The synthesis of thyroxine was relatively slow. A maximum value of 15% of the radioiodine in the thyroid was converted to thyroxine under the experimental conditions employed.

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## Effect of Dihydrocholesterol (Cholestanol) Administration on Plasma Cholesterol and Atherosclerosis in the Rabbit.* (20482)

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We reported recently that the administration of dihydrocholesterol (DHC) to chickens fed a cholesterol-containing diet prevented the development of both hypercholesterolemia and the associated atherosclerosis(1). Since the feeding of this saturated steroid might offer a means of inhibiting the atherogenic effects of dietary cholesterol, it seemed desirable to extend our observations to mammals. The present report deals with the action of DHC in the rabbit.

*Experimental.* Rabbits weighing from 1850 to 2270 g were fed an adequate commercial pellet diet† for 6 weeks before being placed on the experimental diets recorded in Table I. During these 6 weeks, 2 blood samples were obtained from each animal by cardiac puncture, and the total cholesterol content of plasma was determined by the method of Sackett(2). At the end of this period, the rabbits were divided into 4 groups, and fed as shown in Table I. These experimental diets were fed *ad lib.* for a period of 2 weeks at the end of which time—because the supply of DHC was exhausted—all rabbits were placed on the normal pellet diet for the next 5 weeks. At week 13 the level of plasma cholesterol was again determined, and the feeding of the experimental diets resumed (Table I). The experiment was continued for 9 more weeks, and during this time 4 additional plasma cholesterol values were obtained. Each group consumed approximately the same amount of diet.

TABLE I. Composition of Diets. All diets consisted of commercial rabbit pellets to which was added one or more of the following constituents:

| Constituent | Group I | II | III | IV |
|---|---|---|---|---|
| | % | | | |
| Wesson oil | 5 | 5 | 5 | 5 |
| Cholesterol | 0 | 1 | 1 | 0 |
| DHC* | 0 | 0 | 2 | 2 |

* The dihydrocholesterol used in this study was generously supplied by the Schering Corp.

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† This diet contained 60% alfalfa meal, 10% ground barley, 10% cottonseed meal, 10% rice polishings, 4% watergrass seed, 4% rice seed, 1% oyster shell flour, 0.5% cane molasses, and 0.5% salt.

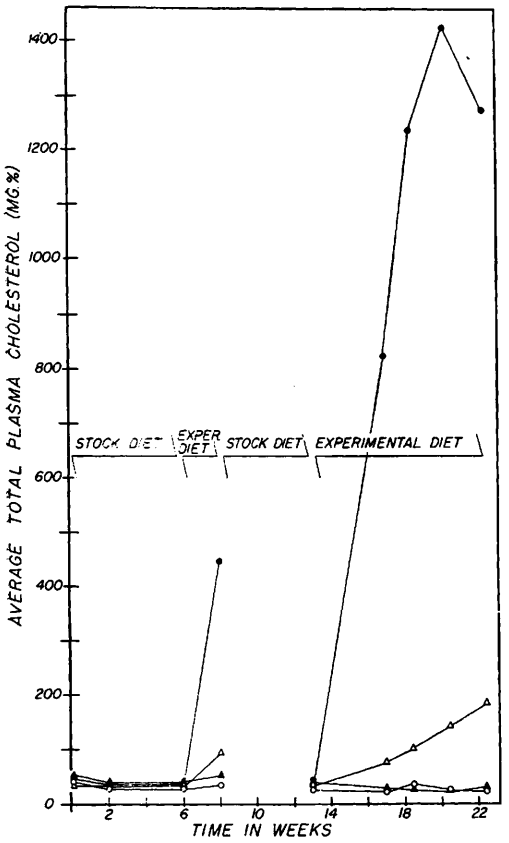


FIG. 1. Influence of DHC feeding upon plasma cholesterol levels of the normal and the cholesterol-fed rabbit. ○—Group I. Average plasma cholesterol levels observed in 8 rabbits fed a basal diet (commercial rabbit pellets plus 5% Wesson oil). ●—Group II. Average plasma cholesterol levels found in 8 rabbits fed a basal diet plus 1% cholesterol. △—Group III. Average plasma cholesterol levels in 7 rabbits fed a basal diet plus 1% cholesterol plus 2% DHC. ▲—Group IV. Average plasma cholesterol levels of 8 rabbits fed a basal diet plus 2% DHC.

The values for total plasma cholesterol are shown in Fig. 1. Although the administration of DHC to rabbits fed cholesterol (Group III) did not completely prevent a rise in plasma cholesterol, it is nonetheless clear that these rabbits exhibited a much less pronounced rise in total plasma cholesterol than did those fed cholesterol alone (Group II). It should be noted that the addition of DHC to a *normal* diet did not depress the level of plasma cholesterol below those found in normal animals. Thus the average values for plasma cholesterol were 28 mg % in the DHC-fed animals and 31 mg % in control animals. DHC apparently acts upon the hypercholesterolemia of exogenous origin for it seems to have no effect upon the cholesterol level of the normal rabbit.

The rabbits were sacrificed at the end of the experiment, and their aortas and livers were removed. Each atherosclerotic plaque was carefully measured, and the total area estimated. These findings are summarized in Table II. Most of the rabbits fed the 1% cholesterol diet developed atherosclerosis. The average area of the atherosclerotic plaques found in this group (II) was 271 $mm^2$. The addition of DHC to the 1% cholesterol diet reduced this figure to 5.1 $mm^2$. Two of the 8 rabbits (Group IV) that were fed DHC with a normal diet developed atherosclerotic plaques. No atherosclerosis was observed in the untreated rabbits.

Total lipids for each liver sample were determined as follows: Liver samples were first hydrolyzed by treatment with a saturated sodium hydroxide-ethanol mixture on a steam bath for 8 hours. The mixture was then acidified and extracted 3 times, each time with a 10-fold excess of petroleum ether. The pooled petroleum ether extracts were transferred to tared flasks and evaporated to dryness on a steam bath. The weight of the residue was then determined. The values for total liver lipids of the untreated rabbits (Group I) averaged 3.0% (2.6-3.3) while those for the DHC-treated rabbits (Group IV) averaged 3.3% (2.1-4.6). The average values for liver lipids were 6.7% (3.2-9.3) for the rabbits fed cholesterol (Group II) and 4.6% (1.1-7.9) for those fed cholesterol plus DHC (Group III).

*Comment.* It is clear that the enteral administration of DHC is effective in inhibiting experimentally-induced atherosclerosis in the rabbit as well as in the bird. Under the conditions of our study, the DHC did not prevent completely the hypercholesterolemia of cholesterol feeding nor did it prevent a small increase of lipids in the livers of rabbits fed *both sterols*. It should also be noted that the formation of atherosclerotic plaques in rabbits fed DHC alone (Table II) is not ruled out. This phase is under further study.

TABLE II. Effect of Dihydrocholesterol upon Degree of Atherosclerosis in the Rabbit.

| No. of rabbits | Diet | Gross atherosclerosis ($mm^2$) | |
|---|---|---|---|
| 8 | No cholesterol (Group I) | 0 | |
| 8 | 1% cholesterol (Group II) | 140 | |
| | | 18 | |
| | | 0 | |
| | | 16 | Avg |
| | | 487 | 271 $mm^2$ |
| | | 318 | |
| | | 1176 | |
| | | 10 | |
| 7 | 1% cholesterol + 2% dihydrocholesterol (Group III) | 25 | |
| | | 0 | |
| | | 0 | Avg |
| | | 0 | 4.9 $mm^2$ |
| | | 9 | |
| | | 0 | |
| | | 0 | |
| 8 | 2% dihydrocholesterol (Group IV) | 8 | |
| | | 0 | |
| | | 0 | |
| | | 0 | Avg |
| | | 150 | 19.8 $mm^2$ |
| | | 0 | |
| | | 0 | |
| | | 0 | |

In the bird, an increase in the lipid content of the liver was observed after prolonged feeding of a diet containing 2% DHC.‡ In the rabbit, however, the feeding of a 2% DHC diet for as long as 2 months failed to affect the lipid content of the liver. The tissues of rats fed a diet containing 10% DHC for 8 weeks were examined histologically by Dr. Stuart Lindsay of the Pathology Department; no abnormalities were observed.

*Summary.* 1. Effects of dihydrocholesterol on cholesterol content of plasma and on development of atherosclerosis were investigated in the rabbit. 2. Rabbits fed a diet containing 1% cholesterol exhibited typical hypercholesterolemia (average, 1041 mg/100 cc of plasma) and atherosclerosis (average estimated plaque area was 271 $mm^2$). Rabbits fed 1% cholesterol plus 2% dihydrocholesterol, the average plasma cholesterol value was 120 mg % and the average atherosclerotic plaque area was 4.9 $mm^2$. 3. Addition of dihydrocholesterol to a normal diet had no effect on the levels of plasma cholesterol, but did result in an increase in the average atherosclerotic plaque area.

The technical assistance of J. Sherokoff is gratefully acknowledged.

‡ The lipid content of the livers of birds fed a diet containing 2% DHC for 12 weeks ranged from 6.5 to 9.7 (average, 7.8%). The range for control birds was 2.9 to 5.7 (average 4.0). (Unpublished observations.)

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## Anterior Pituitary Growth Hormone (STH) and Pancreatic Secretion of Glucagon (HGF).* (20483)

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Houssay and Biasotti(1) were the first to demonstrate that the removal of the pituitary gland ameliorates pancreatic diabetes. This discovery was followed by the observation that injections of crude anterior pituitary extracts(2), or purified growth hormone (somatotrophic hormone or STH(3)) cause temporary hyperglycemia and glycosuria, followed by permanent "metahypophyseal" diabetes. It is believed(4,5) that anterior pituitary hormone(s) first increase the demand for insulin and overstimulate the Islets of Langerhans and, if the injections are continued, cause a progressive degranulation, exhaustion, and degeneration of the beta cells, and a decreased secretion of insulin. In contrast to the beta cells, the alpha cells of animals with metahypophyseal diabetes appear to be normal (4-6). On the basis of evidence recently

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