freshly taken in, as they were unchanged in appearance from those outside the phagocytes.[1] In these three cases, probably the most striking feature was the very large number of ingested *nucleated* red blood cells. The peripheral blood at no time in any case showed more than a very occasional normoblast. The inference is that, in these grossly and microscopically hyperplastic marrows, the abnormally stimulated clasmatocytes were not only ingesting old, and probably functionally mature red blood cells, but immature, young forms as well, which had not yet been delivered into the general circulation. The nature of the stimulus, whether acting directly through the phagocytic cells themselves or indirectly through a hypothetical injury to the red cells, is, of course, problematic. Such an analysis and interpretation, at least, indicate the large part which these phagocytic cells may play in contributing to the anomalous situation recognized in pernicious anemia; *viz.*, that of an apparently hyperactively functioning marrow with, nevertheless, a rapidly progressive, idiopathic anemia.

In the gross, the femoral bone marrow from the cases of pernicious anemia was red throughout, as was also that of the ribs; in consistency, gelatinous and elastic, not friable or granular. It had the appearance of a marrow in which there is a stimulation of the red cell-forming elements.

Microscopically, preparations of the fresh bone marrow showed all of the cells to be viable at the time of examination, since all reacted vitally to neutral red-Janus green, and the polymorphonuclear neutrophils were actively motile. At once one was struck with the frequency of large phagocytic cells. Here and there occasional, less actively phagocytic forms were identified by their clear, round nuclei and a glassy, refractile cytoplasm, with possibly one cell inclusion.

[1] Phagocytized red blood cells undergo chemical changes after ingestion, as is indicated by a difference in reaction to supravital dyes and by observable changes in form and structure. A vacuole, in the terminology of Sabin (6), eventually marks the position of an ingested red blood cell, or cells.

But by far the majority were more than normally engorged, and contained unchanged, nucleated and non-nucleated red blood cells and intact leucocytes, together with vacuoles filled with finer, partially digested debris, and reacting with varying shades to the neutral red. Even when only one phagocytized red cell was included it was invariably found in juxtaposition to the nucleus. No clear centrosphere with a "rosette," typical of the monocyte, was observed in any cell. The mitochondrial content was sparse in all, negligible in most. All stages of maturity and activity in the clasmatocytes were seen, with from none to one to twenty cell inclusions. Some single oil immersion fields under the microscope contained as many as four engorged clasmatocytes. Every phagocytic cell, then, seen in the bone marrow from cases of pernicious anemia has been of the endothelial wandering cell type, which we designate as the *clasmatocyte,* and which, in its range of differential features, has already been described and illustrated elsewhere (2).

Occasional polymorphonuclear neutrophilic leucocytes showed a phagocytized nucleated red blood cell of the macrocyte type; a few showed single phagocytized mature microcytes. The percentages of myelocytes and their degrees of maturity, indicated in the convention A for the youngest to C for the oldest, are presented in the table. In normal bone marrow the great majority of the myelocytes are in the C, or preleucocytic, phase, making a large, readily available, potential reserve of cells, quickly convertible into mature functioning units, both regularly and on unusual demand. It is of interest, in this connection, to note that in the cases of pernicious anemia there was a partial throwback to the B level, or less readily available type of myelocyte, which may be correlated with the well known mild leucopenia with relative lymphocytosis characteristic of the disease.

Erythroblasts of all stages of maturity with a relative, limited increase in the younger forms, as contrasted with a normal marrow, were present. Megaloblasts, the earliest hemoglobinophilic primitive cells (3), were found only in the cases of pernicious anemia. It was quite obvious that the majority of the phagocytized red blood cells had been freshly ingested, and were not necessarily senile cells, for they showed the same reactions as the red cells freely floating about, and, moreover, many of them were nucleated.

Wright's stains of both dried and Zenker-fixed films corroborated the supravital findings, with the exception that most of the phagocytic clasmatocytes were destroyed, apparently through rupture of the cell membrane, so that in the fixed preparations this striking feature of the vital studies did not become so evident. After several hours in the warm box the vital preparations tended to show decreased numbers of the more heavily loaded cells. Whereas the early supravital counts averaged 8 to 10 per cent of these cells, later counts of the identical preparations and fields, without additional trauma to, or manipulation of, the cells, and the fixed preparations, similarly averaged only 3 to 5 per cent. These two observations may indicate either that the clasmatocytes are inherently fragile cells, or that the fully engorged ones are, on the digestion of their content, ready to disintegrate as a part of the process of their normal physiological function as scavengers. The latter seems more probable in the light of certain experimental observations.

Studies of the red cells with brilliant cresyl blue showed many cells with all stages and degrees of reticulum. No abnormal precipitation of the basophilic substance was detected in any instance.

In Cases 1 and 2 the spleens were slightly enlarged, firm, deep red, with the cut surfaces showing some serosanguineous exudate. In Case 3 the spleen was not enlarged. Fresh supravital studies were made as with the bone marrow. The cells reacted uniformly to the dyes with cytoplasmic bodies staining only, the criterion for a true supravital reaction. Small lymphocytes predominated. Occasional myelocytes, type C, were seen. Only a very occasional phagocytic clasmatocyte (1/2 to 1 per cent) was seen. However, those cells present were typical phagocytes, and their cell content paralleled that found in the bone marrow. Wright's stains also confirmed these observations. In contrast to the content of phagocytic cells in the spleens from these cases of pernicious anemia, the clasmatocytes in the other cases of this series ranged from 4 to 8 per cent. Necessarily any actual figures taken from differential counts from the spleen must, in the very nature of the procedure, vary widely. But, when numerous different preparations are made and in each case studied carefully, the impression of the difference in content of phagocytic cells from case to case is suggestively striking.

In Case 4 (pernicious anemia) an interesting result was obtained in the examination of the femoral bone marrow removed at biopsy.

This patient had been going down hill for a period of weeks, despite repeated transfusions at about 10 day intervals. There had been no clinical evidence of any remission in the course of the disease at the time the biopsy was decided upon. In the gross, the appearance of the bone marrow was typical of the condition. Microscopically, it presented a most interesting and suggestive picture. A definite percentage of megaloblasts was seen, but the striking feature was the large number of erythroblasts and normoblasts. The clasmatocytes, while 6 per cent in number, were very small, the majority seeming to contain debris and small vacuoles not identifiable as ingested red blood cells. Only one phagocytized nucleated red blood cell was found in many preparations. No cells with the large numbers of ingested red cells, so characteristic of the marrows from the fatal cases of pernicious anemia, were found. The interpretation from the picture presented by this marrow, and without reference to the clinical findings, would have to be that this patient was making plenty of good red cells, without undue activity on the part of the phagocytic group in destroying them; in other words, that he was in a phase of cell production exceeding cell destruction. Within 48 hours the patient began to show clinically evidences of the inauguration of a real remission, and his peripheral blood count, then and subsequently, corroborated this. 3 weeks previous to the biopsy just reported, a similar removal of bone marrow from the other femur had been made on the same patient. Vital studies were not made but the tissue was fixed and sectioned. Contrasting the stained sections from the first and second biopsy tissue, a definite impression, in the absence of actual differential counts, was gained of greater numbers of phagocytic cells with more marked phagocytosis of red cells, in the former than in the latter. A definite shift from the younger, more immature cells toward increased numbers of normoblasts was also apparent in the sections of marrow from the second biopsy. It may be said, then, that at least 48 hours preceding any clinical evidence of a remission in this case, apparently very definite changes were taking place in the bone marrow, consisting of a decrease in the usual numbers, and more particularly in the activity, of the phagocytic cells, with a decided increase in the numbers and effective developmental activity of the erythroblastic series. This simply confirms by observation that which one would suspect; *viz.*, that changes in the hemopoietic centers must precede an observable change in the clinical course of the disease. The interesting point is the apparent earlier cessation in activity of the phagocytic cells in the marrow, preceding the effective increase in red cell activity and clinical improvement.

Case 5 was one with a questionable diagnosis of pernicious anemia. At biopsy, 2 weeks before death, only fatty marrow, entirely acellular, was secured from the femur. No differential count was possible

at that time. At autopsy the same fatty condition of the marrow was found in all the bones, with the exception of a small, circumscribed area very near the epiphysis of one femur; the differential count made from that one small area is recorded. The clasmatocytes were normal in number, 2 per cent. In numerous other preparations not a single cell of either the myeloid or erythroid series could be found. The spleen showed a normal percentage (5 per cent) of clasmatocytes. In so far as the hemopoietic centers were concerned it was practically an aplastic anemia. It will be noted that the lymphocytes in this case were more numerous (15 per cent) than in any bone marrow studied, which again emphasizes the depression in activity of the myeloid centers.

The control cases, which were of non-anemic conditions, showed nothing particularly striking in their differential counts or in the development of the myeloid or erythroid series. Case 7, acute myeloblastic leucemia, shows the reversion in the myeloid series to the myeloblastic "level" of proliferation (5), with a corresponding disappearance of late myelocytes and leucocytes.

If it should be ultimately found, as it now seems possible, that the endothelium of the adult human is the *sine qua non* for both definitive red blood cells and one group of free phagocytic cells, *viz.* the clasmatocytes, we see in the disease known as pernicious anemia a vicious circle of death within one family of closely related cells. The endothelial progenitor of the essential red blood cell, under some, as yet, unknown stimulus gives rise by multiplication to unusual numbers of a type of cell with phagocytic properties, which might be designated as a first cousin of the red cell, which ingest and disintegrate the red cells, often before they have reached the stage of complete maturation.

CONCLUSION.

In general, there is a reversal of the normal in the ratio of clasmatocytes in the spleen to clasmatocytes in the bone marrow in pernicious anemia, with a marked tendency toward the phagocytosis of young, immature, nucleated red blood cells in the bone marrow. The peripheral blood picture suggests that these cells had never been in circulation. The observations made do not indicate that the spleen

takes any directly active part in an increased destruction of blood in pernicious anemia.

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