REACTIONS OF THE BLOOD AND ORGANS OF DOGS AFTER INTRAVENOUS INJECTIONS OF SOLUTIONS OF METHYL CELLULOSES OF GRADED MOLECULAR WEIGHTS *

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The intravenous introduction of macromolecular substances of varying molecular weights, sizes and shapes (plasma, plasma albumin, plasma globulin, hemoglobin, gelatin, isinglass, polyvinyl alcohol, methyl cellulose, gum arabic, pectin,[1-9] vinyl pyrrolidon polymer[10]), or of colloidal material of different particle sizes (Evans blue, Congo red, inulin, metallic colloids) is a procedure used for therapeutic purposes in shock, hemorrhage and septic infections as well as for diagnostic purposes in the determination of total plasma volume, amyloidosis and renal function. Disturbances of the macromolecular and colloidal status of the proteins, polysaccharides and lipids of the blood are found in immune reactions, myelomatosis, glycogen-storage disease, nephrosis, and diseases with lipemic reactions.

Despite the obvious frequency of abnormal fluctuations in the macromolecular, colloidal equilibrium of the blood, no systematic investigations exist concerning the rôle which the size of the aggregates eliciting such reactions plays in the development and the type of the pathological manifestations produced in the blood and organs. The present investigations were conducted in an attempt to fill this gap by using a series of methyl celluloses which are identical in chemical structure, but differ in the relative length of the dextrose chain and thus in molecular size.

Experimental Procedure

Inasmuch as detailed information on the physicochemical properties of methyl cellulose has been recorded in previous publications (Hueper,[4] Hueper and Ichniowski,[9] Hueper, Martin and Thompson[11]), it may suffice here to point out that methyl cellulose is the methyl ether of cellulose. As a hydrophilic colloid, it forms a viscous solution, from which it can be precipitated by the addition of various salts and which forms a firm, white coagulum upon heating above 65° C., reversible on cooling. It is digested by pancreatin at 40° C. with the development of reducing sugars.[12] When introduced into the blood, it forms an emulsion with the blood plasma. Table I presents the relations which exist as to grade of viscosity, molecular weight and degree of polymeri-

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zation between the seven types of methyl cellulose used in this and preceding studies.

The data on the molecular weights, the degree of polymerization and the grade of viscosity for the 2 per cent solution were supplied by Dr. R. M. Upright, The Dow Chemical Company, from which the various methyl celluloses were obtained. The viscosity of the 0.25 per cent solution was determined with the Hess' viscosimeter and compared with water at 18.5° C.

The present studies were concerned mainly with six members of the series of methyl celluloses, since methyl cellulose of 400 cps. (centipoises) was employed in previous investigations (injected as a 2 per cent solution in amounts of from 25 to 130 cc. into 7 dogs). The data

TABLE I

Physicochemical Properties of Methyl Celluloses

Viscosity grade		Average molecular weight	Polymerization degree
2% sol. cps.*	0.25% sol. H_2O		
15	1.45	32,200	169
25	1.55	36,400	191
50	1.85	53,400	281
100	2.05	60,500	318
400	2.73	77,700	409
1500	3.95	118,200	622
4000	6.00	143,600	756

* Centipoises.

obtained on those occasions, therefore, will be mentioned only briefly for comparative purposes. The solutions of the various methyl celluloses were prepared in 1 per cent sodium chloride solution and had a viscosity of approximately 16 or 8 times that of normal plasma (methyl cellulose, 15 cps., 2.8 per cent; m.c., 25 cps., 2.4 per cent; m.c., 50 cps., 2 per cent; m.c., 100 cps., 1.8 per cent; m.c., 1500 cps., 1 per cent; m.c., 4000 cps., 0.7 per cent). While methyl celluloses of 1500 cps. and 4000 cps. gave a water-clear solution when dissolved at icebox temperature, the solutions of the other methyl celluloses were more or less turbid and had to be filtered through filter paper and Hyflo Supercel* for the removal of coarser floccules. They remained slightly turbid even after these procedures.

For the study of the more immediate hematic reactions, 40 cc. of these solutions were injected intravenously into dogs. There were 2 or 3 dogs in each group. The observation time of these reactions was 24 hours. The prolonged experiment started immediately thereafter

* Hyflo Supercel is a preparation of purified diatomaceous earth, obtained from the Dicalite Co., 120 Wall Street, New York, N.Y.

and consisted in the intravenous injection of methyl cellulose solution 5 days per week in rising amounts. The daily dose was 40 cc. during the first week, and was increased by 10 cc. each week until 130 cc. was reached for those dogs which survived longest. The average weight of the 18 dogs used in these and previous experiments was 9.3 kg. and the weight range was between 7 and 12 kg. The observation time varied for the different dogs from 1 week to 6 months. Examinations of the blood (hemoglobin, number of erythrocytes and leukocytes, coagulation time, sedimentation rate, volume of packed cells, plasma viscosity) were made at intervals of 2 weeks. Autopsies were performed on all dogs.

A. Hematic Reactions after the Intravenous Injection of a Single Dose of Methyl Cellulose Solution

Examinations of the blood were made 5, 15, 30, 60, 120, 240 minutes and 24 hours after the injection, following the standard determinations.

a. Methyl Cellulose, 15 cps. (Two Dogs)

Erythrocytes. During the first 120 minutes there occurred in both dogs moderate to severe downward fluctuations of the number of erythrocytes, amounting to from 1.5 to 3 million cells. Thereafter the number of erythrocytes became stationary at a level slightly to moderately below the original one.

Hemoglobin and Volume of Packed Cells. Variations in hemoglobin and volume of packed cells ran parallel to those of the erythrocytes.

Leukocytes. There was a moderate to severe leukopenia 5 to 15 minutes after the injection, followed by return to approximately the original level within 2 hours.

Clotting Time. There were no significant deviations in clotting time.

Sedimentation Rate. There occurred a marked acceleration of the erythrocytic sedimentation rate before 4 hours after the injection. This was followed by a considerable decrease at the 24 hour test.

Plasma Viscosity. The plasma viscosity was moderately increased (0.5 to 0.1) beyond the normal range immediately after the injection, and returned to normal at the 4 hour test.

b. Methyl Cellulose, 25 cps. (Two Dogs)

The hematic reactions followed closely in pattern those seen in the animals of the previous group, with the exception of the sedimentation rate which was already markedly increased at the 5 minute test, and remained high to the end of the observation period (24 hours).

c. Methyl Cellulose, 50 cps. (One Dog)

Erythrocytes. Following an increase by 1.35 million at the 5 minute level, the erythrocytes remained relatively stationary around the original level during the 24 hour observation period.

Hemoglobin and Packed Cell Volume. The hemoglobin values, as well as the figures obtained from hematocrit determinations, remained below the original level throughout the 24 hours, exhibiting a tendency to drop progressively.

Leukocytes. Transitory leukopenia reached its lowest level at the 15 minute test. The leukopenic crisis had completely disappeared at the 4 hour test.

Clotting Time. No significant changes in clotting time were found.

Sedimentation Rate. Sedimentation was definitely hastened at the 5 minute test, and was moderately accentuated during the 24 hour period.

Plasma Viscosity. There was a marked increase in plasma viscosity directly after injection (1.25). This reaction subsided somewhat during the first 4 hours, at the end of which the increase in viscosity had dropped to 0.35; but this was followed by a renewed increase after 24 hours by 1.6 points (to 3.75) above the original level (2.15).

d. Methyl Cellulose, 100 cps. (Three Dogs)

Erythrocytes. With the exception of 1 dog which showed an initial rise in the number of the erythrocytes at the 5 minute test, amounting to 1.15 million, the erythrocytes remained relatively stationary during the first 2 hours and decreased moderately in 2 dogs from then on.

Hemoglobin and Volume of Packed Cells. There was a definite tendency to reduced values for both hemoglobin and volume of packed cells with the progress of the test.

Clotting Time. No deviations from the normal range of clotting time were noted.

Sedimentation Rate. Sedimentation of erythrocytes was definitely accelerated at the 5 minute test, and was accentuated during the further course of the experiment. The sedimentation rate thus remained persistently high during the 24 hour observation period.

Plasma Viscosity. The plasma viscosity was moderately increased immediately after the injection (0.65 to 1.0 point) and remained elevated during the entire period.

e. Methyl Cellulose, 400 cps. (Three Dogs)

Erythrocytes. There was a mild reduction in erythrocytes during the first 4 hours, followed by a return to the original level at the 24 hour test.

Hemoglobin and Volume of Packed Cells. The hemoglobin values dropped from the 5 minute test on, and remained reduced during the entire 24 hour period. Hematocrit values followed a similar trend.

Leukocytes. Transitory leukopenia lasted from 2 to 4 hours, and was followed by leukocytosis at the 24 hour test.

Clotting Time. There was a lengthening of the clotting time appearing 15 minutes to 2 hours after the injection and persisting in 2 dogs at the 24 hour test.

Sedimentation Rate. Acceleration of erythrocytic sedimentation was marked at the 5 minute test and persisted for more than 24 hours.

Plasma Viscosity. Plasma viscosity was markedly increased immediately after injection and then showed a moderate drop, but remained considerably above the original level during the entire 24 hours.

f. Methyl Cellulose, 1500 cps. (Three Dogs)

Erythrocytes. There was no consistent or appreciable variation in the number of red cells during the first 15 minutes. All dogs showed during some time thereafter a moderate reduction in erythrocytes.

Hemoglobin and Volume of Packed Cells. Fluctuations in hemoglobin and volume of packed cells paralleled in general those in the number of red cells.

Leukocytes. Transitory leukopenia lasted for 4 hours or more.

Clotting Time. There was a minor to moderate increase in the clotting time during the first hour with a return to normal values thereafter.

Sedimentation Rate. The hastening of the erythrocytic sedimentation was pronounced at the 5 minute test and remained high or even increased during the remainder of the observation period.

Plasma Viscosity. Increase in the plasmatic viscosity was mild and did not persist for longer than 30 minutes.

g. Methyl Cellulose, 4000 cps. (Three Dogs)

Erythrocytes. Following a moderate brief reduction in erythrocytes some time during the first 45 minutes, the number of red cells returned to approximately the original level for the remainder of the 24 hour period.

Hemoglobin and Volume of Packed Cells. The hemoglobin was comparatively decreased, particularly during the latter part of the experimental period. The hematocrit values showed a similar movement.

Leukocytes. Transitory leukopenia extended beyond the 4 hour test. A moderate to marked leukocytosis was found in 2 dogs at the 24 hour examination.

Clotting Time. No significant deviations in clotting time were observed.

Sedimentation Rate. The increase in erythrocytic sedimentation was moderate during the first 45 minutes and became considerable and persistent thereafter.

Plasma Viscosity. There was a mild increase in the plasmatic viscosity not surpassing the normal range, which persisted throughout the experimental period.

The hematic reactions observed in dogs following the intravenous introduction of 40 cc. of solutions of the seven types of methyl cellulose prepared in normal saline solution and having the same viscosity (8 times that of plasma) revealed the following pattern and discrepancies. The number of erythrocytes exhibited in all instances a tendency toward a mild to moderate decrease, particularly toward the latter part of the observation period. This movement was displayed more consistently and considerably by the amount of hemoglobin and the volume of packed cells, especially with the methyl celluloses of higher molecular weight. A leukopenic reaction was noted with all types of methyl cellulose, but the leukopenic crisis was least persistent with the methyl celluloses of low molecular weight, while lasting for more than 4 hours with those of higher molecular weights, in connection with which the reaction was not infrequently followed by a leukocytosis at the 24 hour test.

Abnormalities in clotting time were found only with methyl celluloses of 400 and 1500 cps. and especially with the former. The sedimentation rate was increased with methyl celluloses of all types. However, this acceleration showed a delayed appearance with the two low molecular methyl celluloses, compared with the immediate hastening found with the other types. It was noted, moreover, that this response was of lesser duration with the low molecular products, whereas it was of higher severity and greater duration with the higher molecular substances. The plasma viscosity was increased in all instances immediately after the injection. This increase was of relatively short duration and low intensity with the two low molecular methyl celluloses. It was of higher intensity and long duration with the medium molecular substances and of low intensity and long duration with those of high molecular weights, while no pathological increase resulted from the injection of the methyl cellulose of highest molecular weight.

B. Hematic Reactions after the Intravenous Injection of Repeated Doses of Methyl Cellulose Solution

Blood tests were made at intervals of 2 weeks, unless severe changes necessitated a more frequent control of the status of the blood.

a. Methyl Cellulose, 15 cps. (Two Dogs)

One dog received within 2 weeks a total of 570 cc. of methyl cellulose solution, while the second dog was injected within 18 days with 670 cc. Both dogs died.

Erythrocytes. There was a moderate decrease in the number of red cells, ranging from 1.1 to 1.8 millions below the original value.

Hemoglobin and Volume of Packed Cells. The decrease in hemoglobin and in volume of packed cells comparatively surpassed that of the erythrocytes to a minor degree.

Leukocytes. There were no pathological fluctuations in the number of leukocytes.

Clotting Time. No abnormal deviations in clotting were noted.

Sedimentation Rate. There was a marked increase in the sedimentation rate.

Plasma Viscosity. Toward the end of the experimental period the plasma viscosity was elevated by 50 to 100 per cent above its normal level.

b. Methyl Cellulose, 25 cps. (Two Dogs)

One dog received a total of 1580 cc. of methyl cellulose solution within 52 days, whereas the second dog was injected with 130 cc. within 11 days. Both dogs died.

Erythrocytes. The red cells dropped by more than 66 per cent in the dog surviving for 52 days (from 6 million to 1.65 million).

Hemoglobin and Volume of Packed Cells. The reduction in the amount of hemoglobin was approximately equal to that of the erythrocytes, while the decrease in the volume of the packed cells even surpassed the drop of the erythrocytes.

Leukocytes. There was a temporary moderate leukocytosis followed during the latter part of the experiment by a return to normal values.

Clotting Time. No abnormalities in clotting time were noted.

Sedimentation Rate. The sedimentation of the erythrocytes was highly and increasingly accelerated.

Plasma Viscosity. The plasma viscosity rose to a peak of 4.9, 4 weeks after the start and then gradually dropped back to 3.25, rising again toward the end to 4.5.

c. Methyl Cellulose, 50 cps. (Four Dogs)

One dog was injected with 290 cc. of methyl cellulose solution within 10 days, a second with 370 cc. within 12 days, a third with 1320 cc. within 32 days, and a fourth with 2010 cc. within 75 days. All dogs died.

Erythrocytes. In all dogs there was a progressive, and ultimately considerable, decrease in the number of erythrocytes. In the dog sur-

viving 75 days the drop was from 6.21 million to 1.42 million, following a temporary partial recovery during a period in which the injections were discontinued.

Hemoglobin and Volume of Packed Cells. The reduction in hemoglobin was considerable and progressive and reached in the aforementioned dog a level of less than 5 gm. per 100 cc. of blood. The decrease in the volume of packed cells was relatively more pronounced than the decrease of red cells in those animals having a moderate anemia, while it was comparatively less definite in the dog with the severe anemia after having passed previously through the first mentioned relation.

Leukocytes. There was a moderate leukocytosis present during the latter part of the experimental period.

Clotting Time. There were no abnormalities in clotting time.

Sedimentation Rate. There was a very marked acceleration of the sedimentation rate (75 to 79 mm., Wintrobe tube).

Plasma Viscosity. The plasma viscosity was considerably elevated, varying in 3 dogs between 4.9 and 6.0, after having undergone during the early part of the experiment a considerable increase followed during the middle part by a decrease to slightly elevated levels when the injections were discontinued and subsequently again by a marked elevation following the resumption of the treatment.

d. Methyl Cellulose, 100 cps. (Three Dogs)

One dog received 80 cc. within 7 days, a second dog was injected with 4645 cc. within 90 days, and a third dog with 5190 cc. within 97 days. All died.

Erythrocytes. After 2 weeks of treatment there developed a rapidly progressive and severe reduction in the number of red cells, causing after 2 months a drop to one-third and one-fourth, in the respective surviving animals, of the original number. When the treatment was then discontinued, there followed a rapid rise in the number of erythrocytes, which amounted to from 2 to 2.5 million within 2 to 3 weeks. There was, however, a very rapid drop in erythrocytes when the injections were again given, reaching values of 2.4 and 1.35 million, respectively, before death.

Hemoglobin and Volume of Packed Cells. The hemoglobin followed in general the fluctuations of the erythrocytes and sank ultimately below 5 gm. per 100 cc. of blood. The volume of packed cells dropped during the first half of the erythrocytic decrease, comparatively very sharply surpassing the reduction of the red cells, while during the latter part of the experiment the decrease in the volume of packed cells was relatively smaller than that of the erythrocytes.

Leukocytes. There were no significant deviations in the number of leukocytes from the normal range, except during the final days, when a moderate leukocytosis existed.

Clotting Time. No abnormalities in clotting time were noted.

Sedimentation Rate. An early and marked acceleration of erythrocytic sedimentation persisted through the treatment-free period to the end.

Plasma Viscosity. The plasmatic viscosity was increased to slightly above 6 during the major part of the experiment, except during the treatment-free period, when the viscosity dropped to values between 2.5 and 3.5.

e. Methyl Cellulose, 400 cps. (Seven Dogs)

One dog received 260 cc. of methyl cellulose solution within 6 days, a second and a third animal were injected with 1040 cc. within 12 and 13 days respectively, a fourth dog was injected with 2112 cc. within 57 days, a fifth received 2930 cc. within 66 days, a sixth 3375 cc. within 83 days, and a seventh 5720 cc. within 6 months. The first 3 dogs died, while the other 4 were killed. The seventh animal was sacrificed 2 months after the last injection, while the others were sacrificed not later than a few days after the last treatment.

Erythrocytes. There occurred a gradual decrease in the number of erythrocytes, which reached, however, severe degrees only in those animals which died, remaining within a moderate range in those which survived the prolonged treatment for many weeks and were finally sacrificed. It was noted, moreover, that during a temporary discontinuation of the treatment a relatively rapid increase in the number of red cells developed. This was especially striking in the dog which was killed 2 months after the last injection and which showed a recovery to the original erythrocytic level 1 week after cessation of treatment.

Hemoglobin and Volume of Packed Cells. The hemoglobin decreased during the early part somewhat more rapidly than the erythrocytes, but exhibited a normal relation to the cellular elements in the latter part of the experiments. The volume of packed cells underwent a similar movement.

Leukocytes. The number of white cells fluctuated within the normal range, sometimes reaching relatively low figures.

Clotting Time. The clotting time was rather consistently and considerably increased, and decreased slowly after cessation of treatment.

Sedimentation Rate. Erythrocytic sedimentation was considerably hastened during the entire time of treatment and showed a gradual return toward normal values after cessation of injections.

Plasma Viscosity. The plasma viscosity was markedly increased during the course of treatment and particularly so during the last days of those dogs which died spontaneously. In those animals it reached values up to 7.88 after relatively few injections. In dogs with more prolonged and severe treatment this value was often surpassed during the more advanced stages, reaching 9.68. The viscosity remained at a high level for weeks after the cessation of injections, showing a slow decline.

f. Methyl Cellulose, 1500 cps. (Three Dogs)

One dog received a total of 990 cc. of methyl cellulose solution within 4 weeks. A second dog was injected with 2330 cc. within 7 weeks and a third with 4590 cc. within 11 weeks. All died.

Erythrocytes. The number of erythrocytes decreased after prolonged treatment to less than one-third of the original value.

Hemoglobin and Volume of Packed Cells. The hemoglobin was considerably reduced and reached in 2 dogs values below 5 gm. per 100 cc. of blood. The decrease of hemoglobin thus surpassed that of the erythrocytes. The reduction in the volume of packed cells, on the other hand, was somewhat less in degree than that of the erythrocytes.

Leukocytes. The leukocytes were always either at a high normal level or slightly above that range.

Clotting Time. No abnormalities in clotting time were found.

Sedimentation Rate. There was a marked hastening of the erythrocytic sedimentation.

Plasma Viscosity. The plasma viscosity was only moderately increased, reaching as peak value a viscosity of 4.

g. Methyl Cellulose, 4000 cps. (Three Dogs)

One dog received 2290 cc. of methyl cellulose within 6 weeks, a second was injected with a total of 3440 cc. within 8 weeks, and a third dog with 4930 cc. within 12 weeks. All died.

The hematic changes were similar to those seen in the series injected with methyl cellulose, 1500 cps. This was particularly so in regard to the increase in plasma viscosity, which remained below 3.5. It is remarkable that the dog which received 2290 cc. of methyl cellulose developed an anemia of 850,000 erythrocytes and a leukocytosis of 100,000 cells 3 days before death. In the dog which was injected with 4930 cc. the final erythrocytic number was 1.45 million (originally 8 millions) and the final leukocytic number, 42,600 cells.

The hematic reactions noted after repeated intravenous injections of the seven types of methyl cellulose exhibited a uniform pattern in many respects, but certain important deviations in others. There was in all

instances a considerable decrease in the number of the circulating erythrocytes, in the amount of hemoglobin and in the volume of packed cells, which was especially pronounced with the higher molecular types. The reduction of the last two items usually surpassed in degree that of the erythrocytes. It was evident, moreover, from the instances in which the treatment was interrupted for some time, that this anemia-producing effect was relatively readily reversible if the further introduction of the causative agent was stopped. As a rule, the leukocytes stayed within the normal range, but sometimes were slightly to moderately increased during the final stage. This reaction was especially pronounced in the dogs injected with m.c., 4000 cps., one of which exhibited a marked hyperleukocytosis of leukemoid type. The clotting time underwent abnormal changes only in animals injected with m.c., 400 cps., in which it was considerably lengthened. The sedimentation rate was definitely accelerated in all instances and remained so for some time after discontinuation of treatment. The plasma viscosity was always increased. However, this increase was comparatively small with the methyl celluloses of the low and the high molecular weights (m.c., 15 cps., 25 cps., 1500 cps., 4000 cps.), while it was highest with methyl cellulose of 400 cps. having a molecular weight approximating that of serum albumin. It was noted, moreover, that the elevated viscosity dropped rather rapidly to slightly increased values after discontinuation of treatment in dogs injected with methyl celluloses of 50 cps. and 100 cps., whereas it stayed considerably elevated for periods of many weeks after cessation of injections in dogs treated with methyl cellulose of 400 cps.

C. Anatomical Reactions in the Internal Organs Following Repeated Injections of Methyl Cellulose

a. Methyl Cellulose, 15 cps. (Two Dogs)

The 2 dogs of this series received 570 and 670 cc. of methyl cellulose solution respectively, and showed at autopsy practically normal organs with the exception of a large pneumonic induration in the left upper lobe of 1 dog. The spleens were of normal size, of firm consistency and grayish pink.

The histological study of the various organs showed the following findings:

Thyroid; Aorta; Pulmonary Artery; Large Elastic Arteries; Muscular Arteries of Thyroid, Kidney and Adrenal; Pancreas; Stomach and Intestine; Bladder. Normal.

Lung. Purulent bronchitis and large bronchopneumonic foci were present in the lungs of both dogs. There were deposits of a homogene-

ous, vacuolated, light-blue-stained matter in the peribronchial tissue spaces of 1 dog.

Heart. Small bluish-colored homogeneous deposits were found in the myocardial interstices.

Liver. Pericentral fatty degeneration or necrosis of liver cells.

Adrenals. Small bluish deposits were present in the sinusoids of the adrenals of 1 dog.

Spleen. The pulp consisted of accumulations of small foam cells, many of them in a stage of disintegration. Follicles were atrophic.

Kidney. The cortical tubules were distended, cystic, and lined by flattened epithelium. Some of the glomeruli showed grape-like, distended, cystic, capillary loops containing foam cells, or, occasionally were lined by a multinucleated syncytium, which was found also in the lining of some tubules. The interstitial tissue of 1 dog contained bluish homogeneous deposits in the interstices. Renal vessels were normal.

Testes. Arrest of spermatogenesis was present in both dogs. This was associated in 1 dog with the presence of some spermatid giant cells and interstitial edema and was accompanied in the second dog by a severe atrophy of the spermatogenic epithelium, leaving only the spermatogonia and the Sertoli's cells preserved. Some of the tubules were collapsed.

Bone Marrow. There was in the sternum a loose, hyperemic, immature myeloid tissue.

b. Methyl Cellulose, 25 cps. (Two Dogs)

The two dogs of this series received a total of 350 and 1580 cc. of methyl cellulose solution respectively, and showed at autopsy, apart from emaciation, normal internal organs.

The histological examination of the organs revealed the following findings:

Brain; Hypophysis; Parathyroid. Normal.

Thyroid. The follicles contained a thin colloid or were empty. In 1 dog the epithelial lining was desquamated in some follicles. The thyroid of the second dog which received the larger dose of methyl cellulose was normal.

Lung. The lungs were highly edematous, congested and hemorrhagic in the first dog and normal in the second dog.

Heart. The heart was normal in the first dog. The walls of the subepicardial coronary arteries in the heart of the second dog were transformed, particularly in their intimal portion, into a thick foam-cellular tissue considerably narrowing the lumina. Similar lesions were found in the myocardial arteries and arterioles. The subendocardial myo-

cardium of the left ventricle, especially of the papillary muscles, contained large and numerous calcium incrustations of necrotic muscle cells in addition to foci of loose fibrous tissue replacing muscle tissue (Fig. 1).

Aorta. The ascending aorta of the first dog showed a granular necrosis in the thickened intima and adjacent media. In a nearby segment the outer and middle media contained large hyaline areas with an extensive nodular calcification causing a bulging of the vessel wall into the lumen. The subintimal media was edematous. In the thoracic aorta there was perivascular hyalinization around vasa vasorum in the middle media. The aorta of the second dog exhibited similar and extensive medial hyalinization and calcification in the media of the aortic bulb, which showed, moreover, a thin foam-cellular coating and small foam-cellular cushions of the intima. In the thoracic part of the aorta extensive lesions of these types were present. The vasa vasorum of the adventitia were partly obliterated by foam-cellular intimal proliferations, while large portions of the media were hyalinized.

Pulmonary Artery. There were areas of medial calcification above the valves together with some foam-cellular intimal cushions in the second dog.

Large Elastic Arteries. Some large elastic arteries of the second dog showed extensive calcification of the media in some areas and in other parts incrustation of the elastic fibrils together with cyst formation. In several segments these lesions were associated with foam-cellular proliferation of the intima (Fig. 2).

Stomach. There was a diffuse fine granular calcification in the interstitial tissue of the mucosa in the second dog.

Liver. Extreme congestion and extensive fresh coagulation necrosis of the liver cells characterized the lesions of the first dog. The Kupffer cells of the second dog were markedly increased in number and had foamy cytoplasm. The cell cords were pushed apart thereby and the hepatic cells were moderately atrophic.

Pancreas. A medium-sized artery in the normal pancreatic parenchyma of the second dog showed intimal foam-cellular cushions and medial subendothelial calcium deposits.

Spleen. The organ was normal in the first dog and showed a pulp composed of foam cells in the second dog.

Adrenals. These organs were normal in the first dog and contained foam-cellular reticulo-endothelial cells of the medulla in the second dog.

Kidney. The tubules of the cortex were distended, cystic, and lined with flattened cells in both dogs. The glomeruli were hypertrophic and showed extensive hyalinization in the first dog. In the second dog many

consisted of a few cystic structures lined by foam cells or by a multinucleated syncytium. The arterioles in the kidney of the first dog had a distinct, thick, red-stained subintimal layer, while the arteries of the second dog showed a few foam-cellular cushions of the intima undergoing degenerative changes.

Testes. The spermatogenic epithelium of the tubules was reduced in the second dog to the spermatogonia and Sertoli's cells with a few large giant cells. The arterioles revealed a hyaline subendothelial thickening.

Epididymis. The ducts were empty.

Bone Marrow. The sternum contained a dense, immature myeloid tissue.

c. Methyl Cellulose, 50 cps. (Three Dogs)

One dog was injected with 370 cc. of the methyl cellulose solution, a second with 1120 cc. and a third with 2010 cc. The lungs were edematous, hyperemic and studded with dark red, hemorrhagic foci. The spleens of the second and third dogs were enlarged, soft and grayish pink. The livers of these animals were large, and brown with a grayish tint. All other organs were grossly normal.

The histological examination of the organs gave the following findings:

Vena Cava; Stomach and Intestine; Pancreas; Mesenteric Lymph Nodes. Normal.

Brain. The choroid plexus of the third dog, which received the highest amount of methyl cellulose, consisted of a dense and bulky accumulation of foam cells lined by an intact ependyma. The brains of the other dogs were normal.

Lung. The blood vessels in the interalveolar septa and in the peribronchial tissue were frequently transformed in all 3 dogs into small multicystic structures lined by a syncytium, filled with a colorless, slightly refractive substance, and surrounded by granulation tissue containing large round cells.

Heart. The coronary arteries of the third dog had a thick, foam-cellular intima infiltrating into the media.

Aorta. There was only a minor foam-cellular intimal proliferation involving endothelium and subendothelial tissue in the bulb of the first dog, which showed in a thoracic segment an extensive hyaline and collagenous transformation of the media with marked vascularization (Fig. 3). The intimal foam-cellular cushions in the aortic bulb were more highly developed in the second and third dog, and contained in the second dog a few leukocytes (Fig. 4), while the underlying media showed scattered calcium granules. The thoracic and abdominal seg-

ments of the aortas of these 2 dogs exhibited frequent and marked intimal foam-cellular proliferations which sometimes invaded the intima and also involved the vasa vasorum. The media was often highly edematous or mucoid. A mucoid, small-vesicular zone extended between intima and media. Intimal hyalinization was found occasionally, whereas the media contained larger areas of calcification in both the inner and the hyalinized outer medial zone.

Pulmonary Artery. There was a moderate foam-cellular thickening of the intima of the pulmonary artery near the hilum in the second dog.

Large Elastic Arteries. The large elastic arteries were normal in the first dog, but contained foam-cellular intimal thickenings in the other 2 dogs. The nuclei of these foam cells had sometimes grotesque spider-like chromatin.

Liver. Pericentral congestion and necrosis of liver cells were present in the first dog, while there was increase in size and a foam-cellular transformation of the Kupffer cells and of the interstitial tissue histiocytes in the other 2 dogs. The liver cells were moderately atrophic.

Adrenals. Foam-cellular reticulum cells were found in the medulla of the adrenal of the third dog.

Spleen. The splenic pulp consisted in all 3 animals of large masses of foam cells, none of them invading the lumina of the larger vessels.

Kidney. The cortical tubules were distended and lined by flattened cells. The glomeruli of the second and third dogs showed grape-like cysts. A medium-sized extrarenal artery contained a mushroom-like, hyaline, intimal thickening with underlying thickened but fragmented internal elastic membrane.

Uterus. The uterine arteries of the third dog exhibited a marked foam-cellular proliferation of the intima, often obliterating the lumina (Fig. 5).

Bone Marrow. The sternum contained a hyperemic, immature myeloid tissue.

d. Methyl Cellulose, 100 cps. (Three Dogs)

One dog was injected with 280 cc. of methyl cellulose solution, a second dog received 4645 cc., and a third, 5190 cc. The autopsy showed the lungs to be congested and edematous and containing scattered dark red hemorrhagic areas. The livers in the second and third dogs were enlarged. The spleens of these two animals were about two or three times normal size and each weighed 120 gm. They were grayish red and soft. The renal cortices of these 2 dogs contained numerous wedge-shaped white areas. All other organs were grossly normal.

The histological examination of the organs gave the following findings:

Brain; Thyroid; Large Elastic Arteries; Stomach and Intestine; Pancreas; Lymph Nodes; Bladder. Normal.

Hypophysis. The loose and increased stroma of the predominantly eosinophilic parenchyma contained foam cells in the second and third dogs (Fig. 6).

Parathyroid. Interstitial tissue of the parathyroid glands was increased and contained some foam cells in the third dog.

Lungs. The lungs of the first dog were congested and edematous and showed scattered hemorrhagic areas. The lungs of the second and third dogs contained distended cystic vascular lumina in the peribronchial and interstitial septa, surrounded by multinucleated syncytia and large round cells, fibroblasts and foam cells.

Heart. With the exception of some round cell infiltrations in the myocardium of the second dog, the hearts were normal.

Aorta. The intima of the ascending aorta of the first dog was intact, while the media showed a considerable mucoid imbibition causing the production of small cavities filled with mucoid matter, and a wide separation of the muscle bundles. The media of the thoracic part contained several large hyaline areas. The intima of the aortas of the second and third dogs was transformed into a multilayered foam-cellular coat in which cushion-like foam-cellular thickenings were embedded, which in places extended into the media. These changes were most marked in the bulb. There was an extensive subendothelial calcification in some areas.

Pulmonary Artery. Similar, but not as extensive, intimal and medial lesions, especially also muscular calcifications, were present in the postvalvular portion of the second and third dogs, while the media of the first dog revealed increase of the mucoid content (Fig. 7).

Abdominal Muscular Artery. There was a large area of medial necrosis present, associated with calcification of the elastic fibrils (Fig. 8).

Liver. The liver cells of the first dog were atrophic and the organ was congested. In the second and third dog there was also an atrophy of the liver cells. The cords were pushed far apart by proliferated, foam-cellular Kupffer cells.

Spleen. The splenic pulp of the first dog was hyperemic and cellular, whereas those of the second and third dogs were filled with foam cells which were disintegrating in places.

Adrenals. The adrenals were normal in the first and second dog, but showed foam-cellular reticulo-endothelial cells in the medulla of the third dog.

Kidney. The cortical tubules were distended in all 3 dogs. The glomeruli of the first dog were swollen and rich in nuclei, while the arterioles showed focal hyalinization of the media. The cortices of the second and third contained foci of large mononuclear cells intermingled with a foam-cellular matrix. The glomeruli were transformed into grape-like, cystic formations lined with foam cells or multinucleated syncytia.

Testes. In the second and third dogs there were a few spermatid giant cells in the testicular tubular lumina, which were lined by a generally normal epithelium.

Bone Marrow. The sternum contained a loose myeloid tissue.

e. Methyl Cellulose, 400 cps. (Seven Dogs)

One dog was injected with 260 cc. of methyl cellulose solution, a second and third with 1040 cc., a fourth with 2112 cc., a fifth with 2930 cc., a sixth with 3375 cc., and a seventh with 5720 cc.

The spleens were considerably enlarged in all dogs, including the first one. The organ was grayish pink, soft, and had in some instances an almost liquid, dirty yellow pulp. The weights varied from 230 to 400 gm. The livers were also enlarged, brown-red to brown-gray and displayed a yellowish white network. One liver weighed 1015 gm., while the others were around 500 to 700 gm. The kidneys were large with some retractions in a sometimes gelatinous cortex. The lungs were congested.

Histological examination gave the following findings:

Thyroid and Parathyroid; Stomach; Pancreas; Bladder. Normal.

Brain. The choroid plexuses of dogs 5 and 7 consisted of accumulations of foam cells. Numerous small perivascular glial foci were present in the brain of dog 3, while numerous hemorrhages were found in dog 1.

Hypophysis. Large masses of foam cells separated strands of acidophilic cells in the anterior lobe in dog 7.

Lung. Apart from congestion and the presence of numerous, moderately large round cells with an empty cytoplasm in the interalveolar septa, no pathological lesions were seen in the lungs of dogs 1 to 6. Foam-cellular masses filled the capillaries of dog 7 and formed nodules in the interstitial tissue.

Heart. The myocardium was normal in dogs 1 to 6. There were perivascular foam-cellular foci around the myocardial arteries and arterioles in dog 7. Hyaline thickenings of the media of the myocardial arterioles were present in dogs 3, 6 and 7, associated with foam-cellular intimal proliferations in dogs 6 and 7. Foam cells lined the surface of the aortic valves and the endocardium of the left ventricle in dog 7.

Aorta. The aorta was normal in dogs 1 and 2. The intima of the aortas of the other 5 dogs showed an often multilayered foam-cellular coating and cushion-like thickenings which were most strikingly developed in the aortic bulb and decreased toward the abdominal portion. The media underneath these lesions showed hyaline degeneration sometimes associated with scattered small or large nodules of calcification. Other portions exhibited an increase of the intercellular mucoid matter, which occasionally contained some foam cells.

Pulmonary Artery. The intima consisted of 2 or 3 layers of foam cells covering and invading a hyalinized media, in dogs 4 and 5.

Large Elastic Arteries. Intima showed a foam-cellular coating and small cushions in dogs 4, 5, 6 and 7, while a thickened hyaline intimal cushion was noted in one artery of dog 3.

Muscular Arteries. The intima exhibited occasionally small foam-cellular groups in dog 5.

Vena Cava. The intima consisted of foam cells in dog 6.

Intestine. The mucosa contained foam cells in the interstitial tissue in dogs 6 and 7.

Liver. While the liver of dog 1 showed only scattered foam-cellular granulomatous formations and those of dogs 2 and 3 were merely congested, the livers of dogs 4, 5, 6 and 7 revealed an extensive proliferation of Kupffer cells with transformation into foam cells. The liver cells themselves had in part a vacuolated or foam-cellular appearance. In dog 7 the periportal connective tissue participated in the foam-cellular transformation, which affected also, in part, the endothelium of the hepatic veins.

Spleen. The splenic pulps were always foam-cellular and contained clusters of large multinucleated giant cells arranged in circles. There were large areas of necrosis present. The follicles were atrophic.

Lymph Node. Foam-cellular reticulum cells were found only in dog 7.

Adrenal. Foam-cellular reticulo-endothelial cells in the medulla and glomerulosa of the adrenals were found in dogs 6 and 7. The adrenals were normal in the other dogs.

Kidney. There were a few giant-cellular and foam-cellular granulomas in the interstitial, perivascular tissue of the cortex in dogs 1, 2 and 3. A few glomeruli contained small cystic formations with foam cells. The lesions were much more widespread and severe in dogs 4 to 7, where the glomeruli had a multicystic appearance with endothelial foam cells. The tubules were distended and lined either by flattened cells or by foam cells. Foam-cellular accumulations were seen also in

the interstitial tissue. Foam-cellular proliferations of the intima of intrarenal arteries were present in dog 5 together with medial hyalinizations in the main renal artery.

Testes. Arrest of spermatogenesis existed in dog 2; marked degeneration of the spermatogenic epithelium with the appearance of numerous multinucleated giant cells occurred in dogs 3 and 4.

Uterus and Ovary: Normal in dog 6.

Bone Marrow. The sternum contained an immature myeloid tissue in dogs 1 to 6, while a few foam-cellular reticulum cells were present in an immature myeloid and fatty marrow in dog 7.

f. Methyl Cellulose, 1500 cps. (Three Dogs)

One dog was injected with 990 cc. of methyl cellulose solution, a second with 2330 cc., and a third with 4590 cc. At autopsy the lungs were found to be congested and edematous with hemorrhagic spots; the spleens were enlarged, weighing between 174 and 264 gm.; the livers were brown-red and moderately to markedly enlarged (590 to 920 gm.).

Histological examination showed the following findings:

Brain; Hypophysis; Thyroid and Parathyroid; Large Elastic Arteries; Stomach and Intestine; Pancreas; Lymph Node; Adrenals; Bladder. Normal.

Lung. Numerous distended and multicystic capillaries were found in the interstitial tissue of the lungs. The cysts were lined by a multinucleated syncytium and surrounded by foam cells and large mononuclear cells.

Heart. The myocardium was normal. There were marked foam-cellular proliferations in the intima of the epicardial and myocardial coronary branches in dog 1.

Aorta. The ascending aorta showed a foam-cellular lining of moderate extent and thickness in all 3 dogs. The underlying media was thickened by imbibition of mucoid material. In other parts of the aorta there were some cushion-like foam-cellular intimal thickenings in dogs 1 and 3. The deeper parts of the rather thick cushions in dog 3 were composed of spindle-shaped fibroblasts in radiating arrangement and of hyalin containing scattered foam cells (Fig. 9).

Liver. The liver cells were atrophic. The cords were widely separated by distended sinusoids containing desquamated, swollen, spherical Kupffer cells with foam structure.

Spleen. The pulp of the spleen showed extensive foam-cellular transformation.

Kidney. In the cortex of the kidney the tubules were distended. The

glomeruli were swollen and showed cells with a loose, vacuolar cytoplasm. The edematous interstitial connective tissue contained round cells and multinucleated giant cells.

Testes. The testes were normal in dog 2. The spermatogenic epithelium of the other 2 dogs was markedly degenerated and consisted, in some tubules, of hypertrophic Sertoli's cells only, while in others no epithelial lining was present, but a thickened hyaline capsule surrounded an empty lumen. Some tubules contained deep brown pigmented cells.

Epididymis. The empty ducts of the epididymis were lined by hyperplastic epithelium.

Bone Marrow. The sternum contained fatty myeloid tissue.

g. *Methyl Cellulose, 4000 cps.* (*Three Dogs*)

One dog was injected with 2290 cc. of methyl cellulose solution, a second with 3440 cc., and a third with 4930 cc. Autopsies showed that the lungs were edematous and contained some hemorrhagic spots. The livers weighed between 500 and 600 gm., the spleens between 190 and 504 gm. The kidneys were swollen, pale light brown.

Histological examination of the organs gave the following findings:

Brain; Hypophysis; Thyroid and Parathyroid; Pulmonary Artery; Vena Cava; Stomach; Intestine; Pancreas; Bladder. Normal.

Lung. The interalveolar septa were cellular.

Heart. Myocardium and coronary arteries were normal.

Aorta. The aortic intima showed in dogs 1 and 3 a thin foam-cellular proliferation beneath the endothelium. The muscle cells of the media in dog 1 contained fine blue granules or were diffusely incrusted with calcium salts, particularly beneath the intima. There was a more massive foam-cellular intimal proliferation in the ascending aorta of dog 2, associated with imbibition of mucoid material in the media. The other aortic segments were normal.

Large Elastic Arteries. Some scattered small, foam-cellular, intimal proliferations were found, but the majority of the large arteries were normal.

Liver. In the 3 dogs there was an extensive destruction of the liver parenchyma leaving often only a reticular framework and proliferated foam-cellular Kupffer cells.

Spleen. There was extensive necrosis in a foam-cellular pulp in the spleens of dogs 1 and 2, while numerous multinucleated giant cells, arranged in clusters, and massive accumulations of foam cells were present in dog 3.

Adrenals. Some foam-cellular reticulum cells were present in the medulla of the adrenals in dog 2.

Lymph Nodes. The mesenteric nodes of dog 2 showed numerous multinucleated giant cells, mainly in the peripheral sinuses.

Kidney. The renal cortical tubules were distended and lined by a flattened epithelium. The glomeruli were converted into multicystic grape-like formations with foam-cellular endothelium. There were localized round cell infiltrations in the interstitial tissue, associated with foam cells in dog 3.

Uterus. Normal in dog 3.

Testes. The spermatogenic epithelium was highly atrophic and the Sertoli's cells were hypertrophic in dog 2.

Bone Marrow. The sternum contained a dense mature myeloid tissue.

Foam-cellular transformation of the supporting tissue of the choroid plexus of the brain was found only in dogs injected with large amounts of methyl celluloses of 50 and 400 cps., while similar changes in the anterior lobe of the hypophysis were seen in animals treated with large doses of methyl celluloses of 100 and 400 cps. The presence of multicystic, distended pulmonary capillaries and precapillary vessels, lined with multinuclear syncytia and surrounded by large mononuclear cells and foam cells, was noted only in dogs into which methyl celluloses of 50, 100, 400 and 1500 cps. had been introduced. Dogs receiving the two methyl celluloses with lower viscosities and the one with a higher viscosity were free from such lesions. The dogs injected with methyl cellulose of 15 cps. did not exhibit degenerative and foam-cellular vascular reactions, such as were seen in animals treated with methyl cellulose of the other types. Although their occurrence and extent showed a dependence upon the amount of methyl cellulose solution introduced, the foam-cellular intimal response as well as the hyaline and calcifying medial changes were comparatively mild in the dogs which received methyl cellulose of 4000 cps. The livers of dogs treated with methyl celluloses of 15, 25 and 50 cps. showed evidence of direct liver necrosis in the absence of extensive storage of methyl cellulose in swollen, proliferated, foam-cellular Kupffer cells. In dogs injected with the higher molecular methyl celluloses such degenerative hepatic lesions were usually secondary to extensive proliferative reactions in the Kupffer cells, associated with the storage of methyl cellulose in these elements. This type of hepatic parenchymatous destruction was marked in the dogs which received methyl cellulose of 4000 cps. The presence of methyl cellulose within liver cells was noted only in dogs injected with methyl cellulose of 400 cps. In all other dogs the liver parenchyma appeared to be free from methyl cellulose, but sometimes contained fat. The retention of methyl cellulose in the reticulum and reticulo-endo-

thelial cells of the spleen and the transformation of these cells into foam cells and multinucleated giant cells was found in dogs of all series. A similar uniformity was noted in the retention and deposition of methyl cellulose in the glomeruli of the kidneys, in the degenerative changes in the spermatogenic epithelium of the testes, and in respect to the absence of foam cells in the bone marrow.

Comment

From the data recorded it becomes evident that the various intravenously injected methyl celluloses elicit, regardless of their molecular weights, changes in the blood characteristic of the hematologic macromolecular syndrome (primary leukopenia, secondary leukocytosis, anemia, increased sedimentation), as well as organic lesions (foam-cellular masses in liver, spleen, adrenal, kidney), typical of the storage of macromolecular colloidal matter, such as glycogen, lipoids, polyvinyl alcohol, silica, pectin, gum arabic, etc., *i.e.*, substances which form emulsions with hemoplasmatic and cytoplasmatic protein solutions.[3] However, this reactive pattern was not uniformly developed with all methyl celluloses. If cognizance is taken of those deviations which are attributable to the differences in the amount of material injected, such as the appearance of foam cells in the choroid plexus, in the anterior lobe of the hypophysis, and in the adrenals, there remain discrepancies which seem to be causally related to differences in the physicochemical properties of the injected agents.

The shorter duration of the leukopenia after a single injection of methyl celluloses of 15 and 25 cps., the milder decrease in the number of erythrocytes and in hemoglobin and volume of packed blood cells, the delayed appearance and shorter duration of the acceleration in erythrocytic sedimentation, and the more rapid transitory elevation of the plasma viscosity, as compared to the findings after the introduction of methyl celluloses of higher molecular weights, obviously reflect the influence of the relative molecular size upon the intensity and duration of such acute colloidal reactions. This is particularly true, since a comparatively larger amount of the low molecular methyl celluloses was contained in the injected dose of 40 cc. than was present in solutions of methyl celluloses of higher molecular weight. This conclusion is supported by observations made by Bucher [13] in connection with the intravenous injection of colloidal solutions of glycogen into rabbits. This investigator found that the severity and duration of the leukopenic reaction was dependent upon the quantity as well as upon the molecular size of the glycogen injected.

However, this relationship concerning the influence of the molecular

size upon the severity and type of reaction was not consistent in respect to the plasmatic viscosity reactions elicited by methyl celluloses of higher molecular weight, as this response was very mild and of very short duration for treatment with both single and repeated doses. The relatively very small absolute amounts used of methyl celluloses of 1500 and 4000 cps. may have militated against any marked and prolonged elevation of the plasma viscosity. On the other hand, it may be possible that the very long-chained molecules of methyl celluloses of 1500 and 4000 cps., when introduced into the blood, do not preserve their rod-shaped form and their directed arrangement responsible for the high viscosity of their aqueous solution, but follow the example of the protein molecules and curl up into globules, thereby causing a marked diminution of the viscous properties. This suggestion is based on a claim of Lepeschkin,[14] who recently reported the occurrence of such changes for the filamentary gelatin molecules when a gelatin solution is heated from just above the jellying point to higher temperatures at which the gelatin is perfectly liquid and much less viscous. An additional causal relation between molecular size and plasmatic viscosity appears to be reflected in the rapid decrease of plasma viscosity after cessation of treatment in animals injected with methyl celluloses of 50 and 100 cps., in contrast to the long-continued elevation of this factor in dogs receiving methyl cellulose of 400 cps.

A similar parallelism between the action of the two low molecular methyl celluloses and the very high molecular cellulose exists in regard to the absence of intracapillary retention cysts of the lungs after their injection. Such lesions were regularly found with the other types. Primary or secondary differences in molecular size and configuration among the various methyl celluloses seem to offer the most plausible explanation for the discrepancies between these anatomical reactions, the development of which depends to a definite degree upon the width of the pulmonary capillaries.

It is noteworthy in this connection that retention of methyl cellulose within the glomerular filter, associated with foam-cellular and proliferative reactions of the endothelium, was found with methyl celluloses of all types regardless of their molecular weights. This is remarkable since three of these substances have a molecular weight below 65,000, which is supposed to represent the critical level for glomerular filtration of macromolecular substances. The observation shows that molecular size determined for the dry material is evidently not the only factor in determining filtrability, but that the degree of molecular hydration plays an important rôle in this respect. Similar results were obtained by Bott and Richards [15] in tests of the permeability of the

glomerular membrane of the amphibian kidney for serum albumin of duck's and hen's eggs, lactoglobulin, zinc insulin, horse serum albumin, tuberculin protein, salmine, and inulin, as only inulin, with a molecular weight of about 6000, filtered through completely. All other agents studied were retained to some degree.

It must remain uncertain whether the molecular weight and perhaps also the molecular configuration of methyl cellulose of 4000 cps. may be responsible for the unusually severe anemia and the extraordinary hyperleukocytosis observed in two of the three injected dogs. Also remarkable is the relatively slight severity and extent of the foam-cellular intimal, and hyalinizing and calcifying medial, lesions of the aorta and large elastic arteries in dogs treated intensely with methyl cellulose of 4000 cps., as compared to the vascular changes in animals treated with methyl celluloses of lower molecular size.

Other striking relations between molecular size of methyl cellulose and biological effect were found in its storage in the liver and in the coagulation time of the blood. Whereas the Kupffer cells and also, after intensive treatment, the histiocytes of the periportal connective tissue of dogs injected with any of the methyl celluloses exhibited a marked foam-cellular transformation and proliferation associated with atrophy of the liver cells and, with the high molecular methyl cellulose, extensive destruction of liver cells, it was only in dogs receiving methyl cellulose of 400 cps. that the liver cells also retained methyl cellulose. It is significant that dogs of this series only displayed a considerable lengthening of the clotting time, while the dogs of all other series showed no abnormal fluctuations of this factor. The presence of hepatic necrosis did not seem to influence the clotting time.

It may be added that the repeated intravenous introduction of methyl cellulose solutions of the different types resulted in the production of vascular lesions characterized by foam-cellular proliferations of the endothelial and intimal cells, sometimes associated with fibroblastic and hyaline intimal thickenings, increased mucoid imbibition of the media which sometimes resulted in the formation of small, irregular, subintimal cavities filled with mucoid material, and with hyalinization and calcification of the media. These various reactions, which increased in extent and degree with the intensity of the treatment, were found in both elastic and muscular arteries but were more marked and more frequent in the former. Although they frequently occurred together, it was not unusual for intimal or medial changes to be seen alone. The vasa vasorum of the aorta sometimes participated in the foam-cellular reactions. It is noteworthy that occasionally extensive areas of medial calcification without intimal lesions or with foam-cellular intimal pro-

liferation were encountered in medium-sized abdominal branches of the aorta. These observations strongly suggest that also in the development of the degenerative intimal and medial lesions in man, particularly those of the Mönckeberg type, common causal factors are active, and that medial calcinosis of the peripheral arteries does not represent a special type of vascular disease which must be distinguished etiologically from ordinary atherosclerosis.[16-19]

The high frequency of severe testicular atrophy in the dogs of this experiment supports the conception advanced in previous publications that anoxemia is the fundamental common causal factor in the production of degenerative vascular disease, as testicular degenerations are a phenomenon frequently met with in anoxemic states (chronic mountain sickness, lead poisoning, nitrate poisoning, nicotine poisoning, carbon disulfide poisoning, carbon monoxide poisoning, etc.) [17, 20]

Conclusions

1. The intravenous injection of single and repeated doses of seven types of methyl cellulose ranging in molecular weight from 32,200 to 143,000 results in hematic and organic reactions which differ among themselves in some respects, depending upon the molecular weights.

2. Methyl celluloses of low molecular weight cause a shorter duration of a transitory leukopenia, a milder decrease in the number of erythrocytes, a shorter acceleration of erythrocytic sedimentation and a shorter elevation of the plasmatic viscosity than those elicited by methyl celluloses of higher molecular weights.

3. Methyl celluloses of high molecular weight form an exception to the rule in regard to the increase in plasmatic viscosity, as they do not elicit an appreciable elevation of this factor even after the introduction of large amounts.

4. A similar parallelism between molecular weight and anatomical lesions is represented by the fact that the two methyl celluloses of lower molecular weight do not produce intracapillary pulmonary retention cysts, as do all other methyl celluloses with the exception of the methyl cellulose of highest molecular weight.

5. All methyl celluloses were retained at least in part by the glomerular filtration membrane, indicating that here the degree of hydration plays an important rôle by influencing molecular size.

6. Lengthening of the clotting time was found only with methyl cellulose of 400 cps., which has about the same molecular weight as serum albumin and enters the liver cells, which do not store the other methyl celluloses.

7. The degree and distribution of atheromatous intimal, and hyalin-

izing and calcifying medial, lesions in the elastic and muscular arteries increased with the intensity and duration of treatment, but varied somewhat with the type of methyl cellulose injected, being least developed with the very high molecular type.

8. The frequent occurrence in muscular arteries of medial calcification unrelated to atheromatous changes indicates that the same causative mechanism is active in the production of both intimal atheromatosis and medial calcinosis.

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[*Illustrations follow*]

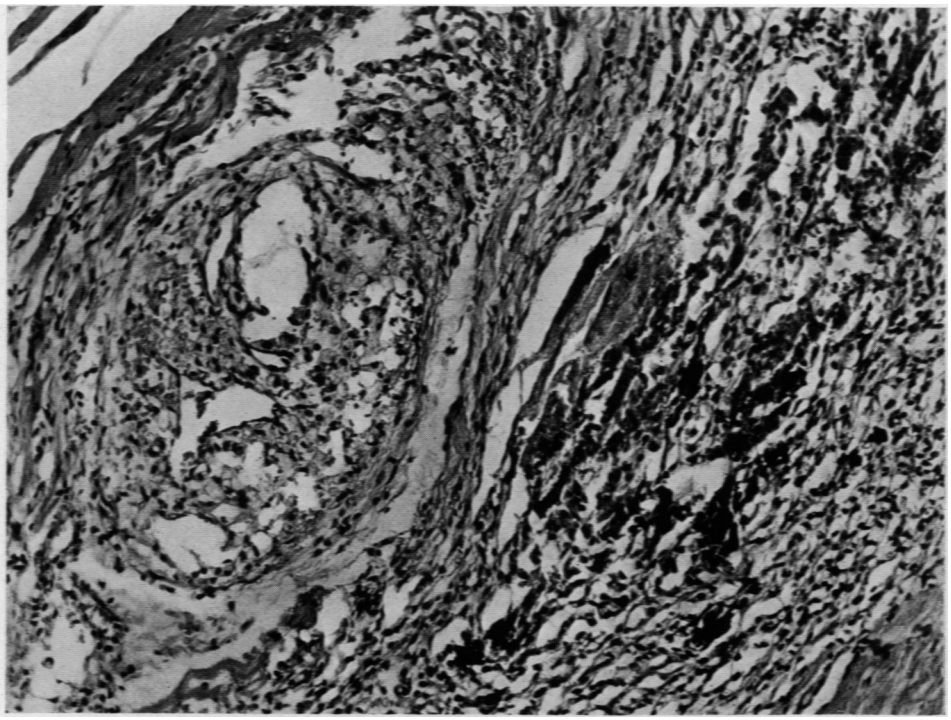
DESCRIPTION OF PLATES

PLATE 141

FIG. 1. Myocardial necrosis and calcification with proliferation of fibroblasts and with infiltration of leukocytes and mononuclear cells. An artery shows considerable proliferation of intimal foam cells. Hematoxylin and eosin stain. × 360.

FIG. 2. Large elastic artery with mild foam-cellular proliferation of the intima and a large focus of necrosis and calcification in the middle media. Hematoxylin and eosin stain. × 360.

1



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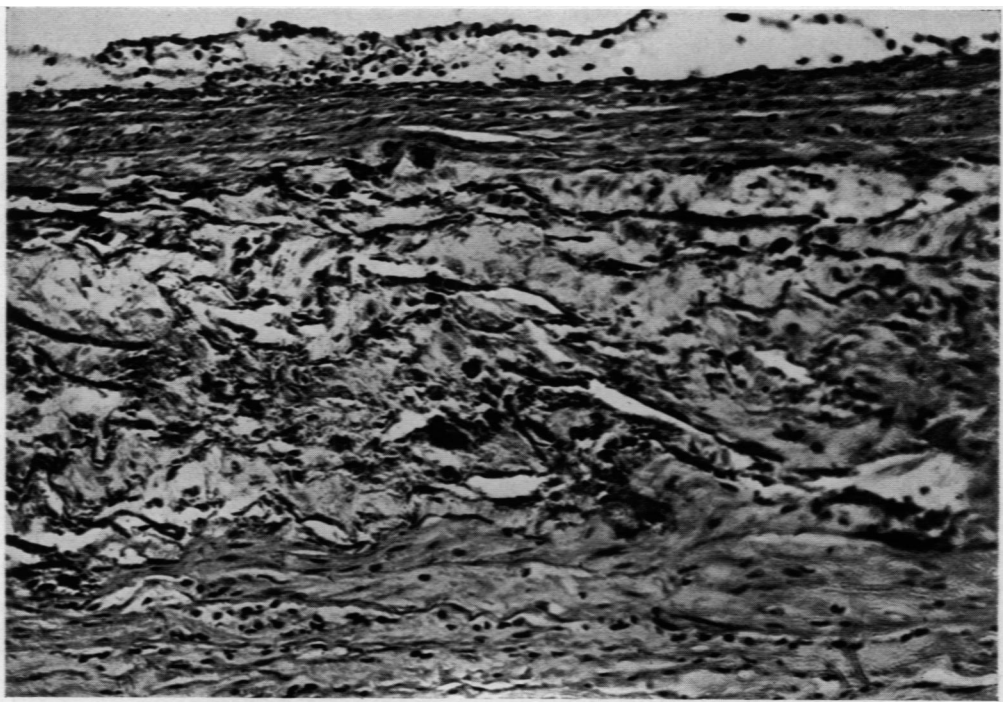


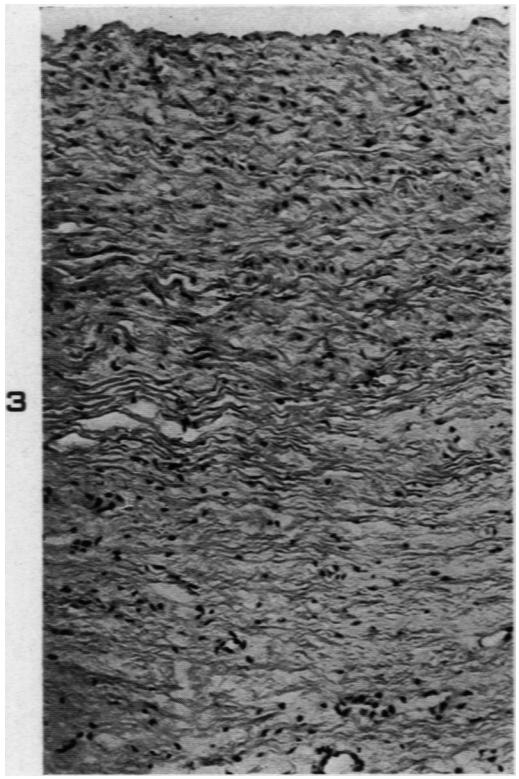
Hueper Intravenous Injections of Methyl Celluloses

PLATE 142

FIG. 3. Extensive area of fibrosis and hyalinization in the outer half of the aortic media (lower part of figure) with marked capillary proliferation and mild infiltration with lymphocytes. Hematoxylin and eosin stain. × 310.

FIG. 4. Cushion-like, cellular intimal thickening of the aorta with invasion of scattered leukocytes into a highly mucoid media. Hematoxylin and eosin stain. × 310.

FIG. 5. Uterine arteries with extensive foam-cellular intimal proliferation almost occluding the lumina. Hematoxylin and eosin stain. × 360.



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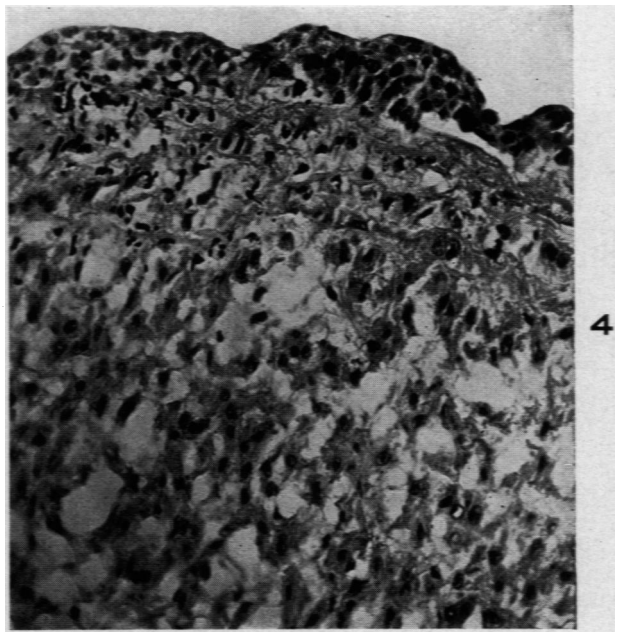

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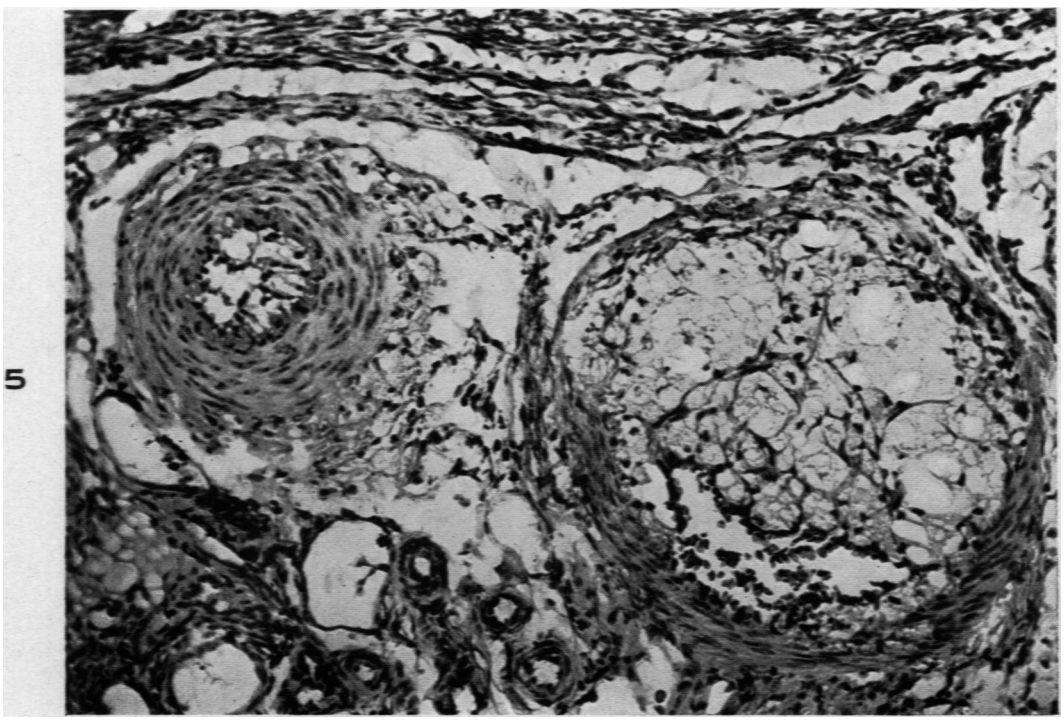


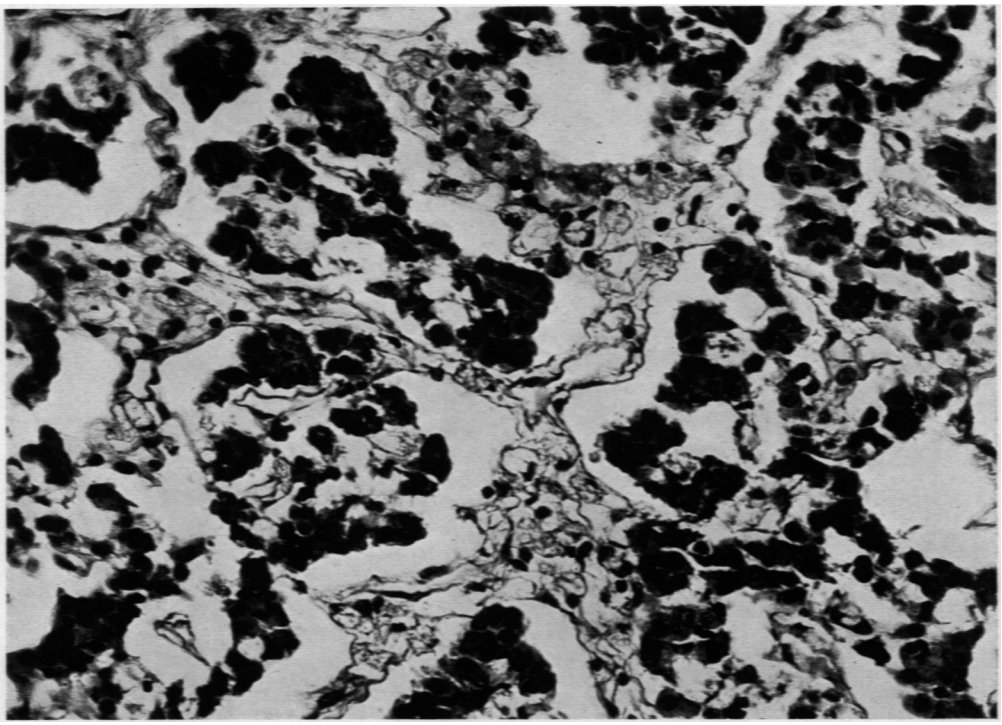
5

PLATE 143

FIG. 6. Anterior lobe of the hypophysis with foam-cellular interstitial tissue. Hematoxylin and eosin stain. × 660.

FIG. 7. Pulmonary artery with foam-cellular thickening of the intima and with hyalinization and calcification of the media. Hematoxylin and eosin stain. × 360.

6



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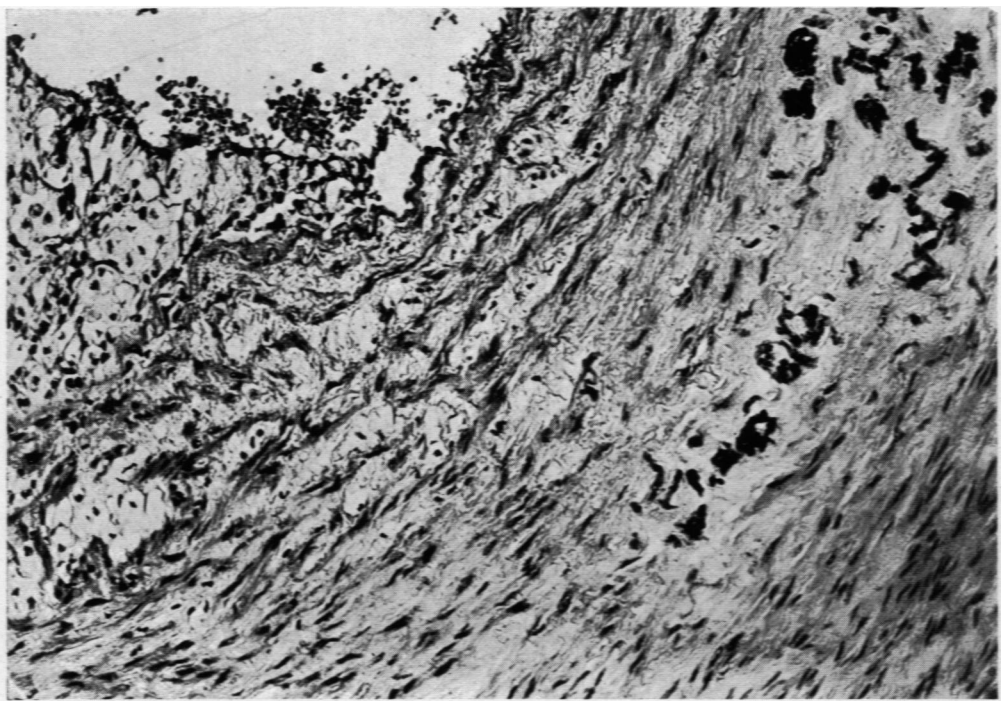
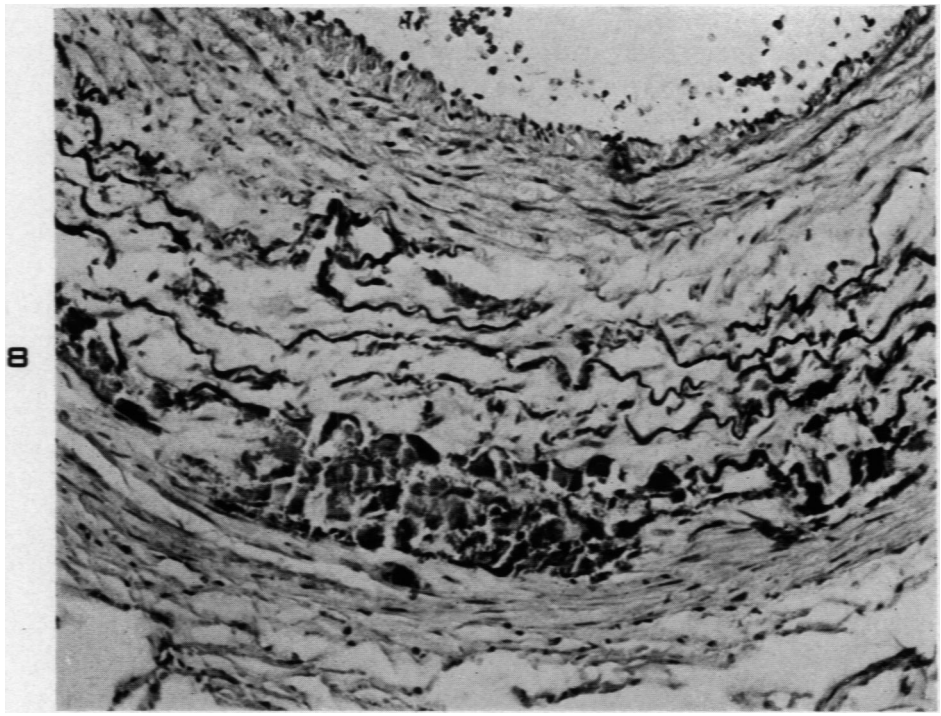


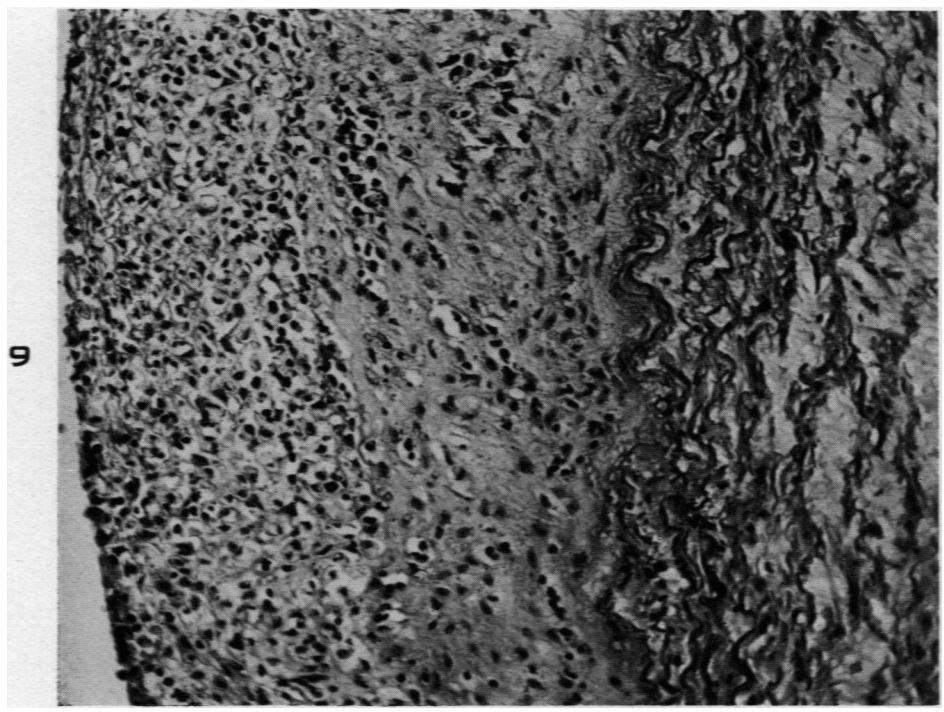
Hueper Intravenous Injections of Methyl Celluloses

PLATE 144

FIG. 8. Large muscular artery with extensive medial necrosis, and calcification of the necrotic matter and of the elastic fibrils. Hematoxylin and eosin stain. × 360.

FIG. 9. Intimal thickening of the aorta consisting of a foam-cellular inner layer and a deeper, partly hyaline, partly fibroblastic layer covering a highly mucoid media. Hematoxylin and eosin stain. × 360.





Hueper Intravenous Injections of Methyl Celluloses