enzyme system. In a calcium free medium, calcium is removed and is probably replaced by one of the competing ions. Cu^{++} ions may compete so avidly for this local constituent that they preferentially combine with this factor even in the presence of large amounts of Ca^{++} ions. On shaking with $CaCl_2$ solution free of the competing ions, the formation of the calcium complex is favored as an initial step to calcification.

Summary. It was possible to demonstrate the reversible inactivation of calcification *in vitro* of the hypertrophic epiphyseal cartilage. When rachitic bone sections are shaken with strontium chloride, sodium chloride, calcification *in vitro* is inhibited. On subsequent shaking with calcium chloride calcification *in vitro* takes place. In addition, inactivation takes place with ½ mE of cupric chloride in the presence of 150 mE of calcium chloride. On subsequent shaking with 150 mE of calcium chloride (in the absence of cupric chloride) reactivation takes place.

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The Influence of Alloxan Diabetes on Cholesterol Atheromatosis in the Rabbit.*† (17335)

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It has long been suspected, largely on the basis of statistical studies, that humans suffering from diabetes mellitus develop arteriosclerosis not only in greater numbers than do non-diabetics, but also develop it earlier. However, conclusive experimental evidence that diabetes enhances arteriosclerosis has been lacking. It seemed to us that it might be instructive to test the influence of alloxan diabetes in the rabbit, despite the fact that the relationship of both experimental entities to their human counterparts are poorly understood.

Methods. The general plan of the experiment was as follows: 3 groups of rabbits, one group of which had been made diabetic with alloxan, were fed a cholesterol enriched diet; and animals from each group were examined at intervals after commencement of the diet to see if there was any difference in the time of onset or character of the vascular lesions in the different groups. Young Dutch rabbits weighing from 900 to 1400 g were given intravenous injections of alloxan monohydrate (Eastman) amounting to 80 to 100 mg per kilo of body weight daily for 2 consecutive days. Animals that were not diabetic on the third day were given similar injections on the third and fourth successive days. Although this method had been found in previous experiences with alloxan to be very effective, nevertheless in this instance a large number of animals died either during the course of the injections or within a week thereafter. Only approximately one half of the animals developed diabetes and survived long enough to be of value in the experiment.

Blood sugar determinations were performed by the method of Somogyi,[1] using a Klett-Summerson photoelectric colorimeter, both before and after alloxan injection. During the period of feeding the diet, the urines of the diabetic group were tested periodically with Benedict's qualitative reagent, and just before each animal was sacrificed, another blood

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† Since preparation of this manuscript the results of G. L. Duff and G. C. McMillan (abstracted in *Am. Heart J.*, 1948, **36**, 469) have come to our attention. These results confirm and extend those presented in this paper in showing that alloxan diabetes inhibits experimental cholesterol atherosclerosis in the rabbit.

[1] Somogyi, M., *J. Biol. Chem.*, 1945, **160**, 61.

TABLE I.
Influence of Alloxan Diabetes on Cholesterol Atheromatosis in Rabbits.
Group A Control Group—Basal diet and cholesterol in olive oil.

Rabbit No.	Duration of diabetes, days	Duration of diet, days	Terminal blood sugar, mg/100 cc	Terminal blood cholesterol, mg/100 cc	Body wt Initial, g	Body wt Final, g	Atheromata Gross	Atheromata Micro.
48-6		38			910	1110	0	0
48-21		40	132	576	1150	1610	0	0
48-25		41	110	864	823	1160	0	+
48-22		48	120	864	1000	1640	++	++
48-20		49			1080	1790	+	+
48-4		58		1120	1060	1535	0	+
48-8		72		1025	1500	2225	+++	+++
48-10		72		1425	1425	1755	++	++
48-15		72		837	1961	2200	+++	+++
Avg		54	121	959	1212	1670		
Avg of control values			120	141		1212		
% change			+1	+580		+38		

sugar determination was performed. Blood cholesterol values were determined on each animal of this group before alloxan injection and before sacrificing according to the method of Bloor, Pelkan and Allen.[2] Those rabbits which manifested a blood sugar of 200 mg% or higher were accepted as diabetic. A total of 8 rabbits satisfying this criterion and showing persistent glycosuria for the duration of the experiment were thus obtained.

Nine rabbits of similar strain, age, and original body weight were used as a control group. Blood sugar values were determined on 3 of these animals before beginning the diet and before sacrificing, and blood cholesterol levels were determined on all except 2 animals at the same times.

A third group consisted of 3 rabbits which had received 2 injections of alloxan and had manifested a temporary glycosuria and hyperglycemia, but the urines of which later became sugar free.

For the cholesterol enriched diet, Pfansteil cholesterol suspended in olive oil to a concentration of 6% was added to Rockland Rabbit Ration in such amounts that each rabbit received between 0.3 and 0.6 g per day.

All rabbits of the 3 groups were then fed this cholesterol enriched diet and animals from each group were sacrificed at various intervals after commencement of the diet. Complete autopsies were performed, with especial attention being paid to the heart, aorta, and great vessels. The presence of atheromata in these organs is summarized in the tables. It may be mentioned here that the pathological changes with respect to the other organs were similar to those previously described for alloxan diabetes and cholesterol atheromatosis separately.

Experimental observations. Table I summarizes the data on the control group. It is seen that there was a consistent increase in blood cholesterol values, the increase roughly paralleling the duration of the diet, and the average increase being approximately seven fold. The weight increase was also consistent. The item of chief interest is the fact that in all animals fed the diet 41 days or longer, there was gross or microscopic evidence of atheromata, the severity of the lesions roughly corresponding to the duration of the diet. The atheromata were in all respects similar to those previously described in this condition.

In Table II are seen the summarized data on the diabetic animals. Among these the elevation in blood cholesterol was very irregular, but the average is higher than the average for Group A, and in 3 cases it is considerably higher than that of any single rabbit in Group A. All of these rabbits except two (48-26 and 48-27) lost weight. Much to our astonishment, when these animals were autopsied, only one showed any evidence of

[2] Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, **52**, 191.

TABLE II.
Influence of Alloxan Diabetes on Cholesterol Atheromatosis in Rabbits.
Group B Diabetic Group—Same as control plus alloxan.

Rabbit No.	Duration of diabetes, days	Duration of diet, days	Terminal blood sugar, mg/100 cc	Terminal blood cholesterol, mg/100 cc	Body wt Initial, g	Body wt Final, g	Atheromata Gross	Atheromata Micro.
47-7	22	18	365	503	940	763	0	0
47-12	20	20	297		1060	550	0	0
47-11	31	31	279	2000	1213	1175	0	0
47-8	41	41	268	665	1360	550	0	0
48-26	43	41	205	960	1000	1300	0	0
48-27	48	48	190	349	692	1380	0	0
48-11	68	65	300	2000	1220	650	0	0
47-10	98	98	319	3820	1010	910	+	+
Avg	47	46	278	1287	1062	910		
Avg of control values			117	132		1062		
% change			+138	+875		—14		

TABLE III.
Influence of Alloxan Diabetes on Cholesterol Atheromatosis in Rabbits.
Group C—Alloxan injected—non-diabetic.

Rabbit No.	Duration of diabetes, days	Duration of diet, days	Highest blood sugar, mg/100 cc	Terminal blood cholesterol, mg/100 cc	Body wt Initial, g	Body wt Final, g	Atheromata Gross	Atheromata Micro.
48-24	?	40	180	368	1070	1530	+	+
48-28	?	52	230	368	910	1570	+++	++
48-3	?	69	157	672	985	1650	++	++
Avg		54	189	469	988	1583		
Avg of control values			120	140		988		
% change			+58	+235		+60		

TABLE IV.
Influence of Alloxan Diabetes on Cholesterol Atheromatosis in Rabbits.
Summary of data on all rabbits fed diet for 40 days or longer.

Group	No. with atheromata / No. in group	Percent positive
A. Control	7/8	88
B. Diabetic	1/5	20
C. Alloxan injected, non-diabetic	3/3	100

atheromata—and this rabbit (47-10) had been fed the diet for 98 days.

In Table III are the data on the alloxan-injected, non-diabetic animals, in which it is seen that they behaved as did the controls in Group A.

Table IV summarizes the incidence of atheromata in all rabbits fed the high cholesterol diet for 40 days or longer. These figures become even more impressive when one recalls that the one diabetic animal had developed only minimal lesions after 98 days on the diet.

Discussion. In criticism it may be pointed out that the number of animals is very small and that there were no diabetic animals kept as controls. These defects were due to the difficulties encountered in inducing alloxan diabetes. However, with respect to the latter point, in previous experiences by one of the authors with alloxan diabetic rabbits several animals kept for as long as 11 months showed

no atheromata and no reports of finding atheromata in alloxan diabetic rabbits have appeared in the literature.

In discussion we would like to mention one observation concerning the food intake of these animals. It was noted soon after the beginning of the experiment that, contrary to previous experiences with regular diets in which diabetic animals consumed much more food than normal rabbits, these diabetic rabbits did not eat the oily diet nearly so enthusiastically as did the controls. A greater amount of cholesterol had to be added per unit weight of ration to insure that each rabbit received at least 0.3 g daily. Facilities did not permit us to determine the exact daily consumption of each rabbit. The control animals, therefore, may have received somewhat more cholesterol per day than did the diabetic animals, but it is certain that they did not receive more than twice as much. It is our opinion that the difference in intake was not sufficiently great to account for the very great difference in time of onset of atheromata.

Whatever the mechanism, and despite the above mentioned criticisms, we feel that these findings clearly indicate that under the conditions of this experiment alloxan diabetes retards in time and degree the development of cholesterol atheromatosis in the rabbit, and that it represents a conclusive demonstration that hypercholesterolemia of itself does not lead to the formation of atheromata.

Summary. Alloxan diabetic rabbits, when fed a cholesterol enriched diet, developed blood cholesterol levels much higher than did normal rabbits fed the same diet. Atheromata appeared consistently in controls (and also in alloxan injected, nondiabetic rabbits) after 41 days on this diet, but only to a slight degree in one diabetic rabbit fed the same diet for 98 days. There was no qualitative difference in the character of the lesions in the 3 groups.

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Studies on the Pathogenesis of Experimental Necrotizing Arteritis.* (17336)

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Previous studies from this laboratory[1-3] have shown that acute necrotizing arteritis can be produced with regularity in dogs by feeding a specified high fat diet for a period of 8 weeks or longer then sacrificing them through renal damage. Three factors, time, diet and renal damage, seem to be essential in the production of these lesions.

Methods have been published in detail previously.[2] Briefly these consist of feeding healthy adult dogs a high fat diet for a period of 8 weeks or longer, then sacrificing them through renal damage. The active "dietary factor" is found in cod liver oil but is apparently not unique to cod liver oil. The oil may be added to a kennel diet of unselected table scraps or to a "standard diet", consisting of calves liver (raw wet weight), 32 parts; cane sugar, 25 parts; corn starch, 25 parts; butter, 12 parts and cod liver oil, 6 parts, with equal results. Renal damage has been produced by any one of 3 ways: 1) uranium nitrate subcutaneously; 2) mercuric chloride intravenously; and 3) bilateral nephrectomy; all equally effective.

In all experiments to date, renal damage has been essential. To determine whether or not damage to tissues and organs other than the kidney would precipitate the arterial le-

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1 Holman, R. L., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 307.

2 Holman, R. L., and Swanton, M. C., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 87.

3 Holman, R. L., *So. Med. J.*, 1949, **42**, 108.