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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raised on a standard rachitogenic diet (U.S.P. Rachitogenic Diet No. 2). When they were 5 to 6 weeks old, they were sacrificed, the tibia was dissected out and sliced longitudinally. These tibial sections were placed in calcifying solution (described below) and incubated at 37°C for 16 to 20 hours. Sections were then washed with water, placed in 2% aqueous silver nitrate and exposed to the light of incandescent lamp for about 10 minutes. Calcification manifests itself as a dark deposit on the surface of the epiphyseal plate. It is measured by extent of this deposit, using numbers from 0 to 4, to designate width of surface of the plate covered and by zero or 1 to 4 plus signs, enclosed in parenthesis, to designate length of plate covered. Range of values extends from 0 (0) to 4 (++++). The action of luteocobalti chloride was studied in 3 ways; (1) incorporation of inhibitor into the calcifying solution itself, (2) prior exposure of sections to inhibitor in the basal salt solution, followed by washing with water and incubation in calcifying solution, and (3) prior exposure of sections to inhibitor in the presence of calcium chloride, followed by washing and incubation in the calcifying solution. The calcifying solution contained the following components: sodium chloride (0.07 M), potassium chloride (0.005 M), sodium bicarbonate (0.022 M), calcium chloride (0.0025 M; 10 mg% calcium), and a combination of NaH_2PO_4 plus Na_2HPO_4 at a molarity of 0.00161 corresponding to 5 mg% phosphorus. The pH of the solution was adjusted to 7.3 with carbon dioxide. The basal salt solution consisted of the following: sodium chloride (0.07 M), potassium chloride (0.005 M), and sodium bicarbonate (0.022 M). The solution was adjusted to a pH of 7.3 with CO_2.

TABLE I. Inhibition of Calcification *In Vitro* by Addition of Luteocobalti Chloride to Calcifying Solution.

Conc. of LCC (normality)	No. of experiments	Degree of calcification (avg)
.0	9	1½(++++)
.0056	3	1 (+)
.009	2	1 (++)
.019	2	0 (0)
.028	4	0 (0)
.056	2	0 (0)
.112	7	0 (0)

LCC = Luteocobalti chloride.

TABLE II. Inhibition of Calcification *In Vitro* by Prior Exposure to Luteocobalti Chloride.

Conc. of LCC (normality)	No. of exps.	Degree of calcification (avg)
.0	5	1(++++)
.0–(B.S.)	5	1 (+++)
.0011	2	½ (+++)
.0022	2	½ (+)
.003	2	0 (0)
.0056	4	0(0), 0(0), 0(0), 1(++++)
.011	4	0 (0)
.056	3	0 (0)
.112	3	0 (0)

B.S. = Basal salt solution.

Results. The results in Table I were obtained by addition of luteocobalti chloride to the calcifying solution in concentrations indicated. Inhibition began in the concentration range 8.96×10^{-3} to 1.9×10^{-2} N luteocobalti chloride. There was a deposition of cobalt ion on the surface of the epiphyseal plate increasing with concentration of luteocobalti chloride employed.

The results in Table II were obtained by prior exposure of tibial sections to luteocobalti chloride in the presence of basal salt solution. The system was shaken intermittently for 2¼ hours. Then the tibial sections were washed with water and placed in calcifying medium for 16 to 20 hours. The sample designated as zero normality of luteocobalti chloride is a control which received no prior treatment; B.S. indicates a control that received prior treatment with basal salt solution. Inhibition of calcification begins in the range 2.2×10^{-3} N to 3.4×10^{-3} N, if one ignores the single case of inhibition found at 5.6×10^{-3} N.

It has been demonstrated by Sobel, Nobel and Hanok(8) that inhibition of calcification *in vitro* by prior shaking with neutral salts may be reversed by subsequent shaking with calcium chloride. In view of this it was decided to determine the effect of calcium chloride on inhibitory action of luteocobalti chloride. Table III shows results of prior exposure of tibial sections to luteocobalti chloride in the presence of calcium chloride for 1½ hours, followed by their exposure to calcifying solution.

TABLE III. Effect on Calcification of Prior Treatment with Both Luteocobalti Chloride and Calcium Chloride.

Conc. of—			
LCC (normality)	$CaCl_2$ (normality)	No. of samples	Degree of calcification (avg)
.00	.0	7	1½ (+++)
.00--($CaCl_2$)	.5	6	1½(++++)
.0056	.5	4	2 (++++)
.011	.5	5	1½(++++)
.0179	.5	4	1½(++++)
.028	.5	5	1½(++++)
.056	.5	4	½ (+++)
.112	.5	6	1 (+++)
.056	.001	6	1 (++++)
.056	.05	7	1 (+++)
.056	.10	5	½(++++)

There is no complete inhibition of calcification. Calcium chloride appears to exert a protective effect on the calcifying system. The amount of cobalt deposited, indicated by depth of yellow color at the surface of epiphyseal plate, depended on the ratio of luteocobalti chloride to calcium chloride. The greater the concentration of the calcium chloride, the less the deposit of the cobalt salt. In this connection it was observed that precipitates, formed from luteocobalti chloride and chondroitin sulfate in aqueous solution, readily dissolved in the presence of calcium chloride.

The most probable binding site of luteocobalti chloride at cartilage surface is either the chondroitin sulfate or its mucoprotein. In view of the possibility of mucoprotein involvement, it was decided to determine the effect of trypsin on the binding of luteocobalti chloride by tibial sections. In these experiments tibial sections were exposed to a solution of Tryptar (lyophilized crystalline trypsin, Armour) at concentration of 10 mg/ml in water for the times indicated in Table IV. The sections were then washed and exposed to luteocobalti chloride (.2%) for one hour. Evaluation of cobalt deposit was similar to that used in evaluation of calcification. However, due to the nature of the cobalt color, evaluation tends to be more subjective. The final value of the cobalt deposit was obtained by multiplying the number for length by the number for width of deposit. The results indicate a complete loss of capacity to bind luteocobalti chloride on exposure to trypsin for 75 minutes.

TABLE IV. Effect of Trypsin on Capacity of Epiphyseal Sections to Bind Luteocobalti Chloride.

Exposure time to trypsin (min.)	No. of sections	LCC deposit	Final value of LCC deposit
Control	1	3(++++)	12
15	1	2(++++)	8
37	1	1(++++)	4
45	2	0 (0)	0
50	1	½(++++)	2
60	1	½ (++)	1
75	2	0 (0)	0
100	2	0 (0)	0

Discussion. The work reported here supports the view that chondroitin sulfate or a mucoprotein containing it may be one component of calcifying systems. The luteocobalti cation, to the extent that it is a relatively specific precipitant for chondroitin sulfate, may be more useful than other cations for studying reversible inhibition of calcification. The fact that nasal cartilage does not calcify *in vitro* under the test conditions, shows that a high content of chondroitin sulfate or its mucoprotein is not a sufficient condition to cause calcification.

Summary. 1. Luteocobalti chloride inhibits calcification *in vitro* when tibial sections are exposed to its action, either prior to their exposure to the calcifying solution, or concomitant therewith. The inhibition is antagonized by calcium ions. 2. That the luteocobalti cation deposits on the surface of the epiphyseal plate can be seen because of its color. This deposit is prevented by calcium ions. 3. The capacity of tibial sections to bind luteocobalti chloride is destroyed by their preliminary exposure to trypsin.

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Absorption in Dogs Following Exclusion of Bile and Pancreatic Juice Using I^{131} Labeled Fat. (22147)

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For the past year this laboratory has utilized I^{131} labeled protein† (albumin) and fat† (glycerol trioleate) in studying absorption and digestion in various disease states, *i.e.* pancreatic disorders, ulcerative colitis, small intestinal disorders such as sprue and Whipple's disease, and in post gastrectomy patients. In comparative studies using radioactive iodinated serum albumin and radioactive iodinated glycerol trioleate in dogs subjected to total pancreatectomy, there was a greater abnormality in digestion and absorption of the radioactive fat than in digestion and absorption of I^{131} labeled albumin as measured by percentage of radioactivity found in blood following the test meal containing these substances(1).

The present study was done to further evaluate the effect of exclusion of bile and pancreatic juice on the pattern of fat digestion and absorption by use of a fat test meal containing I^{131} labeled glycerol trioleate.

Materials and methods. Two types of fat meal were prepared, each identical except that one was emulsified in a Waring blender and contained 2-3 ml of polyoxyethylene sorbitan mono-oleate (Tween 80). The constituents of the meal were: ½ ml of peanut oil‡/kg body weight of the animal, 10 μc of I^{131} labeled glycerol trioleate, the total mixed with equal volume of water. For 3 days prior to the test meal the animals were given .99 g tablet of potassium iodide daily to saturate the thyroid with iodine. All animals were fasted for 12 hours prior to the test. A rubber Levine tube was passed into the stomach of the unanesthetized animal and the test meal given via the stomach tube. 2 ml of venous blood was then drawn at 1, 2, 3, 4, 5, and 6 hours following the test meal into the stomach. Each sample of blood was then assayed for radioactivity in a calibrated sodium iodide thallium activated scintillation crystal well counter. Using measured radioactivity of the 2 ml sample and the calculated blood volume of animal (assumed to be 7.5% of body weight), the total radioactive content of blood was calculated and expressed as percentage of total ingested radio-iodine. From these data a curve was constructed showing the relationship of radioactivity in blood as a function of time. To establish a "normal" pattern of radioactivity of blood following a fat meal of peanut oil containing I^{131} labeled glycerol trioleate, 39 tests were done on 24 normal dogs. In 29 of these tests peanut oil and glycerol trioleate constituting the test meal were emulsified. Ten additional tests were done on 10 dogs in which the constituents were not emulsified. Ten dogs were then subjected to total pancreatectomy under sodium amytal anesthesia, following which they were given a diet as suggested by Markowitz (2) with daily injection of insulin. Two-three weeks following operation these animals

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† Obtained from Abbott Laboratories, Chicago, under A.E.C. authorization.

‡ Obtained from Planters Nut and Chocolate Co., Wilkes-Barre, Pa.