non-absorption of the proteins when injected subcutaneously, which is caused by precipitation at the locus of injection.[6]

At present the quantity of proteose used for intravenous injections for the human varies from 15 to 40 mg. repeated every forty-eight hours. The administration of this small quantity is quite safe. The lethal dose for the albino rat is 125 mg. per 100 gm. body weight. Applying the lethal dose for the rat to man, 87.5 gm. would be the lethal dose for a 70 kilo man. This is more than two thousand times the largest therapeutic dose employed. (See Table I.)

CONCLUSIONS

1. The so-called "reaction" seen in protein therapy is not at all necessary in order to secure beneficial therapeutic action.

2. Shattered hemo-protein has qualities which make it useful in therapy: it is desensitizing; it acts as a non-specific immunizing antibody; and it is of low toxicity, producing almost no "reaction" in therapeutic doses.

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The differentiation of two distinct types of phagocytic cells in the spleen of the rabbit.

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It has come to be the accepted opinion that the phagocytic cells of the tissues, which have been designated as *clasmatocytes, macrophages* or *histiocytes*, and the large mononuclear elements of the blood, the so-called *monocytes* or *transitionals,* are cells which are very closely related if not identical. The majority of observers consider them to be derivatives of the reticulo-endothelial apparatus or of endothelial cells in general; indeed one of us (Sabin[1]) has presented the view that they are identical cells

[6] Opie, E. L., The fate of injected protein in an animal immunized against it, PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 162.

[1] Sabin, F. R., *Contrib. to Embryol.*, 1920, ix, No. 36, Carnegie Institution of Washington, Publication No. 272.

derived from endothelium. A few observers have maintained that there are differences in the two types but even they accept a common ancestry from endothelium or from a hypothetical histiogenic stem-cell. Many observers have noted the great abundance of phagocytic cells in the spleen and have assumed that the spleen is especially favorable for their production.

With this concept in mind, we have studied the living cells which can be obtained from the spleen by puncture and have come to the conclusion, that, however difficult it may be to establish criteria for two distinct types of phagocytic cells in fixed tissues, in living material definite and satisfactory criteria can be established for separating these cells into two distinct types.

The spleen of an anesthetized rabbit was exposed, a capillary pipette plunged into the substance of the organ, and the blood and tissue cells thus obtained were studied in supra-vital films. The stain used for this study was a combination of neutral red and Janus green.[2] With this technique, it soon became apparent that in addition to the lymphocytic and leucocytic strains of white cells which were always present, the remaining cells could be sharply differentiated into two groups. One group consisted of cells to which the names *clasmatocyte, macrophage* and *histiocyte* have been applied. They were usually large cells, 15 to 30 micra in diameter. These cells always reacted intensely to neutral red; the aggregations of the stain varied greatly in size and color (light red to deep maroon) and were scattered irregularly, without any pattern whatever, throughout the cell. Many of these cells contained phagocytized erythrocytes and occasionally leucocytes; the red blood cells, which had been ingested, sometimes had the color of hemoglobin but in other cases were much darker in tint. The smaller cells always resembled the larger in regard to the character and distribution of the dye and phagocytized material; when only a single cell had been taken up it was always placed adjacent to the nucleus on the side where the greatest amount of cytoplasm was found. The nuclei were usually oval in shape, peripherally located, and relatively small

[2] It is a pleasure to state that samples of both these dyes, which have been obtained from the National Aniline and Chemical Company, have been found wholly satisfactory. Their neutral red is a pure stain and must be used in a much more dilute solution than the Grübler dye.

in comparison to the size of the cell. These cells contained very few mitochondria; the larger cells had none at all or only a few which showed a faint reaction to Janus green; the smaller cells, however, quite frequently contained a moderate number of definite mitochondria scattered throughout the cytoplasm.

The other group consisted of cells which could be identified with the typical monocytes of the peripheral blood. These cells were almost always uniform in size, about 15 micra in diameter, and their horseshoe or kidney-shaped nuclei were large in comparison to the amount of cytoplasm. These cells stained quite characteristically in neutral red. Opposite the hof of the nucleus there was a central clear spot surrounded by a rosette of small radiating aggregations of neutral red, the most peripheral of which were considerably larger than the others. The characteristic color of the neutral red enclosures of the monocyte approximated a soft salmon tint, and there was never the wide variation in color-shading which has been described for the clasmatocyte. The mitochondria, which were likewise always present, were brilliantly stained in Janus green and were located in the periphery of the cell. This appearance of a central, clear area with a rosette of small granules radiating from it, surrounded by the larger neutral red bodies, and by the mitochondria, seems to be quite specific for the monocyte and is usually retained up to the point of cell disintegration. The rosette is occasionally obscured temporarily when the cell is in active motion but it can be seen to reappear when the cell resumes a round or flattened-out shape; here the distinctive reaction to neutral red helps to identify it. This cell is also phagocytic and the reason that its pattern is so persistent is that the material which is phagocytized is placed in the zone of the larger neutral red bodies on the periphery of the rosette.

In spleens in which no myelocytes could be found and, where there were no evidences of a myeloblastic reaction, cells were found in large numbers showing every transition between the full-grown monocyte and a characteristic primitive cell containing only mitochondria. This transition between the primitive cell and the adult monocyte is marked by the appearance of a clump of a few, fine neutral red granules among the mitochondria. These granules never disperse throughout the cytoplasm as is the case in the development of the myelocyte, but

remain close to the clear spot or the centrosphere and very early assume the characteristic radiating arrangement noted above. It is also important to note that these neutral red granules never become so numerous as in the developing myelocyte nor so varied as in the clasmatocyte. In the case of the monocytes of the spleen it has been comparatively rare to find one which has phagocytized whole cells, though occasionally we have seen a monocyte containing one or two red blood corpuscles; in these instances it has been striking that the phagocytized cell was peripheral to the rosette, while in the case of the other type of phagocytic cell (the clasmatocyte) the first red blood corpuscle to be taken up was placed in the area comparable with the center of the rosette of the monocyte.

Therefore these two types of cells present this contrast, that, while the one, the clasmatocyte, shows an absence of pattern both in the reaction to neutral red and in the position of the phagocytized cells; the other, the monocyte, has a striking and persistent pattern in its staining with the neutral red. Differential counts of many normal spleens indicate roughly the following average percentages: monocytes 17 per cent, clasmatocytes 1 per cent.

Our observations lead us to conclude that the monocyte arises from a stem-cell of mesenchymal origin which is resident in the pulp, where, according to the observations of one of us, (Cunningham[3]) there is no endothelium whatever; while on the other hand the larger, more phagocytic cell, the so-called clasmatocyte or histiocyte, is a derivative of the endothelium of the sinuses. The question of terminology is a difficult one since all of the terms used have been applied to both types of cells; we retain the term *monocyte* for the type of cell which occurs in the blood-stream and restrict the term *clasmatocyte, histiocyte* or *macrophage* to the endothelial derivative of high phagocytic power.

[3] Cunningham, R. S., unpublished observations.