THE EFFECT OF ALLOXAN DIABETES ON EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS IN THE RABBIT

IV. The Effect of Insulin Therapy on the Inhibition of Atherosclerosis in the Alloxan-Diabetic Rabbit*

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It has been amply demonstrated that an inhibition of the development of experimental cholesterol atherosclerosis occurs in cholesterol-fed rabbits that have been previously rendered diabetic with alloxan. In the original study of this subject Duff and McMillan (1) reported on the effects of feeding cholesterol to diabetic, to normal, and to "alloxan-recovered" rabbits. The last were animals that had been injected with alloxan, had become diabetic for a short time, and had almost completely recovered from the effects of the alloxan before they were fed cholesterol. It was argued that since both the diabetic rabbits and the "alloxan-recovered" animals had received the same intravenous dose of alloxan, the inhibitory effect on the development of cholesterol atherosclerosis observed in the diabetic rabbits only was not dependent on the alloxan *per se* nor on its possible effects on organs or tissues other than the pancreatic islets. At the same time, however, it was recognized that the argument was not perfect since it could well be maintained that the "alloxan-recovered" animals were less susceptible to the general effects of alloxan than the diabetic animals as indicated by the very fact that permanent diabetes failed to develop in them. However, the "alloxan-recovered" rabbits were regarded, at least tentatively, as providing a suitable control of the effects of alloxan injection, *per se*.

Subsequent studies have contributed to an appreciation of some of the mechanisms that may be involved in the inhibition of atherosclerosis in alloxan-diabetic rabbits (2, 3) but the question of whether the inhibitory effect is associated with the alloxan itself or with the induced diabetic state has not been completely resolved. Recently, Cook, Mills, and Green (4) have reported that the effects of alloxan on tissues other than the pancreas do not protect against experimental cholesterol atherosclerosis. The experiments reported here de-

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scribe the effects of treatment of the alloxan-diabetic state with insulin in rabbits fed cholesterol. They establish that, if the metabolic disturbances associated with the diabetic state are mitigated by treatment with insulin, the inhibitory effect of alloxan diabetes on the development of cholesterol atherosclerosis is minimized. Together with the findings of Cook *et al.* they establish that the inhibitory effect is associated with the diabetic state and not with other effects of the injection of alloxan.

EXPERIMENTAL

Two separate but similar experiments were undertaken.

Experiment 1.—Sixty-eight white rabbits of both sexes were studied. The animals each weighed about 2 kilos at the beginning of the experiments. Their exact ages were unknown. The rabbits were allowed to become accustomed to the rabbit house during a period of preliminary study. Their food consisted of a commercial rabbit chow and water *ad libitum*. Blood was drawn from a cut through the central artery and veins of the ear for analysis of its normal concentration of cholesterol and sugar. The concentration of free and total cholesterol in the serum was determined by the Schoenheimer-Sperry method (5); the concentration of sugar in the blood was determined by a modification of Folin's micro method (6). These analyses were repeated at biweekly intervals throughout the duration of the experiment.

When the animals had attained a weight of about 2.5 kilos, they received intravenously an injection of alloxan monohydrate. The alloxan was prepared freshly as a 5 per cent *w/v* solution in distilled water. It was administered into the lateral vein of the ear in a dose of 200 mg./kilo of body weight as rapidly as possible through a 20 gauge needle. A total of 50 animals were injected with alloxan.

Six hours after receiving alloxan the rabbits were given 20 gm. of dextrose in 100 cc. of water by stomach tube. They received no other treatment whatsoever, but were allowed to stabilize their metabolism for a period of about 1 month. During this time many rabbits died in diabetic coma and some recovered from the diabetic state that had followed the injection of alloxan.

Following the period of metabolic stabilization, the diabetic rabbits were placed in metabolism cages for 7 to 10 days. Observations were made daily on the 24 hour urine volume, food intake, weight, and total urinary glucose excretion. Urine volume was determined to the nearest 5 cc., food consumption to the nearest 5 gm., weight to the nearest 100 gm., and glucose excretion was measured by Benedict's quantitative method and spot-tested with a commercial preparation.

On the completion of the preliminary period of observation the surviving diabetic animals and 18 non-diabetic control rabbits were fed cholesterol. Cholesterol was fed by stomach tube daily at midday as a 5 per cent solution in warm corn oil. Fifteen cc. of oil containing 0.75 gm. of cholesterol was administered daily. There occurred a brief period of marked anorexia of 4 to 6 days duration following the initiation of cholesterol feeding. As soon as the diabetic rabbits had recovered their appetite, insulin therapy was begun. Protamine zinc insulin, 40 units per cc., was injected daily at 9 a.m. beneath the skin of the back. The individual doses of insulin were empirical and were determined largely by the glycosuria observed on spot testing the urine. The persistence of a minor amount of glycosuria was considered to indicate the desired therapeutic effect. The food intake of the animals was limited to 150 gm. daily. Throughout the period of cholesterol feeding and insulin therapy

the diabetic animals were studied daily with respect to the volume of urine excreted, the urinary glucose, food intake, and body weight. The duration of cholesterol feeding varied from 57 to 90 days.

The surviving animals were killed and autopsied. Various tissues were examined microscopically. The heart and aorta were removed together, opened, fixed in formalin, and stained *en bloc* in sudan IV. The location and size of sudanophilic intimal lesions of the aorta were charted graphically and graded on a scale of 0 to 4 in accordance with the scheme illustrated by Duff and McMillan (1). Grade 0 indicated no atherosclerosis while grade 4 denoted extensive and severe intimal lesions. The aorta was then coiled longitudinally, embedded, and sectioned in such a way that a single microscopic section included the whole length of the vessel.

Experiment 2.—The procedures and materials of Experiment 1 were employed in Experiment 2. Certain changes and additions to the methods were introduced. Three groups of animals were studied: (1) diabetic, cholesterol-fed, and insulin-treated rabbits; (2) diabetic, cholesterol-fed animals; and (3) non-diabetic, cholesterol-fed rabbits. All animals were female. In addition to studies of the blood glucose and serum cholesterol, determinations of the serum lipid phosphorus (7) and total fatty acids (8) were made and the fatty acids of neutral fat were calculated (9).

During a period of 3 months 74 rabbits were injected with alloxan. Of these, 16 survived, maintained diabetic blood sugar levels in excess of 300 mg. per cent, and completed the experiment. Ten of the diabetic rabbits were fed cholesterol and treated with insulin while 6 were fed cholesterol but were not treated. Thirteen normal control animals weighing approximately 2.5 kilos were fed cholesterol also.

The feeding of cholesterol to all three groups of rabbits was initiated on the same day. The rabbits were fed 100 gm. of commercial chow coated with 1 gm. of cholesterol. The cholesterol was added to the food as a 10 per cent *w/v* solution in ethyl ether. It was rapidly and uniformly mixed with the food pellets which were then allowed to dry until the odor of ether was lost. One hundred gm. of cholesterolized food was given to each rabbit every morning. Every evening the diabetic, insulin-treated rabbits were given a large dish of unadulterated food while the diabetic, untreated rabbits and control rabbits were given 50 gm. of food that did not contain cholesterol. The daily intake of cholesterolized and cholesterol-free food was measured.

Insulin treatment was begun the day before cholesterol feeding was initiated. Protamine zinc insulin was employed once or twice daily, morning and evening, for the first 17 days; thereafter protamine zinc insulin and crystalline insulin were given daily in the morning only.

The duration of the experiment was 76 days of cholesterol feeding. During this time samples of blood for lipid analysis were drawn on days 12, 32, 53, and 74 from the diabetic rabbits. The non-diabetic control animals had blood drawn for cholesterol analyses on the same days, but lipid phosphorus and fatty acid analyses were done on day 74 only. The degree of lipemia in the serum was graded visually on an arbitrary scale of 0 to 4. The content of sugar in the blood of the diabetic rabbits was determined weekly. Daily quantitative analyses of the sugar excreted in the urine were made.

OBSERVATIONS

Experiment 1.—Experimental mortality was severe. Of 50 rabbits injected with alloxan only 6 frankly diabetic rabbits completed the experiment. Of the 42 rabbits that died, 20 suffered fatal diabetic coma, 2 had fatal insulin shock, 6 aspirated oil during feeding by stomach tube, and the remaining 14 died of

miscellaneous causes including infection. Nine of 18 non-diabetic control rabbits died. Six aspirated oil during feeding, 2 developed a fatal diarrhea during feeding, and the cause of death was not determined in 1 rabbit.

The observations made on the surviving rabbits are summarized in Table I.

TABLE I.

Summary of Experimental Data. Experiment 1

Rabbit No.	Experimental type	Duration of cholesterol feeding	Cholesterol consumed	Average daily dose of insulin	Initial body weight	Final body weight	Average daily urinary glucose excretion before treatment	Average daily urinary glucose excretion during treatment	Average serum free cholesterol	Average serum total cholesterol	Average lipemia	Aortic atherosclerosis
		days	*gm.*	*units*	*kg.*	*kg.*	*gm.*	*gm.*	*mg. per cent*	*mg. per cent*	*0–4*	*0–4*
B-50	TD*		37	5.0	2.7	3.6	38.4	10.0	135	368	1.0	1 (trace)
C-44	C	57	40	—	2.7	3.3	—	—	119	348	0.2	1
C-45	C		37	—	2.5	3.7	—	—	142	373	1.0	2
C-57	TD		42	2.5	2.4	2.2	21.7	8.5	98	268	0.4	0
C-3	TD		50	2.1	3.2	3.3	22.2	8.2	233	667	0.8	1
C-2	TD		49	1.8	3.2	3.8	22.0	3.7	258	737	1.0	3
C-54	TD	73	42	0.8	2.6	2.3	25.1	3.5	266	795	1.2	2
D-12	C		45	—	2.5	3.6	—	—	169	450	0.8	2
D-15	C		46	—	3.1	4.1	—	—	247	683	0.4	2
D-13	C		48	—	3.1	3.8	—	—	267	688	1.0	2
D-11	C		49	—	2.7	4.1	—	—	258	700	0.6	1
A-37	TD		63	4.0	3.2	3.6	25.0	0.5	286	683	2.1	3
A-78	C	90	64	—	3.6	4.7	—	—	146	343	0.8	2
A-74	C		64	—	3.6	5.1	—	—	104	351	0.5	2
A-73	C		63	—	3.1	3.9	—	—	278	593	1.1	4

* TD, insulin-treated diabetic rabbits; C, untreated, non-diabetic control rabbits.

There were 3 separate series of animals of experimental duration of 57, 73, and 90 days respectively. All of the diabetic rabbits excreted more than 20 gm. of glucose daily in the urine before insulin therapy was begun. Following the use of from 0.8 to 5.0 units of insulin daily, the daily glucose excretion was diminished on the average from 61 to 98 per cent. The insulin dosage and the diminution of glycosuria were not closely correlated but displayed great individual variation. Two of the treated diabetic rabbits lost a small amount of weight during the experiment, one gained slightly, and the remaining 3 rabbits gained a moderate amount of weight during the experiment. The non-diabetic control rabbits gained more weight than the diabetic rabbits treated

with insulin. The lipemic index and the concentration of cholesterol in the serum tended to be slightly higher in the treated diabetic rabbits than in the control rabbits. It was noted that, with one exception, lipemia did not appear in the serum of the insulin-treated diabetic rabbits with unusual rapidity. The serum of rabbit A-37, which had the highest lipemic index, also tended to manifest quickly its lipemic state *in vitro*.

The differences in the severity of atherosclerosis of the aorta in the control and in the insulin-treated diabetic animals were no greater than might be encountered in groups of normal rabbits fed cholesterol for the same periods of time.

Experiment 2.—The experimental mortality among the animals rendered diabetic with alloxan was severe. Seventy-four animals received one or more injections of alloxan. Of these, 58 died, while 16 were diabetic and completed the experiment. Fifty-one of the rabbits died before cholesterol feeding and insulin treatment were begun and of these 32 died during the first 6 days following the injection of alloxan. The experimental observations are summarized in Table II.

Nine of the 10 diabetic rabbits that were subsequently treated with insulin excreted daily more than 25 gm. of sugar in the urine. The tenth animal (G-41) was ill and anoretic at the time the pretreatment glycosuria was studied so that the value observed (an average daily excretion of 11.0 gm.) was not representative. With insulin treatment a good control of the diabetic state was obtained. The average reduction in glucose excretion was not less than 87.6 per cent, and in one animal it was as much as 95 per cent. The group average daily excretion of glucose in the urine fell from 35.5 gm. to 3.2 gm. during treatment with insulin. The untreated diabetic rabbits lost an average of 70 gm. in body weight, while the treated diabetic rabbits gained 1.03 kilos and the non-diabetic rabbits gained 1.12 kilos.

It was observed that, on the average, the untreated, cholesterol-fed diabetic rabbits had the greatest, the insulin-treated, cholesterol-fed diabetic rabbits had less, and the non-diabetic, cholesterol-fed rabbits had the least concentration of cholesterol in the serum. These differences were not striking. The serum lipid phosphorus values obtained from the untreated, cholesterol-fed diabetic animals were double those observed in the non-diabetic, cholesterol-fed control rabbits and were six or seven times normal. The serum total cholesterol/lipid phosphorus ratio of the non-diabetic rabbits was 18.1; in the treated diabetics it was 16.3, and in the untreated diabetic animals it was 10.4. A normal ratio in the rabbit maintained on a cholesterol-free diet is approximately 10.4 in this laboratory. The serum fatty acids of neutral fat of the untreated diabetic rabbits were markedly elevated. The average content of triglyceride in the serum was approximately 2.5 gm. per cent. This figure included one animal with an average content of neutral fat in the serum of about 8 gm. per cent. However, if this

TABLE II

Summary of Experimental Data. Experiment 2

Rabbit No.	Experimental type	Duration of cholesterol feeding	Cholesterol consumed	Average daily dose of insulin	Initial body weight	Final body weight	Average daily urinary glucose excretion before treatment	Average daily urinary glucose excretion during treatment	Average serum free cholesterol	Average serum total cholesterol	Average serum lipid phosphorus	Average serum fatty acids of neutral fat	Average lipemia	Aortic atherosclerosis
		days	*gm.*	*units*	*kg.*	*kg.*	*gm.*	*gm.*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*m.eq/liter*	*0–4*	*0–4*
F-20	TD*	76	76	17.7	3.86	4.70	48.6	4.8	209	525	25.1	35.5	1.8	3
E-70	TD	76	76	17.0	3.27	4.49	55.5	4.7	44	165	15.0	8.8	0.3	1
F-12	TD	76	76	12.8	4.16	5.30	35.4	4.4	94	278	27.3	13.1	0.3	1
F-2	TD	76	76	8.2	3.04	4.10	28.9	3.6	257	520	43.7	23.1	1.8	2
F-1	TD	76	76	15.2	2.58	3.52	37.7	3.3	232	551	39.4	30.2	2.0	2
E-74	TD	76	75	11.7	3.75	5.20	24.8	2.9	205	542	34.9	10.3	2.5	3
D-99	TD	76	76	10.6	4.13	5.27	26.6	2.4	291	678	26.2	32.0	1.8	3
G-41	TD	76	76	10.7	3.56	5.11	—	2.3	156	401	21.8	12.5	0.5	2
E-43	TD	76	76	4.6	4.67	5.15	25.3	2.0	191	465	34.4	24.8	1.5	3
E-65	TD	74	72	10.8	3.22	3.69	36.8	1.8	170	456	13.6	18.7	0.8	2
E-73	UD	76	76	—	3.05	3.15	47.3	—	161	363	58.0	90.2	2.5	1 (trace)
G-10	UD	76	76	—	3.58	4.01	26.7	—	136	412	55.0	55.8	1.5	1
F-21	UD	76	73	—	3.06	2.51	35.8	—	185	487	24.1	38.9	1.5	1
E-66	UD	76	76	—	3.59	3.72	32.6	—	175	529	35.0	19.3	2.5	2
F-14	UD	76	76	—	2.70	2.19	43.7	—	269	539	72.6	293.2	4.0	1 (trace)
G-11	UD	76	75	—	3.52	3.47	19.4	—	302	570	33.2	36.2	2.5	2
H-76	C	76	76	—	3.31	4.64	—	—	30	91	7.2	5.7	0.3	1 (trace)
H-79	C	76	69	—	3.26	4.28	—	—	94	174	16.9	29.9	0.5	1
H-77	C	76	76	—	2.75	4.25	—	—	84	242	20.2	13.4	0.8	1
H-78	C	76	69	—	2.95	4.34	—	—	102	289	17.3	18.4	0.8	1
G-80	C	76	76	—	2.24	4.14	—	—	127	432	45.7	29.2	1.3	2
G-39	C	76	75	—	2.94	3.75	—	—	174	438	26.1	20.9	1.3	2
G-82	C	76	75	—	2.91	4.98	—	—	189	459	17.0	15.1	1.5	3
G-90	C	76	75	—	3.29	4.83	—	—	221	488	13.3	22.1	1.8	3
G-84	C	76	76	—	3.29	4.08	—	—	222	524	20.0	31.1	2.5	4
G-81	C	76	74	—	3.51	4.11	—	—	232	530	25.5	30.2	1.8	3
G-83	C	76	75	—	3.68	4.45	—	—	230	548	25.3	16.1	2.5	4
G-87	C	76	71	—	3.54	4.53	—	—	231	598	25.4	30.0	2.0	3
G-88	C	76	74	—	2.57	3.61	—	—	293	643	41.0	26.4	2.5	3
Means	TD	11.9			3.62	4.65	35.5	3.2	185	458	28.1	20.9	1.3	—
	UD	—			3.25	3.18	34.3	—	205	483	46.3	88.9	2.4	—
	C	—			3.09	4.31	—	—	172	420	23.2	22.2	1.5	—

* TD, insulin-treated diabetic rabbit; UD, untreated diabetic rabbit; C, untreated non-diabetic control rabbit.

exceptional quantity is omitted, the average serum triglyceride of the untreated diabetic rabbits was about 1.5 gm. per cent or double that of either the diabetic rabbits treated with insulin or the non-diabetic rabbits. It was also observed that the serum of the untreated diabetic rabbits was visibly more lipemic than that of either the treated diabetic or non-diabetic rabbits. In-

deed, the latter two groups manifested about the same intensity of visible lipemia.

In the untreated diabetic group, lesions of grade 1 severity were observed in 4 animals (67 per cent), 2 of which had lesions of minimal degree; lesions of grade 2 severity were found in 2 rabbits (33 per cent). The non-diabetic rabbits manifested lesions of grade 1 severity in 4 (31 per cent), grade 2 in 2 (15 per cent), grade 3 in 5 (38 per cent), and grade 4 in 2 (15 per cent). On the other hand, among the insulin-treated diabetic rabbits, aortic atherosclerosis of grade 1 severity was observed in 2 (20 per cent), of grade 2 in 4 (40 per cent), and of grade 3 in 4 (40 per cent). Thus, while the atherosclerosis of the aorta observed in the untreated diabetic rabbits was of slight degree, that found in the non-diabetic control animals and in the treated diabetic rabbits was considerably more extensive. The difference between the severity of atherosclerosis in the two last groups, while not negligible, was of minor degree.

DISCUSSION

The experiment reaffirms that atherosclerosis of the aorta develops in much less marked degree in rabbits rendered diabetic with alloxan and fed cholesterol than in non-diabetic rabbits fed cholesterol in a similar way. The large content of cholesterol and especially of lipid phosphorus and neutral fat previously observed in the serum of cholesterol-fed diabetic rabbits was observed again. It may be noted that the untreated diabetic animals in the present experiment, unlike those previously reported (1, 2, 10), were not fed additional vegetable oil with the cholesterol-enriched diet.

The development of experimental cholesterol atherosclerosis was inhibited in the diabetic rabbits but not in the diabetic rabbits that were treated with insulin. However, while insulin treatment, which was accompanied by an amelioration of the diabetic state, permitted the development of severe atherosclerosis of the aorta, the lesions did not develop quite as freely, either in size or extent, as in the non-diabetic control animals. At the same time, the control of the diabetic state attained by insulin treatment as judged by the associated decrease in glucose excretion was never perfect. A residual glycosuria persisted in every case. It seems reasonable to suggest that the failure to attain complete control of the diabetic state in the insulin-treated diabetic rabbits accounts for the slight discrepancy in the severity of the atherosclerosis observed in them as compared with the non-diabetic controls. From the experimental data it is concluded that the inhibition of the development of experimental atherosclerosis in rabbits rendered diabetic with alloxan and fed cholesterol is associated with some factor or factors of the diabetic state and is not due to the alloxan *per se* or to its effects on organs or tissues other than the pancreatic islets.

The experiments do not indicate which features of the diabetic state are

responsible for the inhibitory effect. It has been suggested that it may be due to the unusual serum cholesterol/lipid phosphorus ratio and serum neutral fat content of the diabetic rabbits (2), to weight loss or failure to gain weight (11, 12), or to the unusual serum lipoprotein spectrum observed in cholesterol-fed diabetic rabbits (3).

Duff and Payne (2) observed that alloxan-diabetic rabbits fed cholesterol developed a pronounced hyperphospholipidemia and a large concentration of neutral fat in the serum as well as a marked hypercholesterolemia. The serum cholesterol/phospholipid ratio and the serum cholesterol/neutral fat ratio in the diabetic rabbits fed cholesterol were less abnormal in the diabetic than in the non-diabetic cholesterol-fed animals, and they suggested that the inhibitory effect in hypercholesterolemic animals depended upon the maintenance of relatively normal serum lipid ratios. In a subsequent experiment utilizing the surface-active agent tween 80 they obtained evidence that the ratio of serum cholesterol to lipid phosphorus might be closely allied to the inhibitory effect while that between serum cholesterol and neutral fat was not (15). The present data disclosed that the serum cholesterol/lipid phosphorus ratio was about normal in the diabetic rabbits, but was considerably larger than normal in the insulin-treated diabetic rabbits and even larger in the non-diabetic rabbits. Therefore, there was a parallelism between the inhibition of the development of atherosclerosis and the relative normality of the serum cholesterol/lipid phosphorus ratio. A less regular relationship was found between the ratio of serum cholesterol/neutral fat and the development of atherosclerosis. The diabetic rabbits tended to have even lower ratios than normal while the insulin-treated rabbits had slightly higher ratios than the non-diabetic cholesterol-fed control rabbits. The data are compatible with the hypothesis of Duff and Payne (2) but they do not bring any new evidence of importance to bear on the problem.

The data disclose that the untreated diabetic animals generally lost a small amount of weight while the treated diabetic and non-diabetic control rabbits generally gained about 1 kilo. The control rabbits gained slightly more weight than the insulin-treated diabetic animals. Therefore, there was a parallelism between weight loss or failure to gain weight and the inhibition of the development of atherosclerosis in the diabetic rabbits. However, it is our opinion that this correlation merely represents a concommitant variation and is not a causal relationship. In their original paper, Duff and McMillan (1) considered the relationship of body weight and the inhibition of atherosclerosis in alloxan-diabetic rabbits. They concluded by the analysis of arrayed data that the inhibitory effect was not due to variations in body weight. Subsequently, Firstbrook reported an experiment (11, 12) in which cholesterol-fed rabbits were deliberately undernourished so that they did not gain weight normally. It was concluded that this experiment demonstrated that caloric restriction with con-

sequent weight loss or failure to gain weight inhibited the development of atherosclerosis (13). It was suggested (11, 12) that the weight loss associated with the diabetic state was responsible for the inhibitory effect observed by Duff and McMillan (1). Recently we have restudied the hypothesis that weight loss inhibits the development of experimental cholesterol atherosclerosis in undernourished rabbits (14). From the results of this study it was concluded that weight loss or the failure to gain weight in groups of undernourished cholesterol-fed rabbits does not cause them to develop less atherosclerosis than groups of adequately nourished control animals fed the same daily dose of cholesterol, although it does result in a more pronounced hypercholesterolemia in the smaller, undernourished rabbits than in the larger control animals.

It is apparent that insulin treatment has greatly ameliorated the diabetic state in all of its aspects that were observed in this experiment. The excretion of large amounts of sugar in the urine, the weight loss, the hypercholesterolemia, hyperphospholipidemia, and excess of fat in the serum that were observed in the untreated, cholesterol-fed, diabetic rabbits were all reduced in amount by insulin therapy so that the animals were more nearly like the non-diabetic cholesterol-fed rabbits in these respects. At the same time the development of aortic atherosclerosis that attended cholesterol feeding in the insulin-treated diabetic rabbits also approximated that found in the non-diabetic control animals. The experiment does not offer evidence that permits the analytical association of the observed individual components of the diabetic state with the inhibition of the development of experimental aortic atherosclerosis in the alloxan-diabetic rabbit fed cholesterol.

Together with the observations of Cook and associates (4) the experimental data establish unequivocally that the inhibitory effect on the development of experimental cholesterol atherosclerosis observed in alloxan-diabetic rabbits is due to the action of alloxan on the pancreatic islets with a resulting diabetic state and not due to any other possible action of the injected alloxan.

SUMMARY

Experiments were undertaken to ascertain whether the previously demonstrated inhibition of the development of experimental aortic atherosclerosis in alloxan-diabetic rabbits fed cholesterol was due to the injection of alloxan *per se* or to the existence of the diabetic state produced by alloxan.

It was established that, by treating the diabetic state with insulin, the diabetic state could be ameliorated and the inhibitory effect obviated. It was therefore concluded that the inhibitory phenomenon was not due to the injection of alloxan *per se* but that it was associated with one or more factors that characterize the alloxan diabetic state in the rabbit and that are reversible by insulin therapy.

In the course of the experiment it was demonstrated that the inhibitory ef-

fect was apparent in cholesterol-fed diabetic rabbits whether or not their diet was supplemented with vegetable oil. The previously reported metabolic abnormalities of the diabetic animals were confirmed. It was established that suitable treatment of the cholesterol-fed diabetic animals with insulin would bring all the metabolic aberrations, including those of the serum lipids, into reasonably close correspondence with those observed in non-diabetic rabbits fed cholesterol.

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