EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

XI. Thesaurosis and Atheromatosis Produced in Dogs by the Repeated Intravenous Injection of Solutions of Sodium Cellulose Glycollate *

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It was shown in previous investigations that colloidal aqueous solutions of methyl cellulose are effective substitutes for plasma and elicit, when introduced in highly excessive amounts over prolonged periods, symptoms of thesaurosis and atheromatosis (Hueper,[1, 2] Hueper and Ichniowski [3]). When a new water-soluble derivative of cellulose, the sodium salt of cellulose glycollic acid, became available, a toxicopathologic study of this substance appeared to be of interest.

Experimental Procedure

Sodium cellulose glycollate (Collocel S), which is manufactured by the Dow Chemical Co. in three grades of viscosity, is a cellulose ether with a carbomethoxyl ($-OCH_2COOH$) radical. As the degree of substitution is relatively low, there is a predominance of free hydroxyl groups. Collocel S of high viscosity is a white, odorless, granular powder readily soluble in cold and boiling water, giving a slightly turbid, grayish white solution of neutral reaction. After being filtered through Hyflo-Supercel † and filterpaper, a slight haziness remains. According to the statements of the manufacturer, Collocel is compatible with the majority of the acid dyes, but with very few of the basic dyes. Solutions of Collocel do not form foam upon shaking. A 0.25 per cent aqueous solution has a colloidal osmotic pressure of approximately 140 mm. of water.

A 0.25 per cent solution of the high viscosity grade of Collocel in 1 per cent sodium chloride solution was used in the experiments. It has a viscosity of 1.8 at 18°C. This solution was injected into the jugular veins of 5 dogs, 3 of which weighed 8 to 8.5 kg., 1 weighed 10.3 kg., and the fifth, 14.5 kg. Four of the dogs were from 1.5 to 3.5 years old, while the fifth was 6 years old. For the study of the immediate hematic reactions 4 dogs received a single dose of 40 cc. Blood was withdrawn 5, 15, 30, 60, 120, 240 minutes, and 24 hours after injection. The following blood constituents were determined: Amount of hemoglobin, number of erythrocytes and leukocytes, differential count, sedimentation rate, volume of packed blood cells, plasma viscosity. Additional daily injec-

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

tions of the same dose were made in 2 dogs after an interval of 7 days. After having been continued for 3 months, the daily dose was increased to 100 cc. for an additional period of 4 weeks, when the surviving dog was sacrificed after receiving a total of 4800 cc. of the solution containing 12 gm. of sodium cellulose glycollate. The second dog belonging to this set died after having received 7 injections of 100 cc. each.

The other 2 dogs used in the studies of the immediate reactions as well as a fifth dog received, beginning 24 hours after the first injection, daily intravenous administrations of the Collocel solution. The daily dose was 40 cc. for the first month, was increased to 75 cc. during the second month, and to 150 cc. during the third month. One of these 3 dogs was sacrificed after 16 days, the other 2 after 2.5 months, after having received a total of 6,485 cc. of the solution containing 16.25 gm. of sodium cellulose glycollate.

Hematic Reactions after a Single Intravenous Injection of Collocel

The erythrocytes increased from original values, fluctuating between 7.0 and 7.4 million, to values of 8.5 to 8.8 million 5 minutes after the injection of Collocel. This movement was followed by a gradual drop to approximately original levels during the following 2 hours when a second elevation of the erythrocytes was seen in the tests made at the 2 and 4 hour periods. The erythrocytes ranged then from 8.0 to 8.7 millions. Approximately original values prevailed again at the 24-hour test. The hemoglobin values underwent less appreciable changes and showed more often a tendency to decline than to rise. There occurred in all instances a moderate to marked leukopenia 5 minutes after injection, which lasted from 5 minutes to 3 hours in the different animals, but was followed in only one instance by a secondary leukocytosis. The differential counts revealed a mild shift toward the left coinciding with the leukopenic phase, but this development remained, in general, within normal limits. There were no appreciable or significant fluctuations in the viscosity of the plasma or in the sedimentation rate of the erythrocytes during the first 24 hours after injection. The platelets were extraordinarily large and of "parasite-like" appearance due to the frequent presence of flagella-like processes.

Hematic Reactions after Repeated Intravenous Injection of Collocel

The repeated intravenous injection of Collocel solutions was well tolerated. There were no temperature reactions, but immediately after each injection a transitory leukopenia developed. During the third and

fourth months the amount of hemoglobin gradually decreased to approximately 75 to 80 per cent of the original level. No significant abnormalities were found in the number of erythrocytes and leukocytes, nor in the differential count and the viscosity of the plasma. The sedimentation rate, on the other hand, increased progressively during the last 2 months, when it fluctuated between 30 and 40 mm. (Wintrobe-Landsberg tube).

Pathological Observations

The post-mortem examination of the 5 dogs did not reveal any appreciable macroscopic abnormalities of the internal organs. Particularly, the liver and spleen were not significantly enlarged nor did they deviate in consistency and color from normal organs.

Histologic studies were made of the lungs, heart, thyroid, parathyroid, liver, spleen, pancreas, intestine, adrenal, kidney, testis, aorta and its large branches. The aorta was cut for this purpose into 15 to 20 rings, while 2 or 3 rings each were cut from the carotid, brachial, and iliac arteries.

The dog which was sacrificed on the 16th day of the experiment had histologically normal organs with the exception of the presence of scanty amount of bluish homogeneous deposits found intracellularly and extracellularly in the splenic pulp in sections stained with hematoxylin and eosin.

The histologic findings in the other 4 dogs were essentially uniform. The ascending aorta showed occasional, small, localized proliferations of endothelial cells forming bundles running parallel to the longitudinal axis, and large fibroblastic-mononuclear edematous cushions containing numerous multinucleated giant cells. Foam cells filled with homogeneous matter, staining a moderately dark blue, were present in parts of these cushions and, in a few places, beneath the endothelial lining. In some segments of the thoracic aorta scattered small elevations of endothelial foam cells containing bluish matter as well as extracellular bluish material occurred beneath an endothelial lining showing cellular crowding. Such foci were observed at the orifices of branches, where they were found within, or extended into, the funnel-like first part of the branching vessel (Fig. 1). Areas exhibiting foam-cellular swelling of the endothelial lining were scattered here and there (Fig. 2). Similar foam cell lesions were encountered in several vasa vasorum located in the media or adventitia where foam cells were sometimes seen in the perivascular spaces of these vessels. In the lumen of one vas vasis a bluish mass was split up by numerous slender cells (Fig. 3). The inner media was often edematous, while fibro-hyaline foci were occasionally

seen in the outer media. Similar foam-cellular lesions occurred rarely in the large arterial branches of the aorta of only one animal. Atheromatous lesions in general were small and infrequent.

The Kupffer cells of the liver were balloon-like, swollen and proliferating, and contained a blue-stained homogeneous cytoplasm, while the nucleus was crescent-shaped and pushed against the cellular wall. The liver cells were intact. The pulp of the spleen contained many bluish staining foam cells as well as strands of a homogeneous extracellular bluish material. Mononuclear giant cells in a moderate number were present in the spleen of one dog (Fig. 5). The renal glomeruli contained scattered groups of swollen endothelial cells with a bluish content. Similar foam cells were occasionally seen in groups in the interstitial tissue (Fig. 4), particularly in the perivascular areas. Bluish staining casts were found in the tubular lumina of the medulla.

All other organs were without any significant changes.

Comment

The hematic and anatomic changes observed in dogs after the intravenous injection of solutions of sodium cellulose glycollate resemble in many respects those seen in dogs and rabbits after the intravenous introduction of colloidal solutions of other macromolecular carbohydrates such as methyl cellulose, polyvinyl alcohol, pectin, gum arabic (Hueper[2]), inasmuch as there appeared a transitory leukopenia, a decrease in hemoglobin, an increase in the sedimentation rate of the erythrocytes, a deposition of the injected matter in the reticulo-endothelial cells of the liver and spleen and in the glomerular endothelial cells and interstitial cells of the kidney, and the formation of atheromatous deposits in the aorta and its branches. It is remarkable, on the other hand, that these reactions remained within relatively moderate limits when compared with those seen in connection with the other above-mentioned substances, especially the methyl celluloses, if proper consideration is given to the total amount of colloidal material injected. The reason for this discrepancy must remain uncertain as no data are available on the molecular size of this compound and on the rôle which the glycollic acid part of the molecule might have played in its colloidal dispersion, in the colloidal stability of the plasma, and the permeability of the filtration membrane. The presence of bluish staining casts in the tubular lumina of the kidney indicates that the injected material is excreted through the kidney.

In contrast to methyl cellulose, sodium cellulose glycollate is not taken up by the liver cells, but only by the stellate cells. The atheromatous formation evidently occurs, as with other colloidal and macro-

molecular atheromatogenic agents, by the intracellular resorption of this material from the plasma into the endothelial cells and by its extracellular infiltration into the subendothelial spaces of the aorta and its large branches as well as of their vasa vasorum. The localization of these deposits in the region of the orifices of branches furnishes additional evidence supporting the contention that the normal turbulence of the blood, and vibratory lability of the plasma colloids in certain areas produced thereby, represent important factors controlling the distribution of atheromas in the vascular tree (Hueper [4]).

While the reported evidence is not unfavorable as to the suitability of solutions of sodium cellulose glycollate as a plasma substitute, blood pressure measurements made on anesthetized dogs injected with solutions of this substance showed that it exerts a definite depressor effect which obviates its use as a therapeutic agent in the treatment of shock.

Conclusions

Solutions of sodium cellulose glycollate, injected intravenously, elicit in dogs a transitory leukopenia and, upon repeated introduction, a decrease in hemoglobin and an increase in the sedimentation rate.

Sodium cellulose glycollate is stored in the Kupffer cells, the reticulum cells of the spleen, the endothelial cells of the renal glomeruli, and as atheromatous deposits in the aorta and its large branches.

The anatomical character and the distribution of the atheromatous lesions support the concept that in the genesis of these changes the macromolecular colloidal matter present in the plasma is taken up by the endothelial cells, which are transformed thereby into foam cells, and infiltrates extracellularly into the subendothelial spaces in which normally a turbulence of the blood exists and where thereby a vibratory lability of the plasma colloids is produced.

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[*Illustrations follow*]

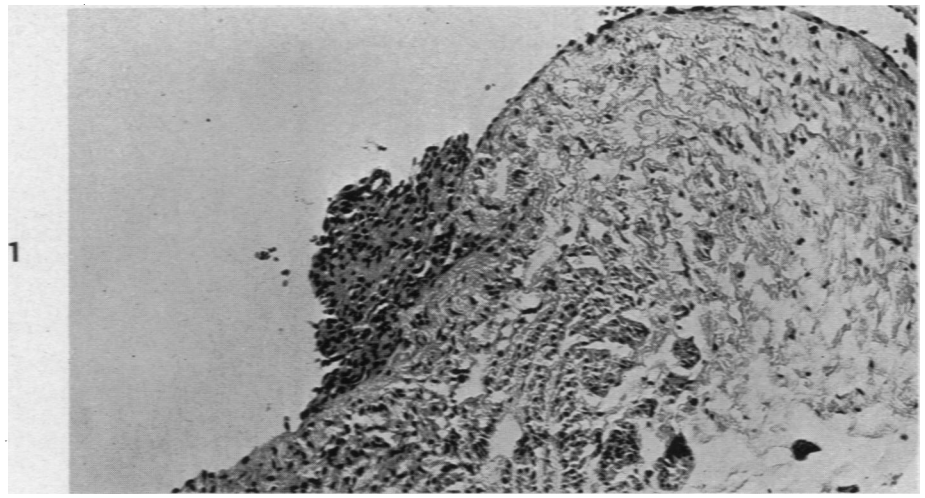
DESCRIPTION OF PLATES

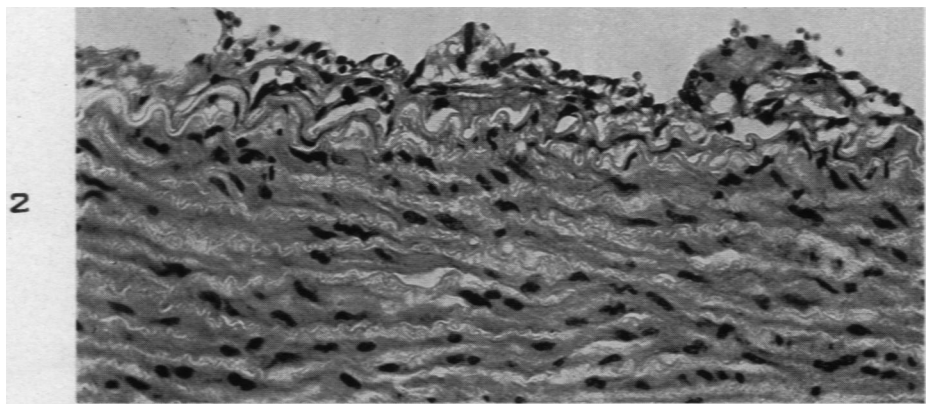
PLATE 182

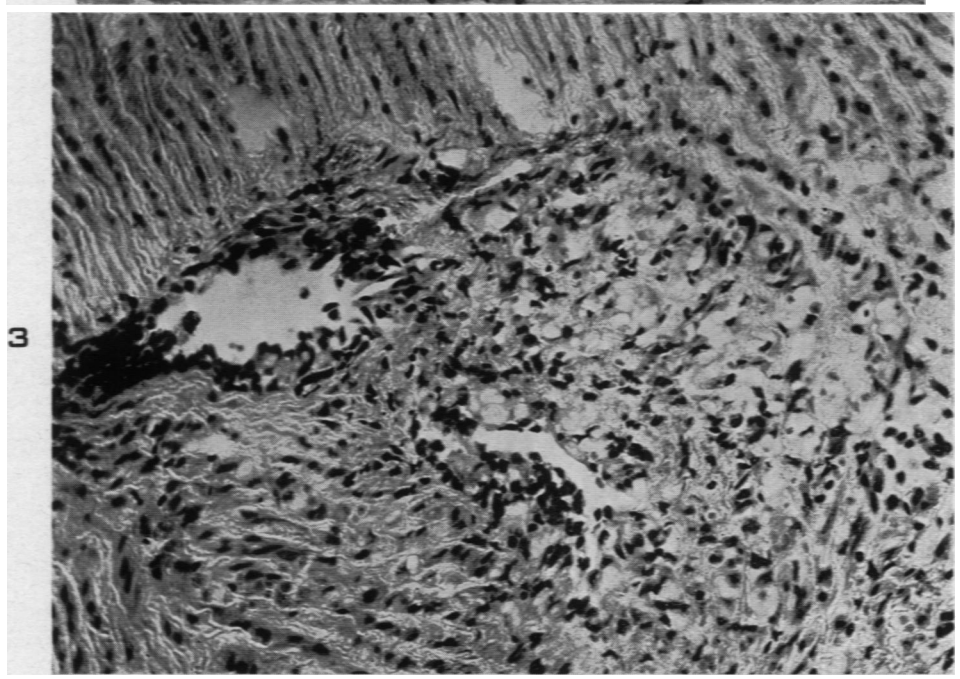
FIG. 1. Broad-based endothelial proliferation at the orifice of a small branch of the aorta. Hematoxylin and eosin stain. × 180.

FIG. 2. Endothelial foam cells coating the wall of the aorta. Hematoxylin and eosin stain. × 270.

FIG. 3. Foam-cellular proliferation in and around vasa vasorum of the aortic wall. Hematoxylin and eosin stain. × 270.





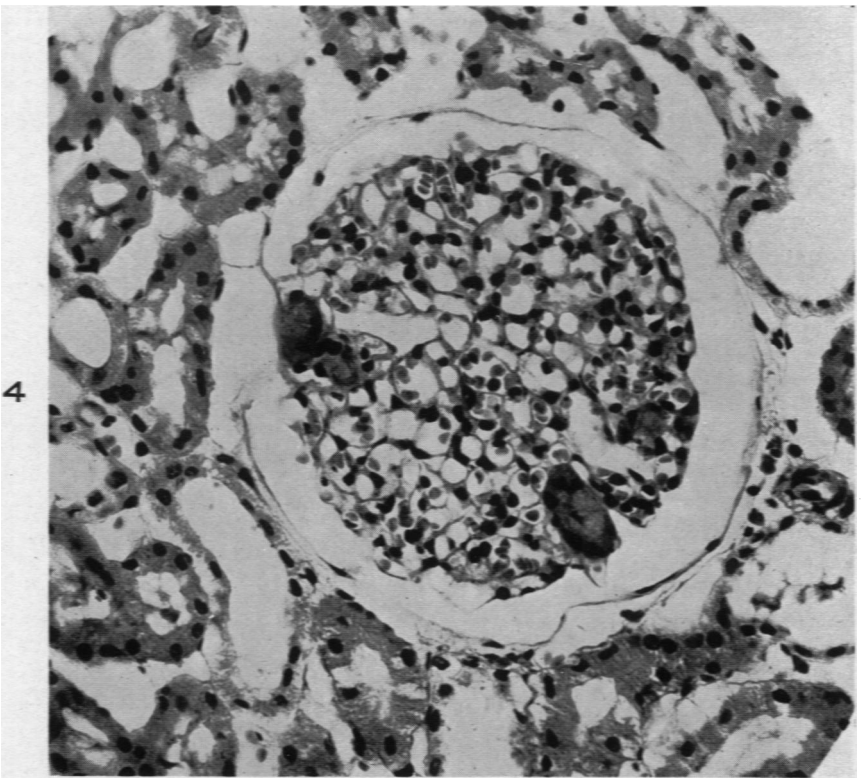


Hueper Sodium Cellulose Glycollate

PLATE 183

FIG. 4. Capillary loops of a glomerulus obliterated by swollen endothelial foam cells. Hematoxylin and eosin stain. × 360.

FIG. 5. Accumulations of reticulocytic foam cells in the pulp of the spleen. Hematoxylin and eosin stain. × 270.



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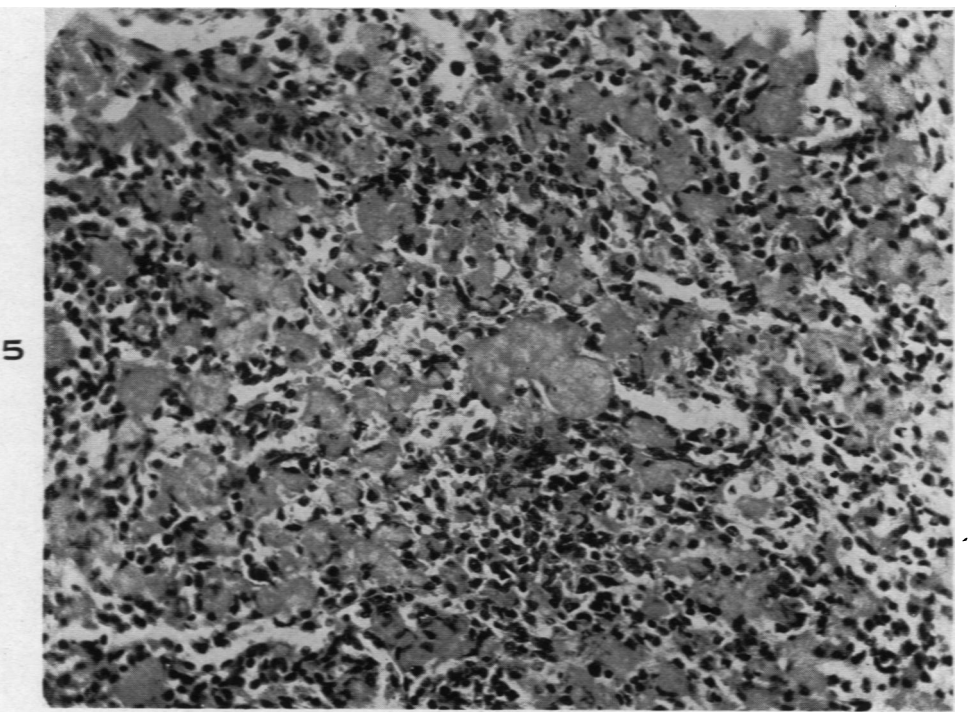

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Hueper Sodium Cellulose Glycollate