THE EFFECTS OF CHOLESTEROL DOSAGE, CORTISONE, AND DCA ON TOTAL SERUM CHOLESTEROL, LIPOPROTEINS, AND ATHEROSCLEROSIS IN THE RABBIT

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(Received for publication, March 13, 1952)

The rabbit has been used in studying experimental atherosclerosis since Anitschkow (1) demonstrated that cholesterol feeding results in hypercholesterolemia and arterial lesions resembling human atherosclerotic lesions. Duff (2) and other investigators have emphasized certain objections to the analogy between experimental atherosclerosis in the rabbit and that in the human being. Recently, Gofman *et al.* (3, 4) and Jones *et al.* (5) reported evidence that the occurrence of "giant" lipid and lipoprotein molecules of low densities (these will be referred to hereafter under the general term lipoproteins) is associated with the degree of atherosclerosis in both the rabbit and the human being and may have etiologic significance. Keys (6) reported that these lipoproteins and total cholesterol levels in the serum of cholesterol-fed rabbits are correlated to about the same degree with the severity of atherosclerosis.

Adlersberg *et al.* (7–10) have reported a consistent elevation of total serum cholesterol, esterified cholesterol, and phospholipids in patients receiving cortisone. Bloom and Pierce (11) observed no trend of change in the S_f 10–20 class of lipoproteins or total cholesterol in human beings treated for short and long terms with cortisone. In rabbits Kobernick and More (12) reported development of lipemia in cortisone-treated rabbits. Rich *et al.* (13) substantiated these results and showed that the lipemia was a result of increases in serum cholesterol and fatty acids. An additional explanation has been proposed by Pierce and Bloom (14). They treated normal and cholesterol-fed rabbits with cortisone for 3 or 4 days and demonstrated increases in lipoproteins of the S_f 80–400 class and smaller quantities of S_f 40–80 but none of the S_f 20 and lower class. They propose that cortisone interposes a metabolic block at the S_f 40–80 class of lipoproteins resulting in an accumulation of lipoproteins of higher S_f rate. In other species Stamler *et al.* (15) reported potentiation of atherosclerosis without alterations in serum levels of cholesterol and phospholipid in cortisone-treated cholesterol-fed chicks. Adlersberg *et al.* (16) reported that dogs did not develop lipemia and hypercholesterolemia. No reports have been noted up to this time on the effects of cortisone on lipoproteins in normal and cholesterol-fed rabbits during long term experiments.

Entenman *et al.* (17) reported DCA to be without effect on blood lipids in birds. Villela (18) claimed that DCA lowered the plasma cholesterol in normal guinea

pigs. Bruger and Lowenstein (19) found little if any inhibiting effect of DCA on the development of hypercholesterolemia or on the deposition of cholesterol in the liver or the aorta of the normal young female rabbit fed cholesterol. In chicks with or without cholesterol added to the diet DCA failed to influence cholesterolemia (20). Gross atherogenesis was not influenced by DCA in birds fed no cholesterol; however, in cockerels given 0.25 per cent cholesterol mash, DCA increased the incidence and severity of atherosclerosis. No reports have been noted on the effects of DCA on lipoprotein constituents.

The present report summarizes the effects of varying concentrations of cholesterol in the diet on total serum cholesterol, serum lipoproteins, and atherosclerosis in the rabbit. It also includes studies on the effects of cortisone and desoxycorticosterone acetate (DCA).

Methods and Materials

Thirty-two adult male rabbits were used in the study. The rabbits were housed in individual cages and given water *ad libitum*. The diet consisted of Rockland rabbit ration modified by the addition of cholesterol in concentrations ranging from 0 to 1 per cent. Each animal was weighed weekly and its food consumption recorded twice per week. Serum lipoproteins and total cholesterol concentrations were determined on venous blood samples taken at intervals during the experiment. Blood glucose levels, when determined, were done on heparinized blood samples taken at the same intervals.

Lipoprotein analyses were made according to the ultracentrifugal flotation method of Gofman (3). The centrifugally clarified serum was brought to a salt-medium density of 1.063 gm. per cc. with sodium chloride. The low density components were then concentrated by flotation. Experimentation proved that quantitative separation was achieved by a 6½ hour run at room temperature with an average field of 152,000 *G* using a preparative rotor (type A) in a Spinco model E ultracentrifuge. Flotation rates and concentrations were measured by analytical ultracentrifugation at 25° ± 2°C., retaining the medium density of 1.063 gm. per cc. In the calculations a value of 0.00185 was used for the specific refractive index increment.

Analysis of the flotation diagrams indicated three general classes of components. The apparently normal class (these gave simple patterns, often single symmetrical peaks) consisted of components floating at rates of 5 through 9 negative Svedberg units (S_f), uncorrected. A second class contained species of S_f 10–15 and a third class fell in the S_f 16–30 range. The patterns produced by these last two classes overlapped and were complex. In general, however, the simplest analysis could be made on the above basis. This classification differs from that of Gofman in that it subdivides his S_f 10–30 class.

Total cholesterol was determined by modifications of the methods described by Riegel and Rose (21) and Foldes and Wilson (22).

The animals were sacrificed and autopsied at the end of 112 days. The degree of atherosclerosis was graded into five groups (0 to 4) according to the area of aortic intima involved by lesions.

Cortisone, when used, was given subcutaneously in a dosage of 5 mg. per day, 5 days per week. DCA was administered by subcutaneous implantation of five 75 mg. pellets at the beginning of the experiment. About 3 mg. per day were absorbed; only traces of the pellets were present at the end of 112 days.

The thirty-two rabbits were subdivided into seven groups which received respectively (*a*) no cholesterol, (*b*) 0.063 per cent cholesterol, (*c*) 0.25 per cent cholesterol, (*d*) 1.0 per cent

cholesterol, (*e*) 1.0 per cent cholesterol plus DCA, (*f*) 1.0 per cent cholesterol plus cortisone, and (*g*) cortisone without cholesterol. Each group consisted of four rabbits except for the 1.0 per cent cholesterol group which contained eight rabbits.

On all statistical tests made in this report a significance level of $P = 0.05$ was used unless otherwise stated.

RESULTS

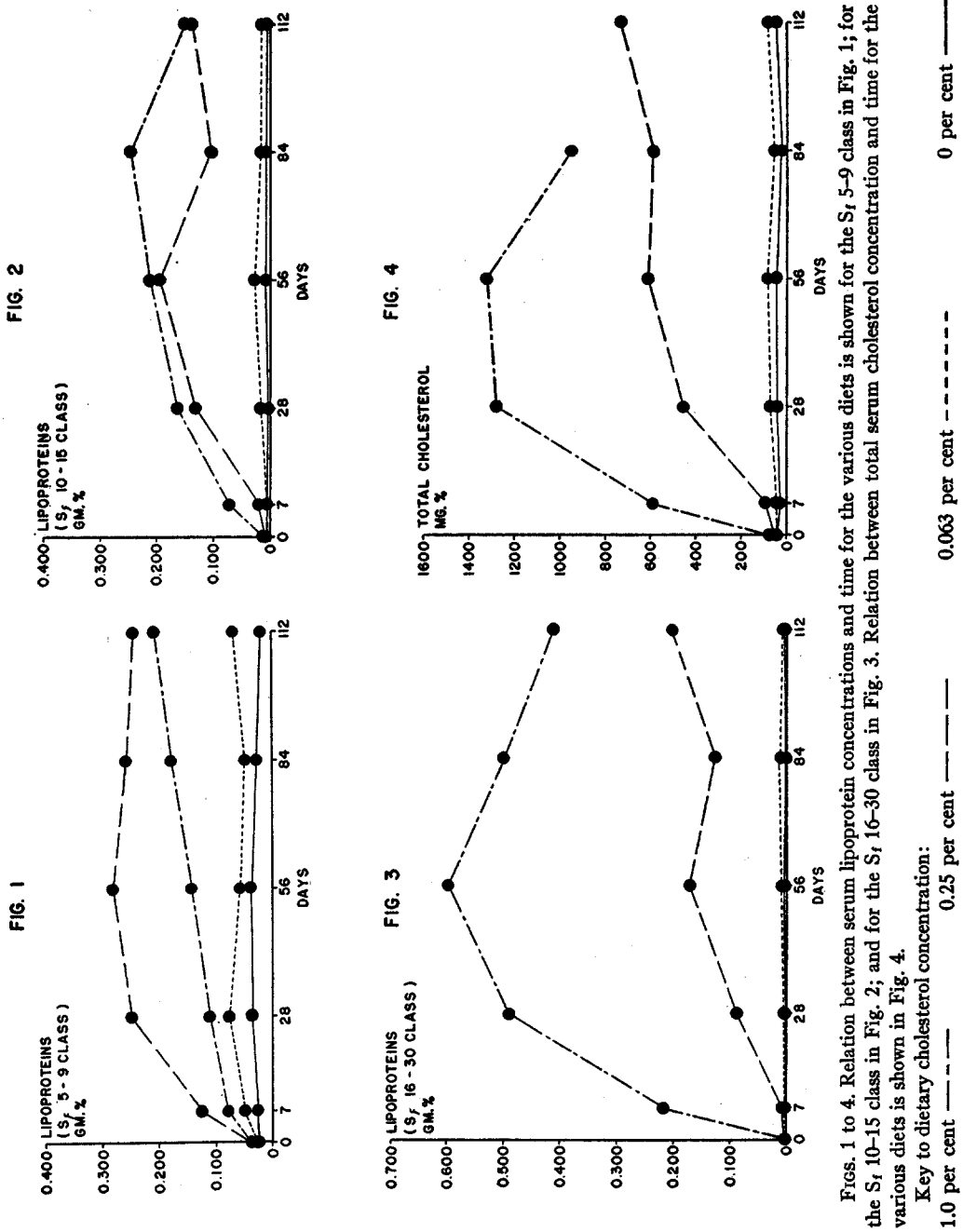
For comparative purposes, a summary of the data is shown in Table I. Three of the cortisone-treated rabbits died during the experiment. One was replaced by a rabbit from the 1.0 per cent cholesterol group on the 23rd day. The other two, one of which was found with a broken back at 50 days and the other of which died on the 65th day of treatment, were not included in the tabulation. The mean and standard error for the lipoprotein and total cholesterol concentrations are based upon average values for each rabbit and do not include the values before cholesterol feeding. A comparison of the degree of atherosclerosis with mean values appears to be valid since the formation of lesions is presumably dependent upon concentrations of these substances throughout the entire period rather than at any given time. The initial weight and ratio of final to initial weight are included, as it has been shown that weight loss can inhibit the development of atherosclerosis (23). The more specific effects with time were as follows:—

Effect of Cholesterol Dosage on Serum Lipoprotein Concentration

(*a*) *Lipoproteins of S_f 5–9 Class (Fig. 1).*—No significant change occurred in the animals which received no added cholesterol in the diet. The 0.063 per cent cholesterol diet produced a slight increase by the end of the 1st week and little change thereafter. The 0.25 per cent caused a large increase over 28 days, and this level remained constant thereafter. The 1.0 per cent diet produced throughout the 112 days a gradual increase which remained below that of the 0.25 per cent diet; this difference was not statistically significant because of the high variability and the small number of animals involved.

It was observed that the flotation rate characterizing the peak (or peaks) of this class, while always within the S_f 5–9 range, appeared to shift with the feeding of cholesterol. This shift was not due to experimental errors in measuring flotation rates; a mixture of sera from the same rabbit before and after receiving cholesterol demonstrated the presence, unchanged, of each component found in the individual serum samples. This point is being investigated further.

(*b*) *Lipoproteins of S_f 10–15 Class (Fig. 2).*—Little or no change occurred in the animals which received no added cholesterol in the diet. Lipoproteins of this class could, however, usually be detected in these animals although in very low concentrations. With the 0.063 per cent cholesterol diet, this class of lipoproteins increased very slightly in 28 days and leveled off. The 0.25



FIGS. 1 to 4. Relation between serum lipoprotein concentrations and time for the various diets is shown for the S_f 5–9 class in Fig. 1; for the S_f 10–15 class in Fig. 2; and for the S_f 16–30 class in Fig. 3. Relation between total serum cholesterol concentration and time for the various diets is shown in Fig. 4.

Key to dietary cholesterol concentration:

1.0 per cent — - - — 0.25 per cent — — — 0.063 per cent - - - - - - 0 per cent ———

per cent diet produced an increase up to 56 days and a decrease thereafter. The 1.0 per cent diet increased this class only slightly above the 0.25 per cent diet throughout 84 days but decreased thereafter.

(*c*) *Lipoproteins of S_f 16–30 Class* (*Fig. 3*).—This class was essentially absent in rabbits which received no cholesterol (only 2 sera out of 24 examined showed any trace of these components). The 0.063 per cent cholesterol diet produced only a slight increase by 84 days, then a decrease. The 0.25 per cent diet gave a very slight rise the 1st week, followed by a steady increase thereafter. The 1.0 per cent diet gave a sharp increase up to 56 days and then a decrease. These values were consistently and significantly higher than those induced by the other cholesterol diets. In agreement with the observations of other workers, the appearance of this class of lipoproteins was accompanied by increased amounts of low density materials of great heterogeneity which floated at rates exceeding S_f 30. These materials were not considered in the centrifugal analyses.

Effect of Cholesterol Dosage on Serum Total Cholesterol Concentration and Degree of Atherosclerosis

An approximately linear relationship existed between total cholesterol and both cholesterol concentration in the diet and the amount of cholesterol consumed in grams per kilogram per day (Table I). A rise of 100 mg. per cent of cholesterol in the serum resulted from an increase of 0.085 per cent cholesterol concentration in the diet, which was equivalent to an increase of 0.025 gm. per kg. in amount of cholesterol consumed.

The 0.063 per cent diet produced only a slight increase in total cholesterol above the controls (Fig. 4). The 0.25 per cent gave a gradual increase throughout the 112 days. The 1.0 per cent diet gave a sharp rise in total cholesterol which decreased after 56 days.

These curves were similar to those for the lipoprotein concentrations previously illustrated. With all four cholesterol diets the correlation coefficients between lipoprotein concentration and total cholesterol were 0.7 or higher for the S_f 16–30 class (statistically significant at $P = 0.01$).

The severity of atherosclerosis was found to be equally well correlated with serum total cholesterol concentration and S_f 16–30 class lipoprotein concentration. This is in agreement with the observation of Keys (6). This does not indicate, however, that atherosclerosis cannot occur independently of one or the other factors as has been reported under certain other conditions (4).

Effects of Cortisone and DCA

Cortisone administration in the normal rabbits (Table I) increased the mean concentrations of the S_f 10–15 and S_f 16–30 classes of lipoproteins significantly above the controls. Total cholesterol was also increased. The initial weights

TABLE I

Summary of Changes Induced by Cortisone, Desoxycorticosterone, and Cholesterol Feeding in the Rabbit

Treatment and diet	No. of animals	Lipoprotein concentration per 100 cc. serum Mean ± S.E.			Total cholesterol per 100 cc. serum Mean ± S.E.	Initial weight Mean ± S.E.	Ratio of final weight to initial weight Mean ± S.E.	Food consumption per day	Degree of atherosclerosis Mean ± S.E.
		Sf 5–9	Sf 10–15	Sf 16–30					
		gm.	*gm.*	*gm.*	*mg.*	*kg.*		*gm./kg.*	
Controls, no cholesterol	4	0.029 ± 0.007	0.006 ± 0.001	0.0003 ± 0.0001	35 ± 4	2.81 ± 0.19	1.56 ± 0.10	43 ± 3	0
0.063% cholesterol	4	0.064 ± 0.016	0.015 ± 0.004	0.005 ± 0.002	64 ± 17	2.85 ± 0.15	1.52 ± 0.05	43 ± 1	0.25 ± 0.25
0.25% cholesterol	4	0.229 ± 0.042	0.120 ± 0.024	0.120 ± 0.037	492 ± 119	2.58 ± 0.19	1.62 ± 0.20	45 ± 5	2.50 ± 0.65
1.0% cholesterol	7	0.145 ± 0.026	0.183 ± 0.019	0.484 ± 0.018	1207 ± 135	3.41 ± 0.19	1.11 ± 0.07	29 ± 2	3.71 ± 0.18
DCA + 1.0% cholesterol	4	0.094 ± 0.014	0.138 ± 0.009	0.269 ± 0.037	846 ± 65	3.70 ± 0.37	1.05 ± 0.06	29 ± 1	3.50 ± 0.50
Cortisone + 1.0% cholesterol	3	0.253 ± 0.050	0.167 ± 0.010	0.400 ± 0.038	985 ± 175	3.42 ± 0.12	1.09 ± 0.04	36 ± 2	2.67 ± 0.66
Cortisone, no cholesterol	3	0.023 ± 0.004	0.021 ± 0.001	0.058 ± 0.023	94 ± 27	2.77 ± 0.37	1.33 ± 0.01	52 ± 1	0

for the two groups were not significantly different. The rate of gain in weight was significantly less for the cortisone group. The serum from two of the cortisone-treated group was strikingly turbid; there was evidence of abnormal amounts of flotation material having S_f values greater than 30. These two rabbits also showed high total cholesterol and blood glucose levels. The mean weight of the adrenal glands was significantly lower than that of the controls for the cortisone group. There was no atherosclerosis in any of these animals.

Cortisone treatment in cholesterol-fed rabbits (Table I) did not significantly alter the serum lipoprotein, cholesterol concentrations, or the degree of atherosclerosis from those observed in the rabbits receiving 1.0 per cent cholesterol alone.

DCA treatment (Table I) lowered lipoprotein and total cholesterol concentrations. This decrease was significant only for the S_f 5–9 class of lipoproteins and for total cholesterol. The degree of atherosclerosis was not different.

SUMMARY AND CONCLUSIONS

The effects of cholesterol dosage, cortisone, and desoxycorticosterone acetate on total serum cholesterol, lipoproteins, and atherosclerosis were studied over a period of 112 days in thirty-two rabbits. Cholesterol was administered by feeding the rabbits diets containing 0.063, 0.25, and 1.0 per cent cholesterol At intervals measurements were made of total serum cholesterol and of low density lipid and lipoprotein components of three classes, S_f 5–9, S_f 10–15, and S_f 16–30.

All three classes of lipoproteins increased with cholesterol feeding. The total serum cholesterol concentration was linearly related to both the quantity of cholesterol consumed and its concentration in the diet. Lipoprotein and total serum cholesterol concentrations were significantly and equally well correlated with the severity of atherosclerosis.

Cortisone administration in the normal rabbit increased the concentrations of total cholesterol and of lipoprotein components of the S_f 10–15 and S_f 16–30 classes, but did not produce atherosclerosis. Cortisone treatment in cholesterol-fed rabbits did not significantly affect the levels of serum lipoproteins, cholesterol concentration, or atherosclerosis produced by a 1.0 per cent cholesterol diet alone.

Values for total cholesterol and S_f 5–9 class of lipoproteins in DCA-treated animals were lower than those in controls but the degree of atherosclerosis was not significantly less.

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