Stability of Serum Cholesterol Concentration in Rats after Injection of Deoxyribonucleic Acid (DNA). (28265)

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Savitsky(1-3) recently reported that a small quantity of heterologous bovine deoxyribonucleic acid (0.05 to 2.5 mg per rabbit) can produce an irreversible fall in serum cholesterol concentration in rabbits. The fall occurs after a latent period approximately proportional to the dose administered—the higher the dose, the shorter the latent period.

If these findings can be confirmed and shown to have general validity, they would be of great importance, practical and theoretical. Apart from the possibility of specific therapeutic application of DNA in cases of hypercholesteremia, it would be possible to develop a direct test for a specific biological effect of DNA in mammals. In this report, we have attempted to repeat the experiments reported in Savitsky(1-3) on rats.

Methods. Female Wistar rats weighing 200-220 g and with serum cholesterol levels of 0.85-1.00 g/l were divided into 4 groups, *viz.*: Group 1, 7 control rats; Group 2, seven rats fed a hypercholesterolemic diet; Group 3, 8 rats receiving DNA; Group 4, 8 rats fed a hypercholesterolemic diet and receiving DNA.

The non-hypercholesterolemic diet consisted of standard biscuits containing maize, oats, barley, fine bran, crumbs of pastry, peanut, cooked soya, brewer's yeast (4%), meat, fish, a mineral concentrate, codliver oil (0.5%) and supplements of Vit. A (55,000 I.U./100 kg) and Vit. D (50,000 I.U./100 kg). The hypercholesterolemic diet consisted of standard biscuits (48.2%), butter (40%), cholesterol (5%), thiouracil (0.3%), cholic acid (2%), codliver oil (0.5%), and brewer's yeast (4%). The rats were given 30 g of either diet per day per animal with water *ad libitum*.

DNA was obtained by the method of Kay, *et al.* (4). It contained less than 3% proteins and less than 1% ribonucleic acid; its water content (constant weight 110°) was 27%; the $(E)_p$ at 260 mμ was 6 720; the hyperchromic effect after deoxyribonuclease hydrolysis was 38.4%; molecular weight determined by light scattering was over 4.10^6. The DNA (0.15 ml of a solution of 1 mg/ml in NaCl 0.15 M) was intravenously administered on the 1st, 4th, 8th, and 12th days.

The animals were individually weighed every day; serum cholesterol concentration was determined every 15 days for 120 days, using the Krieger method(5).

Results. No death was observed among the animals on the non-hypercholesterolaemic diet. On the other hand, rats receiving the hypercholesterolemic diet began to die on the 11th day in the DNA-treated Group 4 and on the 22nd day in Group 2, which did not receive DNA. All of the animals in Group 4 were dead by the 44th day; all of the animals in Group 2 were dead by the 55th day.

DNA injections therefore seemed to enhance the toxic effect of the hypercholesterolemic diet. On the other hand, DNA had no appreciable effect on the general condition or weight of the animals on a normal diet. Serum cholesterol values, for animals in Groups 1 and 3 did not change significantly during the experiment. Data are given for the 4 groups in Table I.

Discussion. Under the experimental conditions employed, DNA did not affect serum cholesterol level in the normal animals, nor did it counteract the considerable increase in cholesterol level seen in the rats fed the diet.

These results are not consistent with the results obtained by Savitsky(1-3). The following explanations can be offered for this disagreement. a) The DNA administered differed either in quality or origin.

The analytical tests given by Savitsky seem satisfactory. However, he does not indicate RNA concentration of the DNA used, which was prepared from liver—an organ particularly rich in RNA. On the other hand, the Biuret reaction is not sufficiently sensitive to exclude with certitude the possibility of pro-

TABLE I. Changes in the Serum Cholesterol Concentration in Rats Treated with DNA and Fed Normal or Hypercholesterolemic Diets.

Days	No. rats	Cholesterol level*	No. rats	Cholesterol level*
	Group 1—Control		Group 3—Control + DNA	
0	7	0.97 ± 0.06	8	0.92 ± 0.01
120	7	1.15 ± 0.05	8	0.98 ± 0.03
	Group 2—Cholesterolaemic diet		Group 4—Cholesterolaemic diet + DNA	
0	7	0.92 ± 0.02	8	0.89 ± 0.02
15	7	3.94 ± 0.30	6	5.22 ± 0.33
30	3	4.65 ± 0.71	1	3.82

* In g/l. Mean ± σ (standard deviation).

tein contamination. Only the Lowry method (6) affords complete certitude in this respect. Apart from this, the origin of DNA cannot be taken into account to explain the results, for the effect described in rabbits was heterospecific.

b) The quantity of DNA injected varied from 20 μg to 1 mg per kg body weight in rabbits; we gave 4 injections (at 4-day intervals) of 750 μg per kg rat. This dose was much larger than the most active dose administered by Savitsky.

c) There may be a species difference in the response of the serum cholesterol level to DNA. In rabbits, the serum cholesterol level is particularly low in comparison with concentrations found in rat and man. Normal values of cholesterol presented by Savitsky varied from 0.18 to 0.90 g/l. Our normal values, however, are less widespread; serum cholesterol concentration in 45 unselected rats is 0.95 g/l ± 0.16 g/l, the 2 extreme values being 0.69 and 1.33 g/l, respectively.

It seems, therefore, that the phenomenon described by Savitsky in rabbits, even if confirmed, is of a very limited character. This observation can be added to a series of negative experimental data which show the great difficulty, with our present knowledge and current techniques, of demonstrating in higher organisms a specific biological action of DNA, so far established only for viruses and bacteria.

Summary. Contrary to data reported by Savitsky in rabbits, intravenous administration of heterologous DNA to rats causes no change in serum cholesterol concentration; nor did DNA alter the increase in cholesterol values seen in rats receiving a hypercholesterolemic diet.

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