THE EFFECT OF CORTISONE ON THE SERUM LIPIDS AND ON THE DEVELOPMENT OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS IN THE RABBIT*

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The importance of the serum lipids and of their interrelationships in the genesis of atherosclerosis has been repeatedly emphasized. In young patients with myocardial infarction (1, 2) and in those diseases that are associated with hyperlipemia and excessive atherosclerosis, such as diabetes mellitus, hypothyroidism and hypercholesterolemic xanthomatosis, hypercholesterolemia, and a decreased phospholipid to cholesterol ratio have been observed frequently.

Experimentally, the expected development of atherosclerosis in rabbits fed cholesterol has been inhibited by rendering them diabetic with alloxan (3–5). The failure to develop atherosclerosis occurred in the presence of marked elevations of the content of cholesterol in the serum together with a proportionate elevation of the serum phospholipids and neutral fat. A similar increase in serum cholesterol and phospholipids has been obtained by the intravenous injection of the detergents tween 80 and triton A 20 into cholesterol-fed rabbits (6–8); there was an inhibition of the expected development of atherosclerosis.

Many clinical observations and experimental studies have revealed that cortisone produces well defined changes in the serum lipids. In the sera of patients receiving prolonged cortisone therapy, Adlersberg and his associates (9–12) noted that there was a consistent elevation of the total serum cholesterol, esterified cholesterol, and phospholipids. In view of his findings and the common association of hypercholesterolemia with atherosclerosis, Adlersberg suggested that atherosclerosis might develop prematurely in patients treated with cortisone. Indeed, Etheridge and Hoch-Ligeti (13) have reported an increase in the severity of atheroclerosis in young patients treated with cortisone.

Kobernick and More (14), while studying the effect of cortisone on tissue lesions produced by foreign serum proteins in rabbits, observed changes in the blood sugar and serum lipids that resembled those of alloxan diabetes and were accompanied by glycogen infiltration of the islets of Langerhans. They reported an increase in the serum lipid phosphorus, total and free cholesterol, and fatty acids of neutral fat which presented a pattern similar to that described by Payne and Duff (5) in the alloxan

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diabetic rabbit. Since alloxan diabetes inhibits the development of cholesterol atherosclerosis in the rabbit, it seemed appropriate to consider whether cortisone might also inhibit the development of experimental cholesterol atherosclerosis. Meanwhile, Cook, Roger, Davison, Feldstein, Calvin, and Green (15) have reported that cortisone does not affect the development of atherosclerosis in the rabbit fed cholesterol, but Oppenheim and Bruger (16) found that cortisone has an inhibitory action on the development of lesions in such animals.

The following experimental study provides further data on the effect of the administration of cortisone on the serum lipids and the development of experimental cholesterol atherosclerosis in the rabbit.

EXPERIMENTAL

Materials and Methods

Experimental Animals.—Seventy-two New Zealand white rabbits of pedigreed stock were employed. These comprised 9 sets of quadruplets and 12 sets of triplets. Each individual set of litter mates was of the same sex; both sexes were about equally represented in the experiment. The rabbits varied in age from 10 to 16 weeks and weighed from 1,640 to 3,650 gm. at the beginning of the experiment. The majority of them weighed from 2,200 to 2,800 gm.

The 9 sets of quadruplets were divided into four groups that received respectively: (1) cholesterol and cortisone vehicle; (2) cholesterol and cortisone; (3) cortisone; (4) cortisone vehicle. One of the quadruplets was compared with a litter mate of the same sex in each of the other experimental groups. The triplets were grouped in a corresponding manner, but the group 4 rabbits that received only cortisone vehicle were not represented.

Experimental Treatment.—The duration of the experiment was about 2 months. Cholesterol was administered by coating 35 gm. of commercial rabbit food pellets with 0.75 gm. of cholesterol dissolved in ether. The ether was allowed to evaporate leaving a film of cholesterol adherent to the food. This amount of cholesterolized food was given daily in the morning to each rabbit of groups 1 and 2 for 6 days each week. When this ration was consumed it was supplemented each afternoon with 65 gm. of food that did not contain cholesterol. 100 gm. of food pellets without added cholesterol was given daily to those rabbits that were not to receive cholesterol and to all rabbits on the 7th day of each week. The cholesterol consumption was measured daily by weighing any uneaten portion of the cholesterolized food ration of each animal.

Cortisone or cortisone vehicle was injected intramuscularly from a tuberculin syringe. The animals of groups 2 and 3 received daily 3 mg. of cortisone per kilo of body weight for the first 3 weeks of the experiment, 2 mg./kilo for the succeeding 3 days, 1.5 mg./kilo for 3 days, 0.5 mg./kilo for 11 days, 1.0 mg./kilo for 2 days, and 1.5 mg./kilo for the terminal period of up to 3 weeks. These variations in dosage were dictated by the general state of the surviving animals. 3 mg. of cortisone per kilo of body weight was not well tolerated by the rabbits after some 3 weeks of treatment and several rabbits died. Indeed, if the dose of cortisone had not been reduced there would have been no surviving rabbits within a short period of time.

Metabolic Observations.—All rabbits were weighed each week. Determinations of the blood sugar (17) were made weekly on all rabbits. Samples of blood for chemical estimations of the serum-free and total cholesterol (18), lipid phosphorus (19), and serum total fatty acids (20) were taken on all rabbits every 2 weeks. Fatty acids of serum neutral fat were calculated from these data (21). Extracts of sera were stored for subsequent analysis since this was not feasible during the course of the experiment.

Assessment of Atherosclerosis.—Surviving litter mates were sacrificed and all animals were autopsied. The aorta was removed, opened and the severity of atherosclerotic involvement was graded as normal or grade 0; trace; slight or grade +; and moderate or grade ++. The size and distribution of the lesions were then recorded on semischematic diagrams. The adventitia of the fresh, unfixed aorta was dissected from the media and intima and discarded. The remaining aortic media and intima of each individual animal were extracted with hot ethyl alcohol in a continuous extraction apparatus for 6 hours and were subsequently extracted with ethyl ether at room temperature for 18 hours. The alcohol and ether extracts were combined. An aliquot of the extract was evaporated almost to dryness, redissolved in acetone-alcohol, and its cholesterol content was determined by the same method used to determine the serum cholesterol. The results were expressed as milligrams of cholesterol content per aorta.

RESULTS

Experimental Mortality.—The mortality among the rabbits of groups 2 and 3 receiving cortisone was severe. 14 of 21 rabbits receiving only cortisone died: deaths occurred on days 13, 24, 26, 26, 39, 40, 41, 45, 49, 54, 55, 55, 57, and 57 after the beginning of the experiment. 13 of 21 cholesterol-fed rabbits that also received cortisone died: deaths occurred on days 21, 24, 24, 24, 25, 29, 29, 29, 41, 44, 46, 55, and 57 of the experiment. 2 of 21 rabbits of group 1 that received cholesterol and cortisone vehicle died on days 22 and 55 respectively. None of the 9 rabbits of group 4 that received only cortisone vehicle died.

Because of the experimental mortality, only certain animals could be usefully considered. These were 7 sets of quadruplets, 4 sets of triplets, and 1 pair of twins. Thus, the experimental data reported below were derived from 12 rabbits that were fed cholesterol, 12 of their litter mates fed cholesterol and treated with cortisone, 11 of their litter mates treated with cortisone, and 7 of their litter mates that received cortisone vehicle only. These animals comprised all those that died on or after the 44th day of the experiment together with the litter mates that were with them.

Sugar Content of the Blood.—Blood sugar levels determined weekly throughout the experiment varied only slightly. The mean of the values for either group 1 or group 4 at any one time was not less than 101 nor more than 123 mg. per cent. The cortisone-treated groups, however, possessed mean levels of approximately 140 to 160 mg. per cent while being treated daily with 1.5 to 3.0 mg. of cortisone per kilo of body weight. When the cortisone dosage was less than 1.5 mg. per kilo the mean blood sugar values of groups 2 and 3 were within normal limits. On only a few occasions was the content of sugar in the blood observed to exceed 200 mg. per cent in individual rabbits. The highest value detected was 398 mg. per cent.

Severity of Cholesterol Atherosclerosis.—The detailed data derived from the 12 rabbits fed cholesterol and their 12 litter mates that received both cholesterol and cortisone are summarized in Table I in which pairs of rabbits are compared. Those rabbits treated with cortisone consumed somewhat less cholesterol

TABLE I

Effect of Cortisone on Serum Lipids and Aortic Atherosclerosis in Litter Mate Rabbits Fed Cholesterol

Rabbit No.	Sex	Duration of experiment	Cholesterol consumed	Cortisone injected	Initial weight	Final weight	Change of initial weight	Average serum lipid content					Grade of aortic atherosclerosis	Cholesterol content of aorta
								Cholesterol			Lipid phosphorus	Fatty acids of neutral fat		
								Free	Total	Ester				
		days	*gm.*	*mg.*	*gm.*	*gm.*	*per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*m.eq./liter*		*mg.*
V-71	F	44	27	—	2293	2717	+18.5	—	—	—	—	—	+	1.75
V-72	F	44	23	230	2810	2345	−16.5	—	—	—	—	—	Trace	0.88
V-97	M	45	28	—	2167	2798	+29.1	49.6	232.8	183.2	8.0	4.18	+	1.25
V-98	M	45	27	189	2445	2720	+11.2	92.8	350.0	257.2	11.6	21.62	Trace	0.65
W-5	M	46	27	—	2042	2595	+27.1	242.0	833.5	591.5	18.4	8.59	++	4.75
W-6	M	46	28	186	2205	2460	+11.6	121.0	330.8	209.8	13.3	21.93	+	1.50
W-1	F	49	30	—	1884	2572	+36.5	60.8	176.8	124.6	7.1	5.74	Trace	1.38
W-2	F	49	30	259	2751	2475	−10.0	58.2	194.4	136.2	8.5	19.97	0	1.20
W-9	F	54	33	—	2310	3035	+31.4	114.6	491.5	276.6	10.2	3.11	++	1.43
W-10	F	54	30	210	2420	2482	+2.6	66.4	212.4	146.0	12.2	27.43	0	0.63
V-84	M	55	32	—	2935	3191	+8.7	55.3	201.0	145.8	6.8	2.48	Trace	0.65
V-85	M	55	32	249	2545	2400	−5.7	57.8	149.5	91.8	12.8	18.05	0	0.85
V-67	M	55	32	—	2968	2975	+0.2	95.2	395.2	300.0	11.7	3.10	+	1.75
V-68	M	55	34	304	2810	2690	−4.3	67.0	158.4	91.4	12.3	46.77	0	0.58
V-52	M	55	31	—	2159	1779	−17.6	71.5	372.0	300.5	13.3	7.29	+	2.05
V-53	M	56	31	313	2852	2627	−7.9	95.2	217.3	122.1	12.0	37.05	Trace	0.75
V-88	M	57	34	—	2860	3440	+20.3	85.4	379.2	294.0	6.6	0.73	++	2.28
V-89	M	57	27	219	2450	1985	−19.0	55.0	146.0	91.0	9.0	25.36	Trace	1.13
V-64	M	57	35	—	3368	3720	+10.5	189.7	770.0	580.3	14.5	2.56	+	2.88
V-65	M	57	30	267	2690	2248	−16.4	54.2	199.0	144.8	7.2	18.96	Trace	0.95
V-77	F	60	35	—	2600	2955	+13.7	54.8	168.4	113.6	6.9	2.68	0	0.95
V-79	F	60	37	246	2315	2540	+9.7	68.2	146.8	78.6	10.7	29.77	0	—
W-29	F	61	32	—	3650	3455	−5.3	129.5	482.7	353.2	12.4	8.82	+	2.50
W-30	F	60	27	278	2888	2435	−15.7	29.2	83.7	54.5	6.9	11.64	0	1.25

than their control litter mates, but this difference was usually slight. On the average the rabbits of group 1 each ate a total of 31.3 gm. of cholesterol while

those of group 2 ate 29.6 gm. In 11 of the pairs of animals the severity of aortic atherosclerosis was judged by visual inspection to be less in the cortisone-treated rabbits; neither of the 12th pair of animals had macroscopically visible atherosclerotic lesions. In 9 pairs of rabbits the cholesterol content of the aorta was

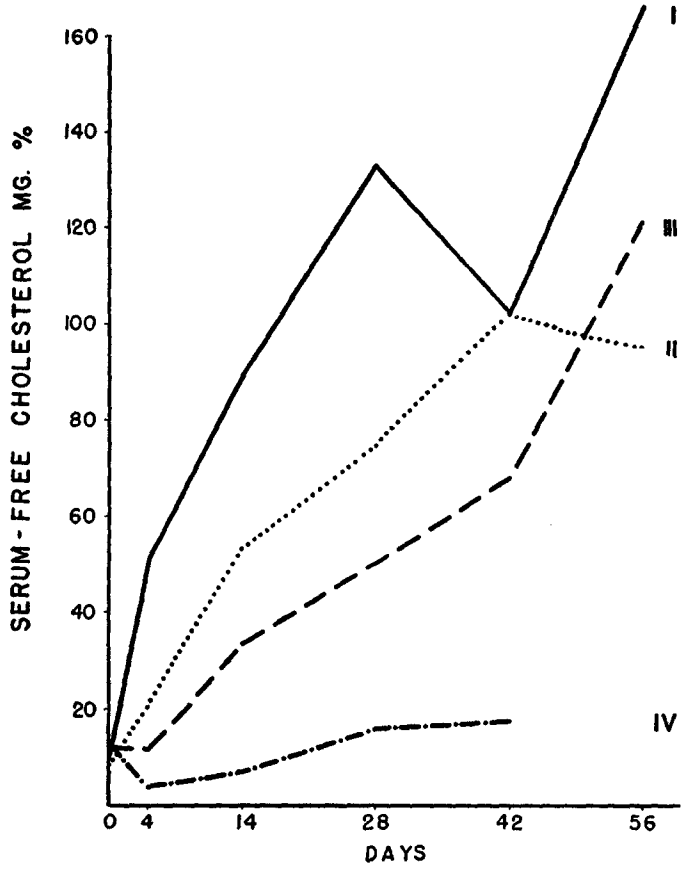

FIG. 1. Graph of serum-free cholesterol plotted against days of experimental treatment. Line I was derived from rabbits fed cholesterol, line II from rabbits fed cholesterol and treated with cortisone, III from rabbits treated with cortisone, and IV from normal control animals.

definitely less by chemical determination in the cortisone-treated litter mates; in 2 pairs the content was about the same; in the remaining pair one of the samples was lost. In all cases the amount of atherosclerosis that developed was small. The mean aortic cholesterol content of the group 1 animals that received cholesterol and cortisone vehicle was 1.97 mg.; that of the rabbits receiving both cortisone and cholesterol was 0.94 mg. The probability of this difference is about 0.01.

Variations in Body Weight.—There was a considerable variation in body weight among the various animals at the beginning of the experiment. This was true not only of unrelated rabbits but also of litter mates. The average initial weights of the rabbits of groups 1 and 2 were 2,603 and 2,598 gm. re-

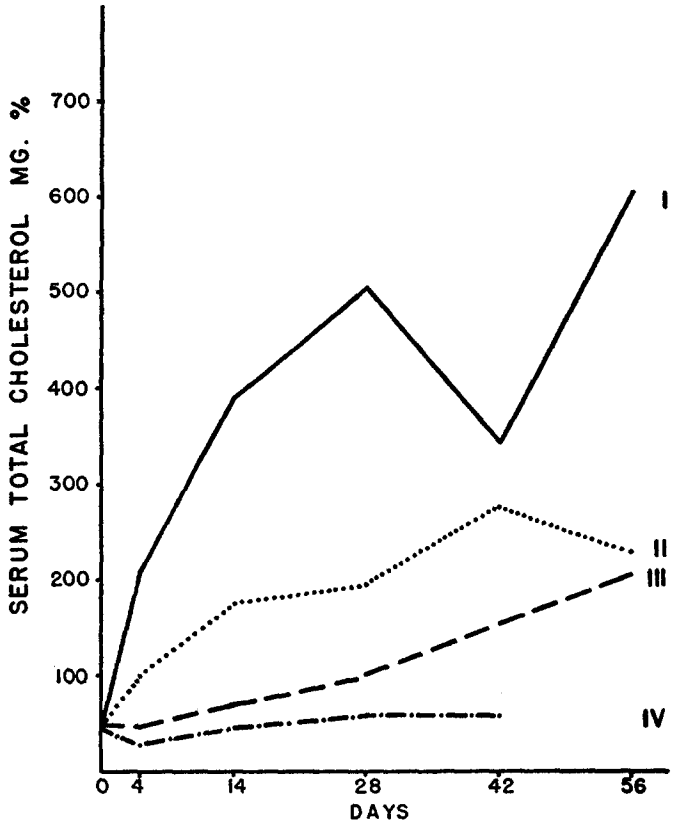

FIG. 2. Graph of serum total cholesterol plotted against days of experimental treatment. Line I was derived from rabbits fed cholesterol, line II from rabbits fed cholesterol and treated with cortisone, III from rabbits treated with cortisone, and IV from normal control animals.

spectively. During the course of the experiment most of the rabbits of group 1 gained weight while those of group 2 lost weight. The mean final weight of the group 1 rabbits was 2,936 gm., that of the group 2 animals was 2,461 gm. The probability of this difference is about 0.02. There were some exceptions to these findings: rabbits W-5 and W-6, and V-97 and V-98 all gained weight although the cortisone-treated rabbits did not gain as much as their litter mates; contrarily, rabbits V-52 and V-53 both lost weight, the cortisone-treated rabbit losing less weight than its litter mate which did not thrive.

Variations in the Content of Lipids in the Serum.—The average contents of lipids in the serum of the individual rabbits of groups 1 and 2 that survived the entire experiment from day 4 to its termination have been tabulated in Table I. It should be noted that the content of lipids in the serum of the surviving animals, in particular those of groups 2 and 3 which were treated with

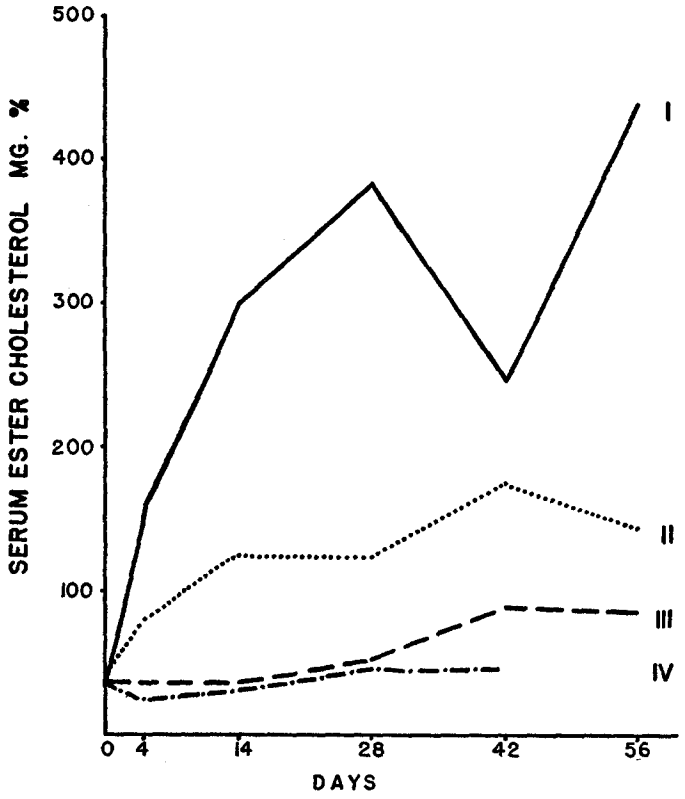

FIG. 3. Graph of serum-esterified cholesterol plotted against days of experimental treatment. Line I was derived from rabbits fed cholesterol, line II from rabbits fed cholesterol and treated with cortisone, III from rabbits treated with cortisone, and IV from normal control animals.

cortisone and suffered a heavy mortality, did not differ materially from the serum lipid content observed at the same times in the animals that died during the experiment. Among the survivors it was observed that in 8 of the pairs of rabbits the litter mate treated with cortisone had considerably lower serum cholesterol levels, in 2 pairs the difference was slight, in 1 pair the cortisone-treated rabbit had an appreciably higher content of cholesterol in the serum. The analyses on one pair were too few to be included as averages in Table I.

It was the esterified cholesterol fraction that showed the greater difference while the free cholesterol fraction did not differ so much. The serum total fatty acids and fatty acids of neutral fat of the cortisone-treated rabbits were always greatly increased over those of their group 1 litter mates. The average lipid

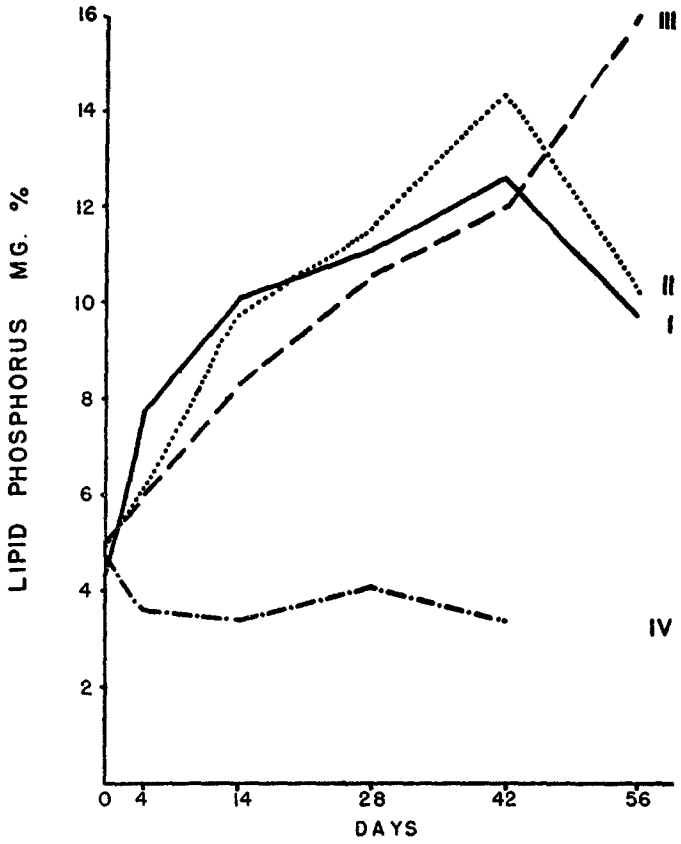

FIG. 4. Graph of serum lipid phosphorus plotted against days of experimental treatment. Line I was derived from rabbits fed cholesterol, line II from rabbits fed cholesterol and treated with cortisone, III from rabbits treated with cortisone, and IV from normal control animals.

phosphorus content of the serum, while variably elevated, was essentially the same for the two groups of rabbits.

The experimental differences in the serum lipids are shown in greater detail in Figs. 1 to 5 in which the mean quantities of serum lipids for groups 1, 2, 3, and 4 are plotted at biweekly intervals during the course of the experiment. The results are noteworthy not only for the differences that are shown, but for their constancy. The differences noted in Table I between groups 1 and 2 are observed throughout the course of the experiment. It is seen that the rabbits

of group 3 that received only cortisone attained a content of neutral fat in the serum as great as that found in their litter mates of group 2 that received both cortisone and cholesterol. Moreover, they had amounts of lipid phosphorus in the serum as great as those of the rabbits that received both cortisone and cholesterol or that had only cholesterol. In addition, the content of serum

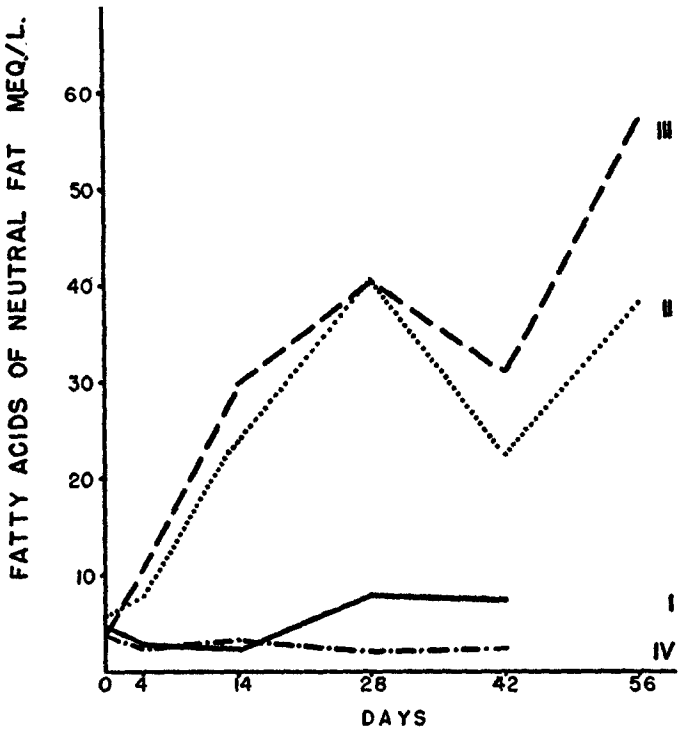

FIG. 5. Graph of serum fatty acids of neutral fat plotted against days of experimental treatment. Line I was derived from rabbits fed cholesterol, line II from rabbits fed cholestero and treated with cortisone, III from rabbits treated with cortisone, and IV from normal control animals.

cholesterol, especially free cholesterol in the rabbits of group 3 gradually increased during the course of treatment with cortisone.

The ratio of the serum total cholesterol to the lipid phosphorus was elevated 2 to 4 times in the cholesterol-fed rabbits of group 1. It was increased by about 1.5 to 2 times in the cortisone-treated cholesterol-fed animals of group 2 and remained within normal limits in the rabbits of group 3 that received cortisone only.

DISCUSSION

The effect on the serum lipids of the repeated daily administration of moderate and large doses of cortisone to normal and cholesterol-fed rabbits was

definite and consistent both in the rabbits that died early in the experiment because of cortisone overdosage and in those that survived because of a reduction in the amount of cortisone administered.

There occurred a moderate elevation of the free and esterified cholesterol fractions in the normal rabbit treated with cortisone. The free cholesterol was elevated relatively more than the esterified fraction. In the cholesterol-fed rabbit the expected elevation of the serum cholesterol attributable to the cholesterol feeding was decreased by cortisone treatment until the free cholesterol was only about 70 per cent of the expected value and the ester fraction was only 40 per cent of the expected amount.

The influence of cortisone on cholesterol metabolism presents a curious paradox. On the one hand it promoted a hypercholesterolemia in which the cholesterol was of endogenous origin; on the other hand it depressed the expected development of hypercholesterolemia due to exogenous cholesterol. In the former case it elevated the free cholesterol relatively more than the ester; in the latter case it depressed the free cholesterol less than the ester. It was apparent that the unesterified cholesterol was more labile than the esterified fraction was. There is no ready explanation of these apparently anomalous effects. Elevation of the serum cholesterol in normal animals can be caused by agents such as tween 80 or triton A 20 or the diabetic state caused by alloxan (3–8), but they do not depress the hypercholesterolemia resulting from cholesterol feeding. Similarly, treatment with thyroid hormone or iodides (22–25) will reduce the hypercholesterolemia of cholesterol feeding but it will not elevate the serum cholesterol of normal rabbits that are not fed cholesterol. The effect of cortisone on the serum cholesterol is singular and its mechanism or mediation remains obscure.

Cortisone caused a moderate elevation of the serum lipid phosphorus. In normal rabbits this elevation was sufficient to maintain a normal total cholesterol/lipid phosphorus ratio. When the rabbits received either 0.75 gm. of cholesterol or both cortisone and cholesterol daily, the serum lipid phosphorus levels were also elevated to almost exactly the same amount as that in the rabbits receiving only cortisone. In the present experiment therefore, the administration of cortisone duplicated the effect of cholesterol feeding, but cholesterol and cortisone together did not have a cumulative effect. This latter fact cannot be explained by the assumption that the serum phospholipids had attained the maximum possible levels since, in our experience, much larger amounts have been found in the serum of rabbits injected with tween 80 (8) or rendered diabetic with alloxan and fed cholesterol (5). It is of interest to note that there is evidence that cholesterol feeding and the alloxan-diabetic state, each of which is capable of causing a moderate elevation in the serum lipid phosphorus levels, may have a cumulative effect when combined. This evidence, while very strong, is not absolute because a small percentage of

diabetic rabbits will have serum lipid phosphorus levels as high as those of cholesterol-fed, diabetic animals (26). The coincidence of the lipid phosphorus levels in the rabbits of groups 1, 2, and 3 is probably fortuitous: it is presumed that a larger dose of cholesterol together with vegetable oil would have increased the serum phospholipids of groups 1 and 2. Whether they would have increased by equal amounts can only be conjectured.

Inasmuch as the average levels of total cholesterolemia attained by the animals of groups 1, 2, and 3 were different while levels of phospholipidemia were the same, the total cholesterol/phospholipid ratios varied among the three groups. It was normal in the animals receiving only cortisone, increased in those receiving both cholesterol and cortisone, and grossly increased in the rabbits that received only cholesterol.

The content of triglyceride in the serum of the cholesterol-fed rabbits increased approximately two times: that of the cortisone-treated rabbits and of the cholesterol-fed, cortisone-treated rabbits was increased 8 to 10 times. The data do not indicate whether cortisone treatment together with cholesterol feeding had a cumulative effect on the serum neutral fat or not. On the contrary, the results show that the animals of group 2 had somewhat less serum-neutral fat than those of group 3, but the data are meager and are not an adequate basis for speculation. The great elevation in the serum triglycerides of the cortisone-treated rabbits is reminiscent of that seen in alloxan diabetes with or without cholesterol feeding. It does not occur with the intravenous injection of tween 80.

Oppenheim and Bruger (16) have reported an experiment similar to the present one. However, the results of their analyses of the serum lipids were not the same as ours. They gave 5 normal rabbits 10 mg. of cortisone three times weekly for 10 weeks. There was no significant effect on the serum cholesterol but there was a definite increase in the serum phospholipids. 13 cholesterol-fed rabbits received cortisone (apparently in the same dosage) and there occurred an accentuation of hypercholesterolemia and a marked depression of the phospholipid/cholesterol ratio. While we cannot explain the different results that occurred in their experiment and in our own, two important differences in experimental method should be mentioned since they may account for the discrepancies. Oppenheim and Bruger seem to have administered approximately the same total dose of cortisone per week as we did, but it was administered 3 times weekly whereas in the present experiment it was divided into 7 doses, one injection being given daily for 7 days of each week. A greater effect may be expected from the latter procedure. Secondly, litter mates of the same sex from pedigreed stock were used in our experiment whereas Oppenheim and Bruger apparently used ordinary, unselected laboratory rabbits.

The amount of atherosclerosis that occurred in the present experiment was

scarcely more than inceptive. However, two different methods of assessing the severity of the aortic lesions, the one morphological and the other chemical, were in substantial agreement and demonstrated that cortisone impeded the development of experimental cholesterol atherosclerosis. While it may be unfortunate that more striking differences between the two groups were not manifest, nevertheless the differences between the rabbits that received cholesterol and their litter mates that received both cortisone and cholesterol were so consistent that there can be little doubt that cortisone inhibited the development of atherosclerosis. This result is in agreement with that of Oppenhiem and Bruger (16) who reported the inhibition of experimental atherosclerosis in 13 cholesterol-fed rabbits treated with cortisone. It does not agree with that of Cook *et al.* (15) who reported that cortisone did not affect the development of experimental cholesterol atherosclerosis in the rabbit. However, because their conclusion is based on a study of 3 cholesterol-fed, cortisone-treated rabbits it must be viewed with some reservation. The result is also in disagreement with that of Stamler, Pick, and Katz (27) who reported an intensification of cholesterol-induced atherosclerosis by cortisone in the chick. However, since cortisone does not affect avian glycocorticoid metabolism in the same manner as that of mammals, the two experiments are not really comparable. The same authors (28) have also studied the action of adrenal steroid compound F in cholesterol-fed chicks and reported an intensified hyperlipemia, hypercholesterolemia, and hyperphospholipidemia. There were no significant changes in aortic atherogenesis. The action of compound F in avian species is glycocorticoid and seems analogous to that of cortisone in mammals. The unconvincing report of Etheridge and Hoch-Ligeti (13) that there is an enhancement of the development of atherosclerosis in children treated with cortisone or ACTH is also contrary to the results reported in the present experiment. It is based on the postmortem visual and microscopic examination of the aortas from 54 patients less than 11 years of age of which 26 had been treated with cortisone or ACTH for varying periods of time.

The mechanism or mechanisms by which cortisone inhibits the development of experimental cholesterol atherosclerosis are obscure. It is possible to postulate several potential impeding mechanisms. The hypercholesterolemia in the cortisone-treated, cholesterol-fed rabbits was significantly less than in their cholesterol-fed litter mates. Their cholesterol/phospholipid ratios were not elevated as much as those of the cholesterol-fed control animals. There may have been an alteration in the serum macromolecular lipoproteins. There may have been an alteration in the aortic endothelial permeability or in the cellular reaction of the aortic intima.

It is reasonable to assume that a lower level of cholesterol in the serum will, of itself, result in a lesser amount of atherosclerosis. It is generally found that there is a moderately good correlation between the level of cholesterol in the

serum and the severity of atherosclerosis in cholesterol-fed rabbits. On the other hand, it has also been observed in a variety of studies that the serum cholesterol/phospholipid ratio is of importance in the genesis of atherosclerosis and the results of the present experiment are explicable on the assumption that the more normal ratio in the cortisone-treated rabbits of group 2 protected them partially against the development of atherosclerosis. The effect on the cholesterol levels is analogous to that occurring in experiments employing thyroid, potassium iodide, and similar substances (22–25). The effect on the phospholipids is analogous to that seen in experiments with alloxan diabetes, tween 80, and similar substances. Apparently cortisone exploits both mechanisms in the inhibition of experimental atherosclerosis. It is of some importance to observe that cortisone in the doses employed was only mildly diabetogenic. The resulting elevation in blood sugar was generally slight and seldom attained levels that were frankly diabetic. On the other hand, its effects on protein metabolism and on lipid metabolism as judged by the body weights and serum lipids were marked. In alloxan diabetes carbohydrate, protein and fat metabolism are all profoundly altered.

A third explanation of the partial suppression of the development of atherosclerosis in the rabbits treated with cortisone may be found in the observation that cortisone alters the serum lipoproteins. Ultracentrifugal analyses have shown that during the administration of cortisone there occurs at first an increase in all types of lipoproteins (29). Later, the elevated elements are found to be those in the S_f 80 to 40,000 classes of lipoproteins while there is a decrease in the classes of lipoproteins below S_f 80 (30). It has been postulated that prolonged treatment with cortisone causes a metabolic block at the level of S_f 80 in the breakdown of the higher S_f series to the lower ones. This alteration in the serum lipoprotein pattern resembles that seen in alloxan diabetes in cholesterol-fed rabbits (31). In each case there is a quantitative increase in the higher S_f lipoproteins, an intermediate block, and relatively minor quantities of the lower S_f series. It has been postulated (32) that the lower lipoprotein classes up to about S_f 400, but especially those from about S_f 12 to 50, are active atherogenic agents while the higher series are not. Unfortunately, there were no ultracentrifugal studies in the present experiment, but it is reasonable to assume the occurrence of variations in the lipoproteins similar to those referred to above. The relative lack of the lower S_f atherogenic classes of lipoproteins would thus inhibit the development of atherosclerosis.

The three foregoing explanations of the manner in which the alterations in the serum lipids may act to inhibit the development of atherosclerosis in cortisone-treated rabbits should not be regarded as entirely independent of one another. Rather, they should be considered, to some extent at least, as mutually interdependent.

The local action of cortisone on the wall of the aorta at the site of the athero-

sclerotic lesions deserves some comment. Many investigators consider that the serum lipids enter and are deposited in the aortic intima by penetrating through the endothelial lining and into the subendothelial tissue where they lead to the formation of atherosclerotic lesions. Variations in the permeability of the endothelial layer may therefore be expected to affect the development of atherosclerosis. There is no direct evidence concerning the action of cortisone on the endothelium of the aorta and other large vessels, but there are studies of its action on the permeability of the capillary endothelium. Cortisone does not appear to affect the permeability of the normal capillary endothelium (33). However, it greatly reduces the increased capillary permeability observed in inflammation (34) or in starvation (35). It is not known whether aortic endothelium of normal permeability will permit the passage of atherogenic material into the intima, nor is it known whether the endothelium is, in fact, more permeable than normal during the development of atherosclerosis. However, if increased endothelial permeability does play a part in the pathogenesis of atherosclerosis, cortisone might be expected to interfere with the development of the lesions. Similarly, it can only be surmised whether cortisone may or may not affect the intimal ground substance and thus inhibit the progress of atherosclerosis. Cortisone is known to have a profound effect on the reactions that are commonly associated with inflammation and healing. Cellular proliferation is depressed and cellular infiltration is diminished by cortisone. It is theoretically possible that the smaller size of the atherosclerotic lesions in the cholesterol-fed rabbits treated with cortisone is due, in some part, to an inhibition of the local cellular reactions that take part in the pathogenesis of the atherosclerotic lesion. Data pertinent to this problem are not provided by the present experiment.

Firstbrook (36, 37) has studied the effect of weight loss or gain on the development of experimental cholesterol atherosclerosis in the growing rabbit. He has observed that the average cholesterol content of the blood, the initial live body weight, and the relative final body weight are of about equal statistical importance in determining or estimating the degree of atherosclerosis that will develop. He finds a high net correlation between relative weight gain during the course of an experiment and the severity of aortic atherosclerosis. It is his conclusion that caloric restriction with consequent weight loss or decreased rate of weight gain inhibits the development of atherosclerotic lesions. However, we have carried out experiments (38) to determine whether undernourished and adequately nourished rabbits fed the same daily doses of cholesterol would manifest different degrees of atherosclerosis at the end of an experiment. It was found that no difference existed in the degrees of atherosclerosis that were produced by equal daily doses of cholesterol in different experiments that tested the effect of undernutrition (1) in young growing animals that failed to gain weight normally, (2) in young rabbits subjected to severe undernutri-

tion both before and during cholesterol feeding and (3) in fully grown, mature rabbits that lost weight during undernutrition. At the same time, undernutrition was found to promote hypercholesterolemia in the rabbit fed cholesterol. The inhibition of atherosclerosis observed in cholesterol-fed rabbits treated with cortisone cannot be explained by the fact that they lost weight. Indeed, the cortisone-treated cholesterol-fed rabbits did not exhibit the degree of hypercholesterolemia that might be expected either in cholesterol-fed rabbits on an *ad lib.* diet and not otherwise treated or, especially, in undernourished rabbits fed cholesterol.

SUMMARY

An experiment was performed to determine the effect of cortisone on the serum lipids and on the development of experimental cholesterol atherosclerosis in the rabbit. Litter mate rabbits of the same sex were employed; both sexes were represented in the experiment. The report is based upon four experimental groups comprising (1) 12 rabbits fed cholesterol and treated with cortisone vehicle; (2) 12 rabbits fed cholesterol and treated daily with cortisone; (3) 11 rabbits treated with cortisone; and (4) 7 rabbits that received cortisone vehicle.

It was observed that: (1) There was less aortic atherosclerosis in the cholesterol-fed cortisone-treated rabbits as judged by both morphological and chemical means than in the rabbits fed cholesterol without cortisone treatment. (2) Cortisone depressed appreciably the hypercholesterolemia resulting from the feeding of cholesterol to rabbits. (3) Cortisone treatment caused a moderate hypercholesterolemia in normal rabbits. (4) Cortisone caused a moderate increase in serum lipid phosphorus equal to that produced by cholesterol feeding alone. (5) The combination of cholesterol feeding and cortisone did not result in a higher phospholipidemia than either one of these agents alone. (6) Cortisone caused a great increase in serum-neutral fat; it was not apparent whether cholesterol feeding affected the neutral fat lipemia due to cortisone treatment alone. (7) The total cholesterol to lipid phosphorus ratio was about normal in the rabbits that received cortisone only. It was doubled in the animals receiving both cholesterol and cortisone, and it was increased about four times in those that received only cholesterol.

The significance of the alterations in the serum lipids induced by cortisone is discussed in relation to the inhibition of the development of aortic atherosclerosis that occurred in the cholesterol-fed rabbits treated with cortisone.

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