THE MECHANISM OF ALLOXAN PROTECTION IN EXPERIMENTAL ATHEROSCLEROSIS

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(Received for publication, September 4, 1953)

A decreased severity of atherosclerosis in cholesterol-fed alloxan-diabetic rabbits, despite marked lipemia and hypercholesterolemia, was reported by Duff and McMillan (1) and Duff and Payne (2). These results were confirmed by Pierce (3) who found a negative correlation between serum cholesterol levels and the degree of atherosclerosis in such animals. Simultaneous measurements of ultracentrifugal patterns of serum showed high concentrations of molecules above S_f 100, indicating a metabolic block in the conversion of lipoproteins to lower S_f classes in the alloxan-diabetic rabbit.

The relative freedom of diabetic cholesterol-fed rabbits from atherosclerosis was considered to be associated with the diabetic state, and Duff and McMillan (1) tentatively concluded that "alloxan-recovered" animals provided a suitable control for the effects of alloxan *per se*.

Although alloxan alters the cytology of the anterior pituitary and adrenal glands (4) and of the kidney and liver (5), it is generally agreed that its diabetogenic action is due to a rapid and selective action on the pancreatic islets of Langerhans. Leech and Bailey (6) first reported that alloxan, after addition to blood *in vitro* or intravenous injection into rabbits, disappears from the blood completely in 5 minutes or less. These results were confirmed by Karrer *et al.* (7). Gomori and Goldner (8) occluded portions of the pancreas from the circulation and found that the diabetogenic potency of alloxan is rapidly lost after injection.

The present studies were designed to determine whether alloxan-injected rabbits in which diabetogenic action of alloxan is prevented, retain resistance to atherosclerosis. The procedure involved a comparison of the effects of cholesterol feeding in normal rabbits, in rabbits previously made diabetic with alloxan, and in rabbits in which alloxan had been administered during temporary occlusion of the pancreatic circulation.

Methods and Materials

Three groups of male rabbits, obtained from Ora Robinson Bunny Ranch, Osceola, Illinois, were the experimental animals. Fifteen rabbits received intravenous injections of

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150 mg. per kg. of alloxan monohydrate (Eastman Kodak Co.), followed immediately by a subcutaneous dose of 10 cc. of 20 per cent glucose solution. The glucose was repeated in the same dose 3 additional times at 2 hour intervals to counteract the acute and transient hypoglycemia which follows the administration of alloxan. Five surviving rabbits showed blood glucose levels greater than 400 mg. per cent and were considered to be in a diabetic state. This group was designated as the alloxan-diabetic group.

The second group consisted of rabbits which survived following administration of alloxan during temporary exclusion of the pancreas from the circulation. These animals were anesthetized with 30 mg. per kg. of sodium pentobarbital given intravenously. Ether was used as a supplementary anesthetic when necessary. The abdominal wall was opened aseptically and the peritoneal cavity exposed. The celiac artery and other arterial branches supplying the pancreas were clamped, and 150 mg. per kg. of alloxan were injected intravenously into a marginal ear vein. After an interval of 5 minutes the clamps were removed and the incision closed. Glucose was administered in a manner identical to that described for the alloxan-diabetic group. Seven out of 21 rabbits survived this procedure and showed normal blood glucose levels. These seven rabbits were considered to be non-diabetic and are referred to as the pancreas-protected group.

The third group consisted of ten control rabbits which received no treatment prior to cholesterol feeding.

Several weeks were allowed to lapse after the alloxan injections so that the transient lipemia and hypercholesterolemia following alloxan would subside before the rabbits were put on the cholesterol diet. Control samples were then obtained from all rabbits. Each animal was housed in an individual cage and given water and food *ad libitum*. The diet consisted of Rockland rabbit ration modified by the addition of cholesterol in a 1 per cent concentration. Each animal was weighed weekly and its food consumption recorded twice per week. Serum lipoproteins and total cholesterol concentrations were determined at monthly intervals during the experiment. Blood glucose levels were measured on heparinized blood samples at 2 week intervals.

Lipoprotein analyses were made according to the ultracentrifugal flotation method of Gofman *et al.* (9) modified by subdivision of the S_f 10–15 and S_f 16–30 classes (10). Blood glucose was determined by the procedure described by Burns (11). Serum total cholesterol was determined by the Foldes and Wilson (12) modification of the Schoenheimer and Sperry (13) method.

All animals were sacrificed at the end of 56 days of the high cholesterol diet. The degree of atherosclerosis was graded into 5 classes (0–4) according to the area of aortic intima involved by the lesions. The lipoprotein and total cholesterol concentrations were averaged for each rabbit over the period of cholesterol feeding and the group means calculated. The degree of atherosclerosis was compared with these mean values, since the formation of lesions is presumably dependent upon the concentrations of lipoproteins throughout the entire period of exposure, rather than at any given time.

The statistical significances of differences between group means were tested by Student's t test (14).

RESULTS

The individual data are presented in Table I, while the group means are summarized in Fig. 1.

The ages and initial weights of the rabbits used for each group were similar. The initial levels of serum cholesterol and lipoprotein did not differ significantly.

TABLE 1

Level of Lipoproteins and Cholesterol in Three Groups of Rabbits Fed Cholesterol

Each value represents the mean of two determinations performed at 4 and 8 weeks during the 8 week period of cholesterol feeding. The degree of atherosclerosis found at the end of the 8th week is also included.

Group	Rabbit No.	Age	Weight		Food consumption per day	Glucose per 100 cc. blood	Lipoprotein concentration per 100 cc. serum			Total cholesterol per 100 cc. serum	Degree of atherosclerosis
			Initial	Ratio of final to initial			*Sf* 5-9	*Sf* 10-15	*Sf* 16-30		
		days	*kg.*		*gm./kg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
Alloxan-diabetic	1	193	3.60	1.07	43	665	295	260	860	1493	2
	2	153	2.79	1.08	53	437	273	238	1631	2140	2-3
	3	153	2.29	1.00	80	600	395	230	1300	2882	0
	4	137	2.84	1.13	79	527	600	190	1400	5112	4
	5	136	3.09	1.17	59	496	265	140	1100	3234	4
Mean ± S.D.*.....		154 ± 23	2.92 ±0.48	1.09 ±0.07	63 ± 16	545 ± 89	366 ±141	212 ±47	1258 ±293	2972 ± 1373	2.5 ± 1.6
Pancreas-protected	6	162	2.91	1.13	39	122	165	180	805	1449	3
	7	180	2.99	1.17	37	122	255	255	920	1403	2-3
	8	148	3.03	1.18	33	131	124	82	295	811	4
	9	144	3.31	1.29	43	131	255	255	880	1365	4
	10	134	3.23	1.14	34	132	87	135	525	1210	4
	11	134	2.42	1.29	35	123	84	125	405	1309	3
	12	134	2.79	1.19	34	115	207	292	788	2096	3
Mean ± S.D.*.....		148 ± 17	2.95 ±0.29	1.20 ±0.07	36 ± 3	125 ± 6	168 ±73	189 ±79	660 ±249	1378 ± 381	3.4 ± 0.6
Controls	13	131	3.45	1.14	35	129	155	200	1100	1484	1-2
	14	148	3.08	1.12	32	134	119	275	520	1236	4
	15	131	3.21	1.17	34	129	370	330	1250	2878	3
	16	131	3.49	1.12	34	123	235	340	855	1651	4
	17	126	3.05	1.16	36	134	355	430	1300	2423	4
	18	126	3.24	1.08	30	128	250	280	990	1561	2
	19	126	3.02	1.20	34	127	215	170	575	1390	1
	20	126	3.08	1.08	35	134	109	168	565	1438	2
	21	126	3.54	1.11	38	131	130	215	470	1395	3-4
	22	131	3.20	1.19	35	124	130	275	650	1822	4
Mean ± S.D.*.....		130 ± 6	3.24 ±0.19	1.14 ±0.42	34 ± 2	129 ± 4	207 ±95	268 ±82	828 ±313	1728 ± 522	2.9 ± 1.3

* Standard deviation = $\sqrt{\frac{\Sigma(x^2)}{(n-1)}}$ in which $\Sigma(x^2)$ represents the summation of the squares of the individual deviations from the mean and n the number of determinations.

The weight gain during the experiment, as indicated by the ratio of the final to initial weight, was slightly less for the alloxan-diabetic group and slightly greater for the pancreas-protected group than for the controls. These differences were not statistically significant—a result of potential importance since it has been shown that weight loss can inhibit the development of atherosclerosis (15).

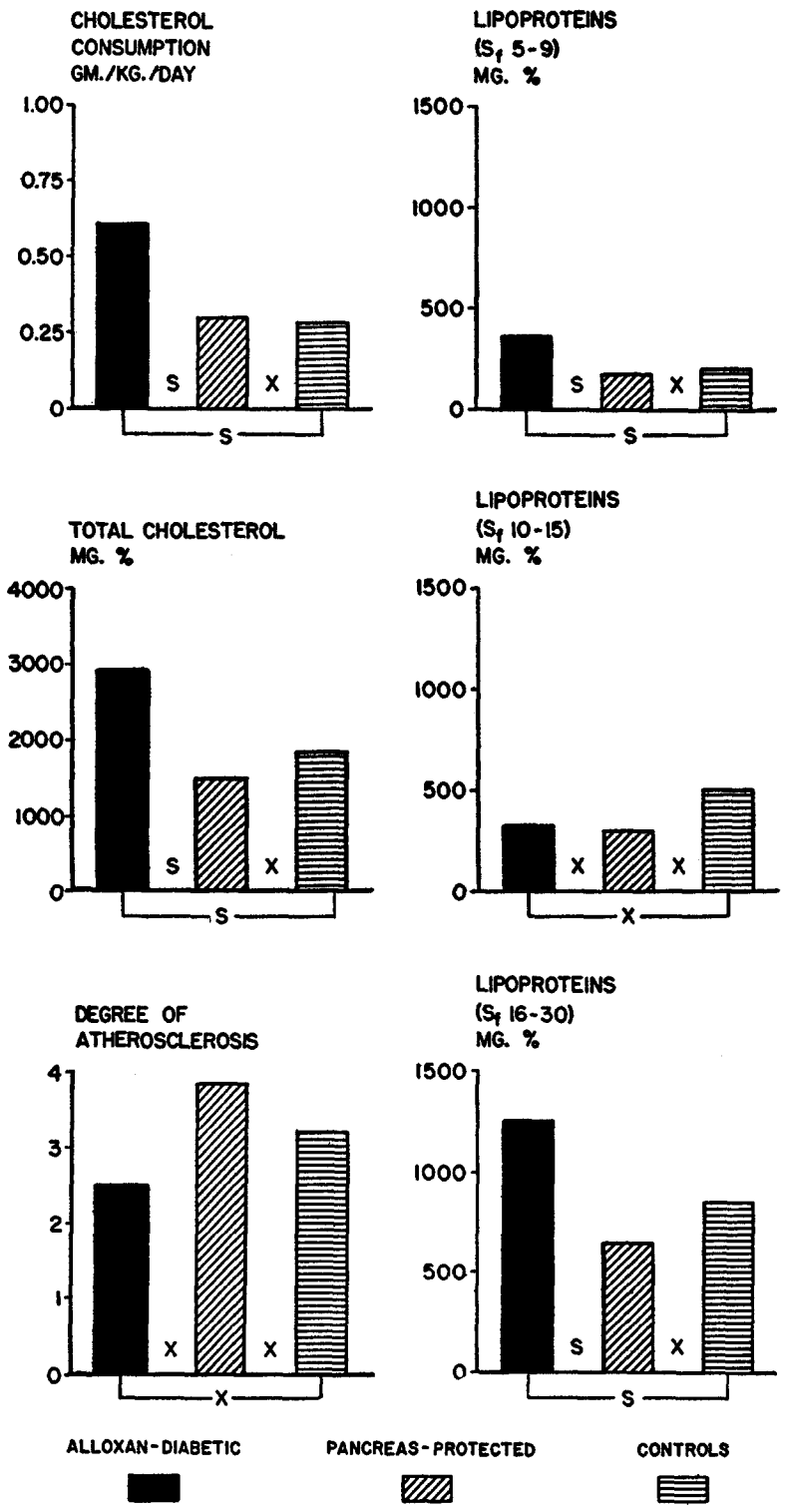

FIG. 1. Summary of the mean cholesterol consumption, serum cholesterol, and lipoprotein levels and degree of atherosclerosis for three groups of rabbits. Large *S* between bars indicates that the difference between the two corresponding means is significant at $P < 0.05$. An *X* between bars indicates no significant difference.

The alloxan-diabetic animals showed a highly significant increase (about double) in cholesterol and food consumption over the pancreas-protected and the control groups. No significant difference in consumption was demonstrated between the latter two groups. The serum cholesterol and lipoprotein (S_f 5–9 and S_f 16–30) levels of the alloxan-diabetic animals were also significantly higher than those of the controls. No significant differences in serum cholesterol or lipoprotein levels were observed between the pancreas-protected and control groups.

The degree of atherosclerosis did not differ significantly among any of the groups. It averaged 2.5 for the alloxan-diabetic animals and 3.4 and 2.9 for the pancreas-protected and control groups respectively.

DISCUSSION

These observations suggest that the exaggerated hypercholesterolemia and lipoproteinemia of the alloxan-diabetic animals resulted from a greatly increased dietary intake, consequent upon the diabetic state.

Results previously reported (10) showed that the degree of atherosclerosis produced in normal rabbits by cholesterol feeding was directly and linearly related to the cholesterol intake and to the serum cholesterol and lipoprotein concentrations. The failure of the alloxan-diabetic group to develop a greater degree of atherosclerosis than the controls, despite provocatively higher cholesterol intake, hypercholesterolemia, and lipoproteinemia, suggests that the atherogenic mechanism was retarded in this group, although not inhibited.

The failure of alloxan to lessen the degree of atherosclerosis relative to the untreated controls when its diabetogenic effect was prevented, indicates that its protective action is associated with the particular diabetic state produced by its action on the pancreas, and not by an action on extra-pancreatic tissues.

SUMMARY

Experiments were performed to compare the effects of cholesterol feeding in (*a*) control rabbits, (*b*) alloxan-diabetic rabbits, and (*c*) rabbits injected with alloxan while the pancreas was temporarily occluded from the circulation.

The alloxan-diabetic rabbits consumed significantly higher quantities of cholesterol and food and had serum cholesterol and lipoprotein (S_f 5–9 and S_f 16–30) concentrations significantly increased over the control levels. They failed to show a commensurate increase in the degree of atherosclerosis.

Rabbits in which the diabetogenic action of alloxan was prevented by temporary occlusion of the pancreas from the circulation during its administration developed grades of hypercholesterolemia, hyperlipoproteinemia, and atherosclerosis not significantly different from the controls.

The results are interpreted as indicating that the effects of alloxan on tissues other than the pancreas do not protect against experimental atherosclerosis produced by cholesterol feeding.

Acknowledgment is made to Dr. Roger Ray, Department of Chemistry, University of Illinois, for the ultracentrifugal analyses reported in this study.

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