Dissecting Aortic Aneurysm in Hamsters Treated with Cortisone Acetate. (22145)

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Dissecting aortic aneurysm has been produced in dogs by thermal coagulation of the aorta(1), and in rats by diets containing meal of the seeds of *Lathyrus odoratus*(2-4). No report has been found of the production of this aortic lesion in any other laboratory animals. During experiments in which hamsters were treated with cortisone to accomplish heterologous transplantation of human fetal lung tissue, many animals died with massive intrathoracic or intraperitoneal hemorrhage. Histologic examination of mediastinal and retroperitoneal tissues revealed that the hemorrhage was usually due to rupture of a dissecting aneurysm of the aorta.

Materials and methods. Seventy-seven golden hamsters 1 to 3 months of age, from the colony at the National Institutes of Health (NIH) were treated with cortisone acetate and inoculated with human fetal tissue. Fifteen additional animals were treated with cortisone but received no transplants. The fetal lung tissue was cut into small fragments and implanted with an 18 gauge trocar, or was minced with small quantity (usually less than 1 ml) of physiological saline and injected by using 1 ml tuberculin syringe and 20 gauge needle. Inoculations were made into submucosa of the cheek pouch of hamsters anesthetized with veterinary nembutal, 0.11 ml/100 g body weight. Cortisone acetate (Cortone,® Merck) was injected subcutaneously into the posterior cervical region in doses of 3 mg at the time of transplantation of human tissues, and 1 mg given twice a week thereafter for remainder of the life of the animal. Hamsters were kept in large plastic cages in air-conditionsd rooms at approximately 78°F. The diet consisted of Purina Laboratory Chow and water *ad libitum,* supplemented with kale, carrots, and apples 3 times a week. Hamsters were examined daily, and dead animals necropsied immediately. Six hamsters were killed when they appeared moribund. The other 71 were found dead, but had not appeared moribund on the preceding day. At first, only the cheek pouch area was examined histologically, since the state of heterologous embryonic tissue was our primary concern. When it was apparent, however, that many animals had died with massive thoracic or abdominal hemorrhage, an attempt was made to determine the origin of the hemorrhage. Entire thoracic contents were then removed at necropsy. Lungs were removed, and remainder of the specimen including thoracic aorta was fixed in Helly's formal-Zenker fluid. The abdominal aorta was also prepared for histological study. When hardened the tissues were cut transversely into 15 to 20 blocks. After tissues were imbedded in paraffin, sections were cut at 6 μ and stained with hematoxylin and eosin routinely. A variety of histochemical technics has also been used, and these will form the basis for a later report.

Results. Massive hemorrhage was the cause of death of 51 of 77 NIH hamsters dying 26 to 162 days after initiation of cortisone therapy and transplantation of human fetal tissue. Of these 51 animals, 37 had hemothorax and 14 had hemoperitoneum. The aorta of 26 of these animals was examined microscopically, and dissecting aneurysm was demonstrated in 23. No inflammatory lesions were found. However, in sections from 3 other animals, necrotizing arteritis of the aorta was observed; this necrosis was so extensive that we were unable to determine whether dissection of the aorta had occurred. In addition, dissecting aneurysm was found histologically in 2 animals in which mediastinal hemorrhage had not taken place. Fourteen hamsters, 2 months old, obtained directly from Lakeview Hamster Colony in New Jersey were treated with

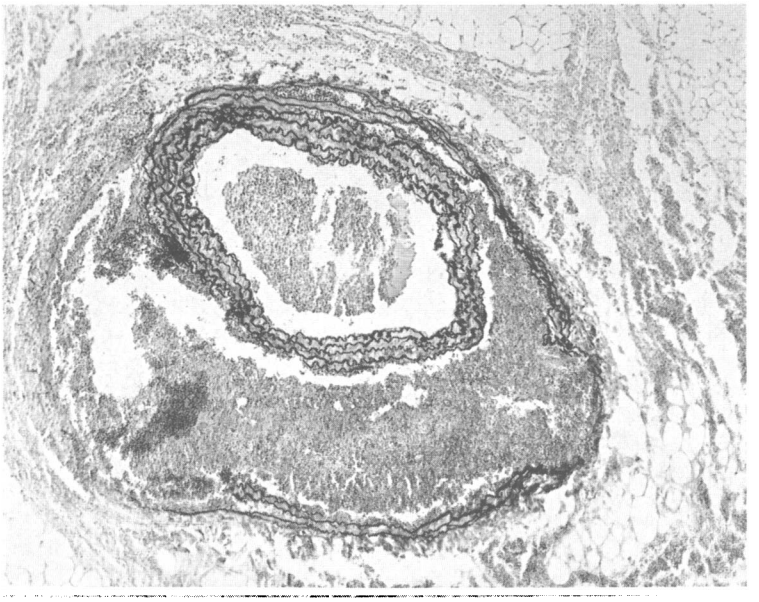

FIG. 1. Thoracic aorta of a cortisone treated hamster showing separation of elastic fibers by hemorrhage. Orcein. 60 ×.

cortisone acetate following implantation of pieces of human fetal lung tissue by the same technics as were applied to the NIH hamsters. Six of these animals died, all with hemothorax or hemoperitoneum. A control experiment to test the effect of cortisone alone is in progress. Five Lakeview and 10 NIH hamsters have been treated with cortisone acetate using the same dosage schedule, but without tissue transplantation. After 80 days 2 Lakeview and 4 NIH hamsters died with hemothorax or hemoperitoneum, and dissecting aneurysm has been demonstrated histologically in all.

Histologically, the aneurysmal dissection of aortic wall appeared to originate at a point lying just above aortic valve. The lesion consisted of separation of layers of the media of the aorta by extravasated blood (Fig. 1). In the sections of aorta from the 2 animals without massive hemorrhage, there was hyalinization of areas of the outer media adjacent to the aneurysmal vascular lumen. In animals with massive hemorrhage, medial necrosis was no longer evident histologically. The structural continuity of the blood-filled intramedial spaces with lumen of the vessel on the one hand or with perivascular tissue on the other, could be demonstrated in some sections. Most of the aneurysms appeared to have developed acutely, since there was little evidence of inflammatory reaction in areas of dissection or hemorrhage.

Discussion. The factors involved in the degenerative lesion that eventuates in weakening of the tunica media and development of these dissecting aneurysms in hamsters, are not clear from these experiments. We have no evidence to suggest that this lesion is related to lathyrism. The diet contained no pea meal, although Purina Laboratory Chow does include legumes alfalfa and soybean. Gross examination has not shown striking abnormalities of bone which have been described in lathyrism(4). The diet has been fed to nearly a thousand other hamsters in our laboratory over long periods of time, and none have shown evidence of aneurysm. Water extraction of Purina Chow according to the method of Dupuy and Lee(5) has yielded an amorphous yellowish substance. The relationship, if any, between this material and the crystals isolated from *Lathyrus pusilis* awaits completion of biological tests now in progress. The possibility of a synergism between cortisone and some dietary factor is still to be considered.

No report of aneurysm in patients treated

with cortisone or corticotrophin has been found, although necrotizing arteritis was described recently following self-administered cortisone overdosage in an arthritic patient (6). Other investigators have found aortas of cortisone-treated children to have a high content of lipids(7). Degeneration of the tunica media of the ascending aorta without hemothorax has been observed in this laboratory in two mice bearing a subcutaneous transplant of a functional adrenal cortical carcinoma(8).

Summary. Massive thoracic or abdominal hemorrhage has been found in 51 of 77 hamsters treated for 26 to 162 days with cortisone acetate. All of these animals bore implants of human fetal tissue. Histological sections of 26 of these animals demonstrated dissecting aortic aneurysm in 23 and necrotizing arteritis in 3 animals. The aorta of the other 25 animals with gross hemorrhage was not examined histologically. Six non-transplant-bearing hamsters treated with cortisone died with microscopically proved dissecting aortic aneurysm.

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Inhibition of Calcification *in vitro* by Luteocobalti Chloride.* (22146)

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There is evidence that chondroitin sulfate or its protein complex may play a role in the process of calcification. Rubin and Howard (1) found an increase in metachromatic staining in regions where active calcification is occurring, which they attribute to increase in concentration of chondroitin sulfate or a change in its degree of polymerization. Neuman(2) showed a correlation between cation binding capacity of cartilage and its sulfate content. Miller, Waldman and McLean(3) found that basic dyes inhibit calcification *in vitro* and that this effect can be reversed in the presence of calcium and phosphate. Finally, it has been demonstrated that protamine, which can precipitate chondroitin sulfate from solution, can cause inhibition of calcification *in vitro*(4,5). Like protamine, the complex luteocobalti cation can precipitate chondroitin sulfate as shown by Matthews and Dorfman(6). It will also precipitate mucoprotein of cartilage(7), as well as ribonucleic acid, heparin and sodium polymetaphosphate, from solutions of low ionic strength and at a pH above 3.5(6). Under similar conditions hyaluronate, neutral polysaccharides and human serum proteins fail to precipitate.

In view of this property of acting as a rather selective precipitant, it was decided to study the effect of luteocobalti chloride on calcification *in vitro*. It was also considered that the orange-yellow color of the cation might make it possible to detect its presence at calcification sites.

Methods. Calcification *in vitro* was studied by a simplification of the method described by Sobel, Nobel and Hanok(8). Twenty-one-day-old weanling rats were

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