An Attempt to Produce Hypersplenism in the Dog, Using Methylcellulose

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THE ROLE of the spleen in the control of production and destruction of erythrocytes in "hypersplenic" disease states is not well understood. The lack of a satisfactory experimental model in a large animal has handicapped laboratory investigations of hypersplenism. Efforts to produce the syndrome by ligation of the splenic vein or by anastomosis of the splenic vein to the inferior vena cava or renal vein resulted in the development of anemia in some studies,¹⁻⁵ but not in others.⁶⁻⁸ Even when these procedures were successful, only a mild and relatively transient anemia was observed. Chronic oral administration of hydroxylamine to rats,⁹ administration of horse antirabbit marrow serum to rabbits,¹⁰ and injection of silica into the splenic vein of the dog¹¹ produced complex pathologic manifestations with splenomegaly as only one component.

The parenteral administration of such macromolecular substances as polyvinyl alcohol,¹² gum acacia,¹³ pectin,¹³ zymosan,¹⁴ and methylcellulose¹³,¹⁵⁻¹⁸ has been reported to produce syndromes including varying degrees of splenomegaly and anemia. The hypersplenism produced by the injection of the metabolically inert polymer methylcellulose has given the most consistent results. After subcutaneous injection of methylcellulose, rats have developed splenomegaly and anemia characterized by a significantly shortened red cell survival as estimated with Cr¹⁹⁻²¹ labeled red cells.¹⁶,¹⁸ Baldin¹⁸ has suggested that the methylcellulose anemia is not fully attributable to an increased rate of red cell destruction and that, in addition, there is a decreased responsiveness of the marrow to stimuli for red cell synthesis. He has demonstrated that newborn rats suckled by mothers given methylcellulose have lower total body hemoglobins than sucklings of normal rats or of splenectomized rats given methylcellulose. He has postulated the presence of a splenic humoral inhibitor of the marrow in the methylcellulose "hypersplenic" animal. No reports of detailed hematological investigations in other species have heretofore appeared, although one group mentions the failure to produce hypersplenism in rabbits.¹⁷ The purpose of the present report is to describe hematological and histopathological changes in dogs given methylcellulose.

MATERIAL AND METHODS

Ten to twenty-kilogram mongrel male dogs in good health and 250-Gm. male Sprague Dawley rats were utilized in the study. Four hundred centipoise methylcellulose powder, obtained from Dow Chemical Company, was used. Two per cent solutions were made by preparing a slurry of the powder in one quarter of the requisite volume of normal saline at 90° C., and then immediately adding the remainder of the fluid as cold normal saline.

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Submitted Sept. 8, 1960; accepted for publication Feb. 23, 1961.
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under sterile conditions. In the dog studies the methylcellulose stock solution was diluted to 1.2 per cent concentration with normal saline immediately prior to injection, yielding a clear, moderately viscous solution.

Hematocrits were determined according to the microhematocrit method of Strumia et al.19 Platelets were counted by the method of Brecher et al.20 The number of red cells per 1000 that contain reticulum were determined by the method of Brecher.21 Blood urea nitrogen was measured by the urease method, and serum iron was determined by the method of Bothwell and Mallett.22

The total red cell volume was determined by injection of autologous red cells labeled with 50 to 100 μc. of Cr51 as sodium chromate. Plasma and red cell iron turnovers were estimated with autologous plasma incubated with 10 μc. of Fe59 as ferrous-citrate. The C14 red cell survival curves were obtained following the intravenous administration of 50 to 100 μc. of glycine 2-C14. These isotopic methods and the findings in the normal dog have been fully described elsewhere.23

Plan: Following a three-week control period, six dogs were given 50-ml. intravenous injections of 1.2 per cent methylcellulose five days a week, until a total dose of 2 grams per Kg. was achieved or until toxicity appeared as manifested by marked drowsiness, anorexia, and unsteady gait. Peripheral blood counts were performed during the control period and at weekly intervals during the period of the infusions and for the following 3 months. The total circulating red cell volume and apparent half-time of disappearance of the red cell-bound chromium were estimated at the termination of the infusions. In two dogs the red cell life span was also estimated at that time with glycine 2-C14. The plasma and red cell iron turnovers were determined on three dogs after methylcellulose treatment.

Twelve rats were injected intraperitoneally with 2.5 cc. of 2.5 per cent methylcellulose twice a week for twelve weeks. One week after the last dose of methylcellulose, the rats were sacrificed and peripheral blood counts, blood urea nitrogens, and necropsies were performed. One dog was sacrificed at 1 week, two at 3 months, and two at 2 years following termination of the infusions. One of the animals died one week following the last injection of methylcellulose. Necropsies were performed on all animals utilized in the study.

RESULTS

Dogs

The dogs exhibited no acute reaction to the injections other than infrequent transient hyperpnea. Three dogs tolerated the entire course of injections. The other three dogs received from 0.5 to 1.0 Gm. of methylcellulose per kilogram before they became severely lethargic. The mean hematocrits of the 6 dogs during the infusions are plotted as the per cent of the control values in fig. 1. There was a 26 per cent reduction from the control mean hematocrit of 48 ± 4 to 35 ± 2 at the termination of the infusions.

The nonisotopic hematological and chemical data are shown in table 1. There was no significant difference in the mean white count, platelet count, reticulocyte count, or serum iron concentration between determinations during the control period and those following methylcellulose administration. The mean blood urea nitrogen rose from 15 mg. per cent during the control period to 96 mg. per cent after the infusions.

The results of the blood volume, red cell life span, and iron turnover determinations are shown in table 2. The total red cell volume following the methylcellulose infusions ranged from 19.5 to 32 cc./Kg. with a mean of 27 cc./Kg., compared with 38.6 cc./Kg. in 40 normal controls. The mean plasma volume of the treated animals was 50 cc./Kg., compared with 47 cc./Kg. in the controls.

The mean apparent Cr51 red cell half-time was 18 days, compared to 24 days
Fig. 1.—The alteration in the mean hematocrit of 6 dogs following methylcellulose infusion.

in the controls. The mean plasma Fe\textsuperscript{59} disappearance T\textsubscript{1/2} was 47 minutes compared to 66 minutes in the controls. The plasma iron turnover was 0.83 mg./Kg./day in the three treated dogs studied, compared to 0.63 mg./Kg./day in the normals. The mean red cell iron turnover was 0.70 mg./Kg./day in the animals receiving methylcellulose, compared to 0.49 in the controls.

The C\textsuperscript{14} glycine red cell survival performed on the dog with the most severe anemia showed a pattern of moderately severe random destruction (fig. 2). The mean red cell life span of a second dog was 80 days, with a C\textsuperscript{14} curve that showed no random destruction.

Table 1.—Studies of Peripheral Blood Counts, Serum Iron, and Blood Urea Nitrogens During the Control Period, and Following Methylcellulose Infusions in the Dog

<table>
<thead>
<tr>
<th></th>
<th>Control period</th>
<th>Postmethylcellulose infusions (1–90 days)</th>
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<tbody>
<tr>
<td>Hematocrit</td>
<td>48 ± 3</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>White blood count</td>
<td>(10 \pm 0.6 \times 10^3/\text{mm.}^3)</td>
<td>(11 \pm 1.4 \times 10^3/\text{mm.}^3)</td>
</tr>
<tr>
<td>Platelets (\times 10^4/\text{mm.}^3)</td>
<td>(30 \pm 7 \times 10^4/\text{mm.}^3)</td>
<td>(30 \pm 4 \times 10^4/\text{mm.}^3)</td>
</tr>
<tr>
<td>Reticulocyte</td>
<td>0.54 ± 0.14/100 RBC</td>
<td>0.62 ± 0.20/100 RBC</td>
</tr>
<tr>
<td>Serum iron ((\mu g. \text{ per cent}))</td>
<td>102 ± 30</td>
<td>99 ± 32</td>
</tr>
<tr>
<td>Blood urea nitrogen ((\text{mg. per cent}))</td>
<td>15 ± 3</td>
<td>96 ± 20</td>
</tr>
</tbody>
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Table 2.—Blood Volume, Plasma and Red Cell Iron Turnover, and Red Cell Life Span Data in Dogs Following Methylcellulose Infusions

<table>
<thead>
<tr>
<th></th>
<th>Controls (23)</th>
<th>Post-methylcellulose infusions (1–30 days)</th>
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<tbody>
<tr>
<td>Cr⁵¹ red cell volume (cc./Kg.)</td>
<td>39 ± 3</td>
<td>27 ± 4</td>
</tr>
<tr>
<td>Cr⁵¹ plasma volume (cc./Kg.)</td>
<td>46 ± 4</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>Cr⁵¹ RBC T½ days</td>
<td>24 ± 3</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>Plasma Fe²⁵ T½ min.</td>
<td>66 ± 14</td>
<td>47 (43–56)*</td>
</tr>
<tr>
<td>Plasma iron turnover (mg./Kg./day)</td>
<td>0.63 ± 0.09</td>
<td>0.83 (0.63–1.02)</td>
</tr>
<tr>
<td>Red cell iron turnover (mg./Kg./day)</td>
<td>0.49 ± 0.08</td>
<td>0.70 (0.50–0.81)</td>
</tr>
</tbody>
</table>

*Observed range.

Pathologic Observations:—Grossly, the spleens of the dogs receiving methylcellulose were pale and uniformly mottled grey-white. After expressing blood, the mean ratio of spleen to body weight was 6.3 Gm./Kg., compared to 4.8 Gm./Kg. in 6 normal controls. Microscopically, the spleens of the animals sacrificed one week following methylcellulose infusions were almost completely replaced by cells with pyknotic nuclei and with their cytoplasm distorted by large clear vacuoles (fig. 3). The malpighian corpuscles were hypoplastic. The spleens of the dogs sacrificed 5 months to 2 years following the infusions contained fewer vacuolated cells, but were fibrotic. Mild subcapsular sinusoidal engorgement with red cells was noted in all spleens and was most prominent.
Fig. 3.—Section of spleen of a dog sacrificed 1 week following termination of methylcellulose infusions (hematoxylin and eosin stain $\times 285$), demonstrating foamy methylcellulose-containing cells throughout the splenic pulp.

in the dog with the C$^{14}$ red cell curve that showed severe random destruction.

The kidneys were hard and pale. Those from dogs sacrificed at 2 years after infusions had irregularly scarred surfaces. Yellow granular calculi were noted in the renal pelvis of one animal.

Microscopically the specimens from animals sacrificed 1 week after termination of the infusions showed foamy cytoplasmic distention of the glomerular endothelial cells with reduction of the capillary lumens. The tubules were dilated and cystic in some areas (fig. 4). There was cloudy swelling and focal vacuolization of the tubular epithelium. Numerous foamy macrophages were present throughout the interstitial tissue. Progressive interstitial fibrosis and lymphocytic infiltration was noted in the kidneys of the dogs sacrificed 3 months to 3 years following the infusions. In one dog interstitial calcification was present. The glomeruli of these dogs showed subcapsular fibrosis, at times progressing to complete hyalinization. The lungs contained small nests of distended macrophages and some septal thickening, but no vascular thrombosis or edema. The livers of the animals sacrificed 1 week to 3 months following
Fig. 4.—Section of kidney of a dog sacrificed 2 years following methylcellulose infusions (hematoxylin and eosin × 130). Note the fibrotic hyalinized glomerulus, the dilatation of the tubules, and the fibrosis and lymphatic infiltration of the interstitial tissue.

Infusions contained Kupffer cells with large cytoplasmic vacuoles. There was minimal atrophy and vacuolization of the parenchymal cells. Only minimal vacuolization and no fibrosis was seen in the livers of animals sacrificed 2 years after the infusions. In the bone marrows there were very few foamy macrophages; occasional vacuolated multinucleated giant cells were the only abnormalities.

**Rats**

Following the methylcellulose injections the mean hematocrit of the rats fell from 48 ± 3 to 35 ± 2. The mean blood urea nitrogen following the infusions was 17.5 ± 2, compared to 12.5 ± 1 mg. per cent during the control periods.

**Pathologic observations:**—The histological changes in the methylcellulose-infused rat are different from those seen in the dog. The rat spleens following the infusions were greatly enlarged to 8 ± 2 Gm., compared to 0.9 ± 0.2 Gm. in the control animals.
Fig. 5.—Section of spleen from a rat sacrificed 1 week following termination of methylcellulose infusions (hematoxylin and eosin × 210), demonstrating islands of methylcellulose-filled cells surrounded by a rim of lymphocytes and an engorged red pulp.

Microscopic examination of the spleens revealed numerous small nodules consisting of large pale cells with indistinct cytoplasmic borders, surrounded by a rim of small lymphocytes (fig. 5). These cells were distended with many small cytoplasmic vacuoles and had large finely reticular nuclei, in contrast to the large vacuoles and pyknotic nuclei seen in the dog spleens. The rat spleens were strikingly engorged with red cells in comparison with those of the dog.

The kidneys of the rats contained vacuolated glomerular cells, but there was no significant tubular atrophy and cyst formation (fig. 6). In contrast to the dog kidneys, neither foamy macrophages nor interstitial fibrosis were prominent.

Sections of the liver and lung contained foamy vacuolated reticuloendothelial cells similar to those seen in the dog.

**Discussion**

Although it has been possible to produce a moderate reduction in the hematocrit, total red cell volume and red cell life span following methylcellulose
infusions in the dog, this is clearly an unsatisfactory model for the study of hypersplenism.

The severe renal damage and resultant uremia provide an adequate explanation for most of the hematological findings and obscure any manifestations of hypersplenism. No severe pulmonary lesions were noted.

The hematological and pathological changes seen in the rat are consistent with those noted in the literature.16,17 The more marked splenic enlargement and engorgement seen in the rat may explain the more severe anemia and shortening of the red cell life span in this species, compared to the dog.

The inability of the rat with splenomegaly due to methylcellulose to increase the rate of erythropoiesis to the theoretical maximum may be on the basis of the observed pathology of the kidney, the currently accepted site of erythropoietin production.24

**Summary**

(1) Hematological and histopathologic changes were studied in dogs and rats after infusions of solutions of methylcellulose.
(2) After 4 to 8 weeks of daily intravenous infusions of 0.6 Gm. of 400 centipoise methylcellulose, the dogs developed moderate anemia with a reduction of the mean red cell volume to $27 \pm 4$ cc./Kg., compared with $38.6 \pm 3$ cc./Kg. in the controls, and a reduction in the apparent red cell life span studied with $\text{Cr}^{51}$ from $24 \pm 3$ days in the controls to $18 \pm 3$ in the treated animals.

(3) Following the infusions in the dog there was an increase of the mean blood urea nitrogen from 15 mg. per cent to 96 mg. per cent, with only a small increase in the rat.

(4) At necropsy there was foamy cytoplasmic vacuolization throughout the reticuloendothelial systems of both rat and dog. In the dog, the spleens were pale and moderately enlarged. Large cells with vacuolated cytoplasm largely replaced the normal structures of this organ. In the markedly enlarged rat spleen there were islands of methylcellulose-filled cells, surrounded by a rim of lymphocytes and strikingly engorged red pulp.

In the rat there was vacuolization of the renal glomerular cells but no significant change in the tubules or interstitial tissue. In the dogs sacrificed one week after the termination of the methylcellulose infusions, foamy cytoplasmic distention of the glomeruli and interstitial cells and tubular dilatation and cyst formation were noted. Progressive fibrosis and hyalinization of the glomerulus and fibrosis and calcification and lymphocytic infiltration of the interstitial tissue were seen in the kidneys of dogs sacrificed from 3 months to 3 years after termination of the methylcellulose infusions.

(5) Although methylcellulose infusions produce splenomegaly and anemia in the dog, the associated uremia precludes this preparation as a model for the study of hypersplenism.

**Summario in Interlingua**

1. Alterationes hematologic e histopathologic esseva studiate in canes e rattos post infusiones de un solution de methylcellulosa.

2. Post 4 a 8 septimanas de diurne infusiones intravenose de 0.6 g de methylcellulose de 400 centipoises, le canes disveloppava moderate grades de anemia con un reduction del volumine medie de erythrocytos ad $27 \pm 4$ cm$^3$/kg, comparate con $38.6 \pm 3$ cm$^3$/kg in animales de controlo, e un reduction in le apparente longevitate erythrocytic (studiate con $\text{Cr}^{51}$) ad $24 \pm 3$ dies in le animales de controlo ad $18 \pm 3$ dies in le animales tractate.

3. Post le infusiones il habeva in le canes un augmento del nivello medie de nitrogeno de urea del sanguine ab 15 mg pro 100 ml ad 96 mg pro 100 ml. Le correspondent augmento in rattos esseva micre.

4. Al necropsia un spumose vacuolisation cytoplastic esseva constatate in omne partes del systema reticuloendothelial in rattos e etiam in canes. In le canes, le splen esseva pallide e allargate a grades moderate. Grande cellulas con vacuolitate cytoplasma reimplaciava in alte mesura le structuras normal de iste organo. In le marcatemente allargate splen del rattos, insulas esseva trovate que consisteva de cellulas plenate de methylcellulosa e que esseva circundate per un margine de lymphocytes et de frappantemente turgide pulpa rubie.

5. Although methylcellulose infusions produce splenomegaly and anemia in the dog, the associated uremia precludes this preparation as a model for the study of hypersplenism.
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In le ratto ii habeva vacuolisation del cellulas reno-glomerular sed nulle significative alteration in le tubulos o le tissu interstitial. In le canes que esseva sacrificate un septimana post le terminacion del infusions de methylcellulosa, un spumose distention cytoplasmic del glomerulos e cellulas interstitial e dilatation tubular e formation de cystes esseva notate. Un progressive fibrosis e hyalinisation del glomerulos e fibrosis e calcification e infiltration lymphocytic del tissue interstittial esseva observate in le renes de canes sacrificate inter 3 menses e 3 annos post le terminacion del infusions de methylcellulosa.

5. Ben que infusions de methylcellulosa produce splenomegalia e anemia in canes, le associate uremia not permitte le uso de iste preparato in le studio experimental de hypersplenismo.

ACKNOWLEDGMENT

The authors wish to thank Dr. John H. Edgcumbe for his help in the interpretation of the histological sections and in the preparation of this manuscript.

REFERENCES

The experimental production of splenomegaly, anemia, and leukopenia in albino rats. Blood. 8:72, 1953.


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