

Plasma Cholesterol of Roosters under Various Conditions. (19252)

J. C. FORBES AND O. PETTERSON.

From the Department of Biochemistry, Medical College of Virginia, Richmond, Va.

Gofman *et al.*(1,2) and Duff and Payne(3) suggest that the physical state in which cholesterol occurs in the plasma may be an important factor in the development of atherosclerosis. Previous reports from our laboratory(4,5) have shown that only a relatively small percentage of the total cholesterol of lyophilized normal human serum can be extracted by cold chloroform in a 3-hour period. It has also been shown that in some cases the amount of this fraction may be considerably increased even though the total cholesterol may be within normal limits(6). Its concentration is invariably high in rabbits rendered hypercholesterolemic from the administration of high cholesterol diets as well as in nephrotic patients with hypercholesterolemia (4). It was suggested that the degree of extractability under these conditions was influenced by the physical state of the cholesterol present.

Since atherosclerosis can be readily produced in roosters by addition of cholesterol to their diet(7) or by administration of estrogenic substances(8,9) it was decided to study changes in the concentration of this "readily extractable" cholesterol fraction following administration of estrogenic substances or of a high cholesterol diet to 3-month-old cockerels. Concomitant studies were carried out on the effect on the cholesterol fractions of the addition of lecithin or of neutral fat to the basal diet.

Methods. Rockland laying mash was used as the basal diet in all cases. Its fat content is about 3.5%. Two estrogenic preparations were employed, diethylstilbestrol and Dianisylhexane.* The diethylstilbestrol was implanted subcutaneously in pellet form.† Each pellet contained 12 mg of the estrogenic material

and one pellet was implanted at a time. The Dianisylhexane, which was used at a level of 20 and 40 mg per kilo of diet, was dissolved in Mazola oil and added to the basal diet. The Mazola oil constituted 5% of the finished diet. The cholesterol was dissolved in chloroform, mixed with sufficient Mazola oil to make up 10% of the finished diet, and the whole thoroughly mixed with the laying mash. The chloroform was allowed to evaporate before the diet was given to the experimental animals. Blood was obtained from the alar vein, heparin being used as an anticoagulant. Serum was analyzed in a few cases and the results were in good agreement with those obtained on heparinized plasma from the same blood. The analytical procedures have been described(4).

Results. Typical results obtained with diethylstilbestrol are shown in Table I. It will be seen that its administration caused a marked increase in the concentration of the "readily extractable" fraction, as well as in total cholesterol. Animals receiving Dianisylhexane showed the same general changes but the individual variations were much greater. Some of the animals were very susceptible to the drug while others were resistant. It is possible that this difference in susceptibility may have been due to variations in the absorption of the estrogenic material from the gastrointestinal tract. The effect of cholesterol feeding on the various fractions is shown in Table II. It will be seen the concentration of the "readily extractable" cholesterol as well as that of the total cholesterol showed a decided increase during the cholesterol feeding and dropped to normal within a relatively short period of time after discontinuation of the cholesterol administration. A comparison of the data on the individual animals with those obtained when diethylstilbestrol was administered showed that, for moderate cholesterol concentrations, the increase in the "readily extractable" cholesterol was much

* Dianisylhexane was supplied by White Laboratories, Inc., Newark, N. J., through the courtesy of Dr. C. W. Sondern.

† Capette pellets, manufactured by Wick and Fry, Inc., Cumberland, Ind.

TABLE I. Effect of Diethylstilbesterol Administration on Plasma Cholesterol of Roosters.

No. of animals	Avg concentration per 100 ml—			Remarks
	R. E. chol., mg	Total chol., mg	N. F. + chol., mg	
11	8 (5-12)	87 (34-127)	273 (172-416)	Control animals on laying mash
5	168 (24-413)	244 (157-445)	1173 (553-2415)	41 days after starting diethylstilbesterol inj.
	249 (83-421)	283 (114-417)	2240 (527-5210)	74 days after starting diethylstilbesterol inj.

Range in parenthesis. R. E. chol. = readily extractable cholesterol. N. F. + chol. = neutral fat + cholesterol.

TABLE II. Effect of Cholesterol Feeding on Plasma Cholesterol of 5 Roosters.

R. E. chol., mg	Avg concentration per 100 ml—		Remarks
	Total chol., mg	N. F. + chol., mg	
200 (18-536)	344 (200-598)	600 (377-960)	After 13-25 days on .5% followed by 8 days on 1% cholesterol diet
172 (41-689)	368 (223-701)	785 (557-1345)	After 22 days on 1% cholesterol diet
213 (26-772)	448 (236-829)	856 (356-1783)	" 92 " " " " " "
8 (6-12)	72 (16-104)	218 (131-303)	25 days after cessation of cholesterol feeding

Range in parenthesis. R. E. chol. = readily extractable cholesterol. N. F. + chol. = neutral fat + cholesterol.

TABLE III. Effect of Addition of Either Neutral Fat or Lecithin to Diet on Plasma Cholesterol of Roosters.

No. of animals	Avg concentration per 100 ml—			Remarks
	R. E. chol., mg	Total chol., mg	N. F. + chol., mg	
7	8 (5-10)	84 (34-127)	285 (172-416)	Control period, laying mash diet
	43 (7-115)	117 (36-161)	277 (54-384)	After 27 days on 20% Crisco diet
	27 (9-60)	81 (23-131)	209 (86-306)	" 40 " " 20% " "
	9 (6-13)	67 (31-120)	263 (131-356)	" 39 " " 10% " "
5	11 (5-20)	116 (86-129)	342 (273-452)	" 22 " " 10% " "
	10 (5-15)	137 (116-160)	367 (231-475)	" 68 " " 10% " "
	6 (3-8)	67 (32-115)	132 (83-201)	After low-fat diet 10-13 days
	5 (3-7)	77 (35-113)	109 (59-154)	" " " " 28 days
	11 (7-15)	116 (91-154)	315 (270-364)	After 13 days on 10% Crisco diet
7	5 (2-8)	67 (37-85)	171 (107-208)	" 28 " " 10% lecithin diet
	5 (3-6)	41 (24-62)	121 (53-255)	" 83 " " " " "

Range in parenthesis. R. E. chol. = readily extractable cholesterol. N. F. + chol. = neutral fat + cholesterol.

less than when the total cholesterol was raised to the same level by the administration of estrogenic substances. However, when the cholesterol content of the plasma was high (about 700 mg or above per 100 ml) in the cholesterol fed animals, nearly all of it was present in the "readily extractable" form. A similar condition was found in the plasma of the animals receiving estrogenic substances at a much lower total cholesterol level.

There is evidence that, in order to lower the plasma cholesterol of human subjects by dietary means, it is usually necessary to reduce to a low value the amount of fat in the diet

as well as its cholesterol content. Consequently, it was considered of interest to determine if, in the cockerel, the level of fat in the diet would influence the concentration of either the total cholesterol or the "readily extractable" fraction. The experimental results are shown in Table III. It will be seen that when the diet contained 20% Crisco, making a total fat content of approximately 23.5%, the concentration of the "readily extractable" fraction showed on the whole a definite rise while that of total cholesterol and of neutral fat showed no consistent change. Only one of the birds failed to show a distinct rise in

the "readily extractable" fraction during the experimental period. When the diet contained 10% Crisco the concentration of the "readily extractable" cholesterol fraction closely approximated the levels found in the cockerels on the basal diet itself. When the basal diet, repeatedly extracted with carbon tetrachloride to remove fat and then supplemented with vitamins A, D, E, thiamine, riboflavin, pyridoxine, niacin and calcium pantothenate was fed, the concentration of the "readily extractable" fraction showed a uniform drop. Similarly low values were obtained when the basal diet was supplemented with sufficient soybean lecithin[‡] to constitute 10% of the diet. It will be seen that in this case the total cholesterol concentration dropped to a very low value by the time the birds were on the diet for 83 days. This effect of the soybean lecithin is of considerable interest in view of the fact that Steiner and Domanski(10), Gross and Kesten(11), Adlersberg and Sobotka(12) obtained a drop in the serum cholesterol of patients with various pathological conditions following oral administration of a soybean lecithin preparation.

Summary. Subcutaneous administration of diethylstilbestrol to cockerels caused a marked increase in the concentration of the "readily extractable" cholesterol of the plasma as well as in total cholesterol and neutral fat. Cholesterol feeding exerted a similar effect, but at moderately elevated plasma cholesterol levels the concentration of the "readily extractable" fraction was not as great as when the total cholesterol was raised by the administration of estrogenic substances. However, when the

total cholesterol was high, about 700 mg %, practically all of it was present in the "readily extractable" form. Roosters receiving a high fat diet for several weeks usually showed a definite increase in the "readily extractable" fraction without a consistent increase in total cholesterol. When the diet contained 10% soybean lecithin the concentrations of both the total cholesterol and of the "readily extractable" fraction dropped below those found in animals on the basal diet.

1. Gofman, J. W., Lindgren, F., Elliott, H., Mantz, W., Hewitt, J., Strisower, B., Herring, V., and Lyons, T. P., *Science*, 1950, v8, 166.
2. Gofman, J. W., Jones, H. B., Lindgren, F. T., Lyons, T. P., Elliott, H. A., and Strisower, B., *Circulation*, 1950, v2, 161.
3. Duff, G. L., and Payne, T. P. B., *J. Exp. Med.*, 1950, v92, 299.
4. Forbes, J. C., Dillard, G. H. L., Porter, W. B., and Petterson, O., *PROC. SOC. EXP. BIOL. AND MED.*, 1948, v68, 240.
5. Forbes, J. C., Dillard, G. H. L., Porter, W. B., and Petterson, O., *PROC. SOC. EXP. BIOL. AND MED.*, 1949, v71, 26.
6. Fowlkes, R. W., and Forbes, J. C., *Arch. Derm. and Syph.*, 1950, v62, 6881.
7. Dauber, D. V., and Katz, L. N., *Arch. Path.*, 1943, v36, 473.
8. Horlick, L., and Katz, L. N., *J. Lab. Clin. Med.*, 1948, v33, 733.
9. Lindsay, S., Lorenz, F. W., Entenman, C., and Chaikoff, I. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1946, v62, 315.
10. Steiner, A., and Domanski, B., *PROC. SOC. EXP. BIOL. AND MED.*, 1948, v68, 240.
11. Gross, P., and Kesten, B., *Arch. Derm. Syph.*, 1943, v47, 159.
12. Adlersberg, D., and Sobotka, H., *J. Mt. Sinai Hosp.*, 1943, v9, 955.

[‡] A dry granular material obtained from Fries Bros., Inc., N. Y. City.