Splenomegaly and Hemolytic Anemia Induced in Rats by Methylcellulose — An electron microscopic study

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ABSTRACT Parenteral administration of methylcellulose causes massive splenomegaly and hemolytic anemia in rats. The red pulp of the spleen is markedly cellular due mainly to: (1) large numbers of voluminous free macrophages containing methylcellulose-induced vacuoles, (2) an increase in the number of plasma cells and (3) stasis of blood evidenced by a large number of erythrocytes and platelets in vessels, sinuses and cords. White pulp changes are usually less marked. Here the major change is the presence of macrophages containing methylcellulose-induced inclusions. The slow circulatory time in the spleen and the increase in macrophages may cause the hemolytic anemia observed in these animals.

Methylcellulose, a water soluble methyl ether of cellulose, like many non-metabolizable macromolecules, is accumulated in reticuloendothelial organs. The spleen is especially avid in the uptake of methylcellulose, thereby enlarging to many times its normal size (Hueper, '42).

With repeated parenteral injections of methylcellulose into rats, a mild hemolytic anemia develops. This anemia, however, is associated with a pronounced reticulocytosis indicating considerable erythrocyte destruction compensated by heightened marrow output (Giblett et al., '56). Machado, et al. ('66) and Rowley et al. ('62) studied life spans of Cr51 tagged erythrocytes and found that a maximum, very marked shortening of half life occurred when erythrocytes from methylcellulose-treated animals were transfused into methylcellulose-treated animals, while there occurred only a moderate shortening of half life of normal erythrocytes transfused into methylcellulose-treated animals. If the normal or methylcellulose-treated animals into which the erythrocytes were transfused were previously splenectomized, however, the half life of the erythrocytes was normal. Further, erythrocytes from methylcellulose-treated animals had normal survival rates if transfused into nontreated rats (with or without splenectomy). These data indicate that though the hemolytic process in an animal treated with methylcellulose is primarily due to changes induced in the spleen, erythrocyte too are altered in such a way as to make them more prone to destruction by the altered spleen.

Our interest was attracted to this phenomenon because of the combination of massive splenic enlargement and hemolytic anemia. Our objectives are to characterize the splenic changes and to obtain insight into the mechanisms of hemolytic anemia and of the splenic sequestration of macromolecules and cells.

MATERIALS AND METHODS
The animals employed were young male albino rats weighing between 150 and 300 gm.

Methylcellulose (Methocel, viscosity 400 cps, USP grade, Dow Chemical Co.) was dissolved as follows: 1.5 gm were suspended in 20 cm3 of normal saline at 90°C and immediately thereafter ice-cold saline was added to obtain the desired concentration (methylcellulose is soluble in cold but insoluble in hot water). A group of rats received an intravenous injection of 1 cm3 of 1.5% methylcellulose and spleens from these animals were removed one hour after injection. Other rats received

1 This work was supported by grants HemCA 5375-06 of The United States Public Health Service and a Training Grant of the National Institute of General Medical Sciences.

2 Senior Investigator of the United States Public Health Service.
twice weekly intraperitoneal injections of 2 cm³ of a 2.5% methylcellulose solution and tissues were removed after 2 to 9 administrations.

Several rats were also given intravenously 0.3 cm³ of a thorium dioxide suspension stabilized by dextrins (Thorotrast, Heyden Chemical Co., New York) at 37°C, 15 minutes before removal of the spleen.

The spleens were removed under ether anesthesia by cutting the vascular pedicle. Two cubic millimeter blocks were fixed in 1% osmium tetroxide buffered in collidine to pH 7.2–7.4 (Bennett and Luft, '59), dehydrated in graded ethanols and toluene and embedded in araldite (Richardson et al., '60). Sections for electron microscopy were cut with glass knives on a Porter-Blum ultratome, mounted on bare copper grids, stained with lead hydroxide (Karnovsky, '61) and examined with a Siemens Elmiskop I. Sections 2-3 μ in thickness were also cut from each block and stained with 1% toluidin blue for light microscopy.

**OBSERVATIONS**

**Light microscopy**

The main feature observable by light microscopy is the extreme cellularity of the red pulp compared to the normal. This is evident one hour after methylcellulose administration. In the chronic experiments there is a progressive increase in the number of vacuolated cells which tend to be arranged in clumps and are much more frequent in the red than in the white pulp. There is a relative decrease in the number of sinuses and these, as the cords, are generally filled with cells (figs. 1, 2).

**Electron microscopy**

The main changes one hour after administration of methylcellulose are to be found in the number and kind of cells in the red pulp. The cords are strikingly hypercellular, with little if any intercellular spaces. There is a great increase in number and size of macrophages, almost to the exclusion of other elements. Another abnormal feature is the presence of considerable numbers of plasma cells and platelets. Reticular cells are also altered (vide infra).

In rats which received repeated methylcellulose injections over a period of days these changes are further accentuated.

**Macrophages.** These are mostly free cells located in cords (figs. 3, 4, 7, 12). A single voluminous cell may engorge a whole cord, reaching from sinus to sinus and even extending cytoplasmic processes into the lumen of a sinus (fig. 12). The endoplasm is often but not always rich in membrane-bounded inclusions varying from small vesicles to large electron-lucent vacuoles. Some cells also show tubular structures filled with a homogeneous dense material resembling hemoglobin (figs. 3, 12). The inclusions induced by methylcellulose are at first completely bounded by membranes and contain a clear portion and very dense amorphous matter (fig. 12) but in the chronic experiments the macrophages are filled with clear vacuoles giving the cell a foamy appearance (fig. 7). The inclusions may be partially surrounded by membrane or show no membrane at all and many of them have dense patchy granular material at the edge of the clear inclusion. The macrophages also have very well developed ectoplasm with numerous pseudopodia (figs. 3, 12) which insert themselves between contiguous cells. We have observed several plasma cells and one erythroblast within the cytoplasm of the macrophages (fig. 12). These cells are intact and may be temporarily enclosed (emperipolesis).

**Plasma cells** (figs. 3, 4). There is an unusually large number of plasma cells present in the cords. These often show dilated cisternae of the endoplasmic reticulum which on occasion contain an amorphous and moderately dense material.

The plasma cells bear a close physical relationship to the macrophages. They are almost always in contact with macrophages and in several instances are within or partially surrounded by the cytoplasm of the macrophage without apparent damage to the plasma cell. In the long-term experiments plasma cells are also seen in large numbers in white pulp.

**Platelets** (figs. 3, 9, 12). Platelets are increased in number and are seen in moderately sized clusters in sinuses and cords. In several instances (fig. 9) they were ob-
served in fibrin clots in the sinuses. In such cases they are degranulated.

Reticular (or fixed) cells. Those lining sinuses (figs. 3, 8, 9, 12) are normal in their appearance as described by Weiss ('62). They contain occasional small methylcellulose-induced vacuoles (figs. 8, 9). They vary in thickness from being very attenuated at the margin to having considerable breadth in the area of the nucleus. Their cytoplasm varies in organelle concentration from place to place (figs. 3, 12).

A second type of reticular cell is seen in the cords (fig. 7) and in the white pulp (fig. 11). These are abnormal and tend to have a very electron dense cytoplasm with many irregular tubules and small vacuoles. They also contain larger vacuoles which we have interpreted as being due to methylcellulose. They lie upon variable amounts of patchy extracellular reticulum.

We have also observed sheets of richly vacuolated reticular cells associated with extracellular reticulum which contains variable amounts of collagen (fig. 6).

**DISCUSSION**

Methylcellulose causes two changes pertinent to splenic function: massive splenomegaly and hemolytic anemia.

The large spleen is due to the accumulation of cells possessing inclusions induced by methylcellulose. Those cells with the largest inclusions are free cells, the histiocytes or macrophages. Only one hour after the administration of methylcellulose the cords of the spleen become populated by these voluminous cells with their extensive ectoplasmic processes. In chronic methylcellulose administration large portions of the red pulp are entirely made up of large nests of these cells which now are extensively vacuolated. The mechanical effect of these cells on the circulation through the spleen must be considerable. Circulatory time through the spleen would appear also to be increased and indeed this has been shown by Motulsky ('58). Most arterial vessels in red pulp terminate in cords (Weiss, '62) and there is ready access of terminal arterioles to the sinus wall (Weiss, '63). After methylcellulose, however, the cords are packed with large active macrophages and a functionally direct cordal channel from arterial vessel to sinus would not appear to exist.

The source of methylcellulose macrophages is not established. It seems to us unlikely that they are derived from the reticular cells contributing to the reticular meshwork or the wall of the vascular sinus. Reticular cells are morphologically different, they are all in place, and there are no intermediary forms. We can provide no evidence from our study that reticular cells, which have been claimed to be multipotential, differentiate into other forms. Their main function appears to be supportive, contributing to the sinus wall and reticular meshwork, although cordal reticular cells are somewhat phagocytic. The red pulp macrophages may in part come from the free cells of white pulp migrating through the spleen. Such cells may be differentiated histiocytes or an indifferent cell capable of undergoing histiocytic transformation. But it seems to us that the blood is the most likely source of the macrophages. We would postulate that monocytes, having phagocytized some methylcellulose in the blood, seek out and settle in the red pulp of the spleen or that as a result of the presence of methylcellulose in the spleen, monocytes are trapped and then readily undergo their transformation into macrophages (Sutton and Weiss, '66). Once begun, the process is self-perpetuating for the impedance to the circulation caused by the presence of a number of these methylcellulose cells provides the conditions for further splenic sequestration of both methylcellulose and monocytes. This mechanism can well account for the rapid accumulation of free cordal cells and underscores both the powerful splenic function of selectively removing cells from the blood and also the role of the blood as a source of cells.

The hemolytic anemia after methylcellulose treatment is directly related to pathologic changes in the spleen and could be analogous to the situation in such clinical entities as Gaucher's disease and Banti's syndrome. But there is the added feature that red blood cells previously treated (in vivo) with methylcellulose are destroyed more rapidly in methylcellulose animals than normal cells (Machado et al., '66).
Rowley et al. ('62) and Giblett et al. ('56) both tested the antigenicity of methylcellulose-coated erythrocytes by the Coomb's technique and found no antibodies in the serum. Despite this, we note a large number of plasma cells in the splenic cords. These plasma cells lie against or within macrophages but we are uncertain of their role in relationship to methylcellulose.

In methylcellulose-treated animals, the erythrocytes may undergo a greatly increased "wear and tear" in their passage through the red pulp of the spleen. Rand and Burton ('64) describe how normal cells, when forced through micropipettes, lose portions of their membranes and progressively become microspherocytes. Such spherocytes are less deformable than normal biconcave cells and display increased mechanical and osmotic fragility. Erythrocytes passing through the normal spleen may be forced through narrow passages with resultant loss of membrane and spherocytosis. After methylcellulose this function of the spleen may be exaggerated. Slowing down of intrasplenic circulation, moreover, may be similar to that occurring in cases of increased portal venous pressure which causes the anemia of Banti's syndrome. In Gaucher's disease, as in methylcellulose-treated animals, there is a large number of cordal macrophages with an increased phagocytizing surface. In amyloid disease in which there is also an accumulation of non-metabolizable substance in many organs including the spleen, there is, interestingly, no counterpart hemolytic anemia. In this disease, the amyloid accumulates extracellularly, mainly in basement membranes of vessels (Cohen et al., '60). Thus it does not encroach upon the lumen of vessels until quite late. Indeed, the changes in basement membranes may actually hinder contact of phagocytes and erythrocytes and protect the latter from destruction.

In methylcellulose-treated animals (as in Gaucher's disease and Banti's syndrome) the anemia is mild until the late stages, when chronic renal disease supervenes with concomitant marrow depression (Rowley et al., '62). This indicates that despite passage through more tortuous vascular pathways beset with macrophages, the inherent viability of the erythrocyte is the major factor governing its life span.

The destruction of blood cells by the spleen is active in proportion to (1) the fragility of the erythrocyte, and (2) the opportunity for erythrocytes and phagocytes to come in contact with each other. In methylcellulose-treated animals both these variables may be increased, the first by increased transit time and difficulty of passage of the erythrocytes through the red pulp and the second because there are more and larger phagocytes in the cords.

**ACKNOWLEDGMENT**

We are indebted to Dr. David Bodian for his advice in the preparation of this work.

**LITERATURE CITED**


PLATES
PLATE 1
EXPLANATION OF FIGURES

1 Light micrograph. Foamy macrophages most evident at the upper border of the field fill this splenic cord. A sinus filled with many cells, including a plasma cell (P), is at lower right of the field. Six intraperitoneal injection of methylcellulose. × 1,500.

2 This light micrograph is from a spleen one hour after administration of methylcellulose. The cord is hypercellular, containing many vacuolated macrophages. A sinus (S) is at upper right. × 1,500.
PLATE 2

EXPLANATION OF FIGURE

3 A sinus is filled with masses of platelets (P) and erythrocytes. The sinus lining reticular cell (R) introduces a stout spur into the cord (arrow). A macrophage (M) with numerous ectoplasmic processes filling a large part of the cord is seen in direct contact with the sinus lining cell in an area where neither reticulum nor cordal reticular cell is seen (star). The cord also contains clumps of platelets (P₁), red blood cells and a darkly stained plasma cell (PC) which may be aged or dying. Red pulp, one hour after intravenous methylcellulose. ×6,000.
PLATE 3
EXPLANATION OF FIGURE

4 The cytoplasm of a macrophage (M) with several phagosomes abuts on and partially surrounds three plasma cells (P), one of which has widely distended cisternae. Red pulp one hour after methylcellulose. $\times 20,000$. 
This macrophage (M) contains both thorium dioxide (arrows) and methylcellulose (stars) in separate vacuoles. Two lymphocytes are seen at the bottom of the field. White pulp. Nine methylcellulose intraperitoneal injections. $\times 20,000.$
Splenomegaly due to methylcellulose

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Plate 4

5
Layers of reticular cells (RC) alternating with extracellular reticulum rich in collagen (C) occupy most of this field. The reticular cells contain many small clear vacuoles (arrows). Smooth muscle (SM) cells are present. One hour after intravenous methylcellulose. × 10,000.
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PLATE 5

Image of a diagram with labeled structures: C, RC, Sm.
The cytoplasm of several macrophages (M) is widely distended by varying sized methylcellulose-induced accumulations. An elongate cordal reticular cell has only two vacuoles (arrows). Red pulp. Nine intraperitoneal methylcellulose injections. $\times 20,000$. 
PLATE 7

EXPLANATION OF FIGURE

8 A small sinus with two erythrocytes (E) in its lumen. The cord surrounding it illustrates the tightly packed character of methylcellulose spleens. The cells lining the sinus contain some vacuoles which may contain methylcellulose (arrows). Note the spur of extracellular reticulum (star) cloaked by cordal reticular cell, which extends from the basement of the sinus. Red pulp. One hour after intravenous methylcellulose. $\times 20,000$. 
The lumen of a sinus is occluded by a clot composed of dark amorphous fibrin, numerous platelets (p) which are degranulated and a trapped erythrocyte (E). The sinus lining reticular cells (R) show numerous vesicles. Red pulp. Three intraperitoneal methylcellulose injections. $\times 30,000$. 
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Plate 8

Image description:
- E: Region labeled as E.
- P: Regions labeled as P.
- p: Smaller regions labeled as p.
- R: Region labeled as R.
A portion of red pulp shows a macrophage filled with methylcellulose (M). Another macrophage (M₁) has a small methylcellulose inclusion (star) and a phagocytized erythrocyte (E). Red pulp. Six intraperitoneal methylcellulose injections. × 15,000.
PLATE 10

EXPLANATION OF FIGURE

11 An abnormal, stellate reticular cell shows very dark cytoplasm and shrunken nucleus. No extracellular reticulum is seen adjacent to it. The two lymphocytes in the lower portion of the photograph appear normal. A portion of a macrophage (M) containing phagocytic vacuoles of varying density is seen in the upper corner. White pulp. One hour after intravenous methylcellulose. × 20,000.
These electron micrographs of contiguous fields show a large macrophage (M) with an erythroblast (E) within its cytoplasm. The macrophage possesses many ectoplasmic processes, one of which (star) is introduced into the sinus lumen through a gap in the sinus wall. Several platelets (P) and erythrocytes fill the intercellular spaces in the cord. The cytoplasm of the macrophage shows a multivesicular body, many methylcellulose-induced inclusions and several vesicles containing a substance resembling hemoglobin (arrow). One hour after intravenous methylcellulose. × 10,000.