CHOLESTEROL BREAKDOWN BY LIVER HOMOGENATES FROM MONKEYS WITH EXPERIMENTAL ATHEROSCLEROSIS

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The blood cholesterol concentration in monkeys and rabbits receiving cholesterol with their diet is inversely proportional to the ability of the liver tissue to split cholesterol.

Since the classical work of Anichkov and Khalatov [1] on rabbits, the method of alimentary cholesterol loading has been successfully used to produce atherosclerosis in other animals: birds [5], dogs [9], monkeys [10], and so on. However, some experimental animals are resistant to cholesterol: 13% of all rabbits [3] and individual dogs [6] and monkeys [4].

Since the liver plays an important role in cholesterol synthesis and utilization, it was decided to investigate the possible link between the resistance of individual monkeys to cholesterol feeding and the activity of the liver enzyme systems decomposing cholesterol.

EXPERIMENTAL METHOD

The experimental animals consisted of 15 rabbits and 26 monkeys (14 of the species Papio hamadryas and 12 of Macaca rhesus). Crystalline cholesterol, dissolved in butter, was fed to the monkeys as an addition to their porridge. The monkeys' daily ration consisted of 50 g meal, 25 g butter, vegetables, fruits, mixed salts, and vitamins. Vegetable oils and, as far as possible, all products containing them (nuts, seeds, etc.) were excluded from the diet. Periodically, the monkeys were given eggs, and sugar to increase the total calorific value of the diet. For monkeys weighing 8 kg this was 1200 cal, and for those weighing more than 8 kg, 1800 cal. With this diet, P. hamadryas received 5 g cholesterol daily, and M. rhesus 3 g cholesterol daily.

The rabbits were kept on the usual atherogenic diet for 5 weeks and received 0.5 g cholesterol/kg body weight daily. The blood cholesterol was determined every month in the monkeys and every 10 days in the rabbits [7].

The enzyme activity of the liver was investigated in relation to its ability to decompose cholesterol in vitro. Into glass homogenizers 0.5 ml of a 30% cholesterol emulsion in 1% Tween-60 solution in 0.05 M phosphate buffer, pH 7.4, was added. The homogenizer with the prepared mixture was immersed in a water bath at 38°C; 100 mg of liver puncture material taken from the living monkey was placed in the prepared homogenizers, minced vigorously for 1 min with the cholesterol emulsion, and incubated for 10 min. The reaction was stopped by the addition of 10 ml 15% KOH solution. The homogenate of a weighed sample of liver in the control tubes was preliminarily boiled (the quantity of cholesterol covered in the control tubes was 99.8-100%). The difference between the control and experimental samples gave the quantity of split cholesterol. The ratio between this value and the cholesterol content in the control sample, in percent, reflected the enzyme activity of the liver. Cholesterol was determined by the method of Sperry and Webb [8]; the time for which the samples were boiled was shortened to 30 min.

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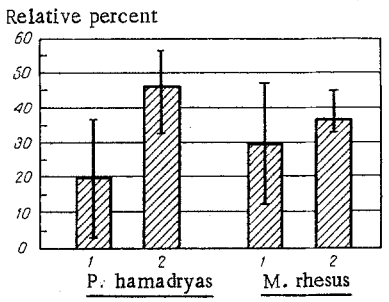

Fig. 1. Quantity of cholesterol (in percent) split by monkey liver homogenates (M ± m): 1) control; 2) experiment.

EXPERIMENTAL RESULTS

Prolonged (about 3 years) administration of an excess of cholesterol with the diet was accompanied by a definite pattern of changes in the blood cholesterol concentration of the monkeys. During the first 4-5 weeks of the experiment, the blood cholesterol level varied within normal limits, not exceeding 200 mg%, and for the next 5-6 months a clear hypercholesteremia (300-400 mg%) was observed. Subsequently the blood cholesterol concentration fell again [2]. In monkeys sacrificed at the height of the cholesteremia, no morphological changes of atherosclerosis were found macroscopically.

In tests on P. hamadryas, the liver enzyme activity of the control animals averaged 18.5% of split cholesterol, compared with 43.5% for the experimental animals. The mean drug cholesterol concentration of the experimental monkeys at this time was 90 mg%, the same as in the control monkeys. In the experiments on M. rhesus, the liver enzyme activity of the monkeys receiving cholesterol also was higher (34.9%) than in the controls (26.7%), but unlike with P. hamadryas, the difference was not statistically significant (Fig. 1). The blood cholesterol concentration in the experimental rhesus monkeys (140 mg%) was undistinguishable from the control. These results show a definite relationship between the state of activity of the liver enzyme system decomposing cholesterol and the blood cholesterol level. This corresponds to the well-known stimulating effect of the substrate on its enzyme, and it suggests that the absence of hypercholesteremia in the experimental monkeys may be associated with increased activity of the corresponding liver enzymes. In fact, in 2 monkeys receiving cholesterol the liver tissue did not split cholesterol at all, and the blood cholesterol concentration at the time of investigation was high (1000 and 340 mg% respectively).

Comparison of the enzyme activity of the liver in rabbits with different degrees of hypercholesteremia confirmed the observations made on monkeys.

In some rabbits fed with cholesterol, hypercholesteremia (average 306 mg%) was observed for 2 months. Their liver enzyme activity was low (mean 19.1%). In the remaining experimental rabbits the cholesteremia was moderate (142.5 mg%) while the liver enzyme activity was high (42.1%). Differences between the groups are statistically significant. The liver enzyme activity in the control rabbits (not receiving cholesterol) varied considerably. In some animals it was high (up to 43.9%), in others it was low (10.2%). Rabbits with high initial liver activity may perhaps correspond to that proportion of animals resistant to alimentary cholesterol loading frequently observed among experimental groups.

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