PRIMARY SELECTION OF HYPOCHOLESTEREMIC AGENTS (SIMULATION OF ENDOGENOUS HYPERCHOLESTEREMIA IN ANIMALS)

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The effect of diethylstilbestrol on the blood cholesterol and phospholipid levels was studied in cocks, and the effect of the surface-active agent triton WR-1339 on the blood cholesterol was studied in mice. The endogenous hypercholesteremia developing as a result of these measures is a convenient model for primary selection of hypocholesteremic agents.

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The search for pharmacological agents controlling cholesterol metabolism in the body continues to attract the attention of pharmacologists [2, 3] and clinicians [1, 10]. However, progress in this direction is impeded by the absence of a model of experimental endogenous hypercholesteremia which would enable the hypocholesteremic activity of compounds to be evaluated in a short time. Administration of exogenous cholesterol to animals sharply modifies their lipid metabolism, and completely inhibits the endogenous biosynthesis of cholesterol, so that the model of hypercholesteremia produced by administration of exogenous cholesterol cannot be used for pharmacological investigation of substances blocking its biosynthesis. In the Western literature there are reports that administration of female sex hormones and surface-active agents to experimental animals stimulates the synthesis of endogenous cholesterol in these animals [5, 6].

The object of the present investigation was to study the effect of a single dose of diethylstilbestrol on lipid metabolism in cocks and the effect of a single dose of the surface-active agent triton WR-1339 (phenol aryl-alkyl polyester) on the blood cholesterol level in mice.

EXPERIMENTAL METHOD

Experiments were carried out on cocks aged 1.5-2 months and on albino mice. Diethylstilbestrol was injected intramuscularly as a 0.5% oily solution, and triton WR-1339 intraperitoneally as a 3-12.5% solution in 0.34% NaCl solution. The blood cholesterol was determined by Todorov's method [4] and phospholipids were estimated as inorganic phosphorus.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of different doses of diethylstilbestrol on the blood cholesterol level was studied in cocks (Fig. 1). The results show that the most marked effect was observed when the dose of diethylstilbestrol was 5 mg/kg; reducing the dose to 2.5 mg/kg caused a marked fall in the level of the developing hypercholesteremia, while an increase in the dose to 10 mg/kg did not significantly increase the effect.

The most marked changes in lipid metabolism after administration of diethylstilbestrol in a dose of 5 mg/kg occurred on the 3rd day (the blood cholesterol level was increased by 2.8 times and the phospholipids by 5 times), after which these indices began to return to normal, so that by the 8th day the blood

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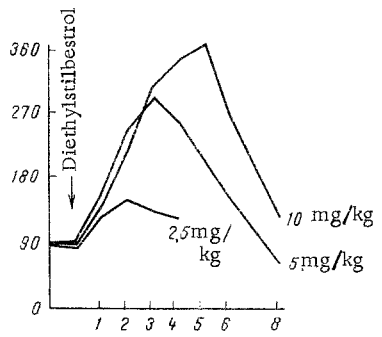

Fig. 1.

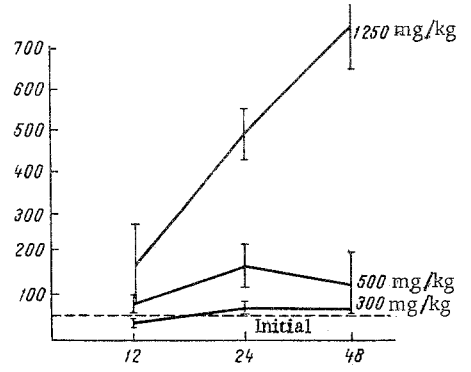

Fig. 2.

Fig. 1. Effect of diethylstilbestrol on blood cholesterol level in cocks. Abscissa, days after administration of diethylstilbestrol; ordinate, cholesterol concentration (in mg %).

Fig. 2. Effect of triton WR-1339 on blood cholesterol in mice. Abscissa, time after administration of triton WR-1339 (in h); ordinate blood cholesterol (in mg%).

cholesterol level had returned to its initial values but the phospholipid concentration still remained a little high.

In the experiments of series II the reproducibility of the changes thus produced was studied after repeated administration of diethylstilbestrol. For this purpose, cocks were injected three times (at monthly intervals) with diethylstilbestrol in a dose of 5 mg/kg. The hypercholesteremia and hyperphospholipidemia developing as a result were not significantly different from those observed after the first injection (on the 3rd day after the first injection the cholesterol level was 260 ± 37 mg%, after the second injection 261 ± 40 mg%, and after the third injection 249 ± 29 mg%, the phospholipid contents being 944 ± 144 and 1006 ± 166 mg% respectively).

In the experiments of series III the effect of p-chlorophenoxyisobutyric acid, which possesses hypocholesteremic properties in intact animals [11], on the level of the endogenous hypercholesteremia produced by administration of diethylstilbestrol was studied. For this purpose 5 cocks were injected intramuscularly with diethylstilbestrol in a dose of 5 mg/kg. The same animals received diethylstilbestrol one month later together with p-chlorophenoxyisobutyric acid (100 mg/kg by mouth once daily for 3 days).

The results obtained showed that p-chlorophenoxyisobutyric acid inhibits the development of hypercholesteremia in cocks after administration of diethylstilbestrol (the mean level of the hypercholesteremia following administration of diethylstilbestrol was 300 ± 24 mg, compared with 223 ± 25 mg% following administration of diethylstilbestrol together with p-chlorophenoxyisobutyric acid to the same animals).

In the second group of experiments the effect of triton WR-1339 on the blood cholesterol level was studied in intact mice.

The results given in Fig. 2 show that triton, in a dose of 300 mg/kg, did not affect the cholesterol level 12 and 48 h after administration, but increased it slightly after 24 h. In a dose of 500 mg/kg it had its maximal effect after 24 h; later the blood cholesterol fell, although it had not regained its initial values 48 h after administration.

Triton, in a dose of 1250 mg/kg, caused a marked hypercholesteremia after 12 h. The blood cholesterol continued to increased after 24 h, reaching a maximum after the end of 48 h, after which it began to fall.

These results indicate that administration of a single dose of diethylstilbestrol to cocks raises their blood cholesterol. The optimum dose of deithylstilbestrol for pharmacological investigations is 5 mg/kg and after administration of this dose the greatest changes in lipid metabolism of the animals occurred by the end of the 3rd day. This agrees with data in the literature showing that lipid metabolism in birds is under the influence of ovarian hormones, so that repeated and prolonged administration of estrogens to

cocks causes them to develop atherosclerosis, differing from cholesterol atherosclerosis simply in the degree of development of the "biochemical syndrome" and the relative proportions of the lipid components infiltrating the aorta [6]. The increase in cholesterol concentration thus taking place in the liver, aorta, and other organs indicates that in this case the hypercholesteremia does not result from a redistribution of lipids between the tissues, but from stimulation of their biosynthesis [10].

The hypercholesteremia developing in cocks after receiving diethylstilbestrol is inhibited by p-chlorophenoxyisobutyric acid.

Administration of triton WR-1339 to mice raises their blood cholesterol, in agreement with results obtained in other species of animals [5, 8]. According to data in the literature, triton causes hyperlipidemia by its action on the lipoproteins and of their interaction with lipoprotein lipase [8] and the rate of cholesterol synthesis in the body [5, 7].

The results show that experimental endogenous hypercholesteremia produced by administration of diethylstilbestrol to cocks and of triton WR-1339 to mice can be used as a model for pharmacological investigation of substances capable of influencing cholesterol metabolism in vivo. The advantages of this model are the speed of reproduction of hypercholesteremia and the small dose of the preparation required for the investigation, making it particularly valuable for the primary selection of agents capable of exerting a hypocholesteremic action. It should be emphasized that the elevation of the blood cholesterol level in this case is attained without administration of exogenous cholesterol, so that this model can be used to study hypocholesteremic agents which block cholesterol biosynthesis.

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