an initial benzene extraction in presence of ammonia. A 250-fold purified preparation from this extract also produced the malformations. It is theorized that the teratogenic material may be an alkaloid of the *Veratrum* series, likely a glycoside or parent alkamine.

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Effect of Injected DNA on a Mild Hypercholesterolemia in Rabbits.* (29179)

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It has been noted in this laboratory that a single injection of purified, undenatured deoxyribonucleic acid (DNA) produced in rabbits a prolonged decrease in serum lipids, with no return to control levels for at least 2 years(1,2). DNA preparations from heterologous, homologous and autologous mammalian tissues were all effective, indicating that no new, specific, transferable, genetic information was introduced into the animals by the DNA(3). The lowered mean serum cholesterol levels largely reflected the significant decrease in those rabbits which maintained high normal values. Varying the amount of DNA injected over a 50-fold range showed a dose-response relationship in length of time required for the changes to appear(2).

It would be interesting to know what effect the DNA injections produce during an experimental dietary hypercholesterolemia. One such attempt has been reported from this laboratory(4). The feeding of a usually innocuous 0.5% cholesterol diet to rabbits, injected with DNA 4 months before, resulted in death of 100% of the DNA-injected animals. This unexpected lethal effect was not understood but it was hoped that a milder form of hypercholesterolemia might permit experimental study.

Several investigators have induced significant hypercholesterolemias by feeding rabbits saturated fats along with synthetic or semi-synthetic diets(5,6). Steiner and his colleagues(7) produced a more moderate elevation of serum lipids in rabbits by feeding them a standard laboratory diet (non-synthetic, containing small amounts of fat,

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mostly unsaturated) supplemented with 12% hydrogenated coconut oil. This mild hypercholesterolemia appeared, possibly, to be the proper tool for the experiments planned. A preliminary pilot study showed that DNA-injected rabbits tolerated the saturated fat diet comparably to normals. The present report describes the changes in serum cholesterol and phospholipids in rabbits injected with varying amounts of DNA and fed a supplement of saturated fat.

Methods. DNA was prepared from frozen rabbit liver by methods previously described (1), *i.e.,* extraction with high ionic strength salt and deproteinization with chloroform-amyl alcohol. The fibres were dissolved and stored for 2 weeks (—15°C) in a .015 M sodium citrate plus .15 M sodium chloride buffer (pH 7.1). Immediately before use, the DNA was precipitated with 2 volumes of ethanol and redissolved in isotonic saline for injection. The physical and chemical properties were those of a highly purified, polymerized, undenatured, 2-stranded DNA preparation, as reported previously(1-3).

Total serum cholesterol was determined by the method of Abell *et al*(8). Serum lipid phosphorus was determined by the Fiske-Subbarow method(9) on serum extracted with Bloor's reagent. The lipid phosphorus was multiplied by a factor of 25 to obtain the phospholipids.

At weekly intervals, the saturated fat feed was prepared by adding warmed, liquid hydrogenated coconut oil to powdered standard rabbit feed (Rockland) to a final concentration of 15% by weight. This was mixed thoroughly, stored in a refrigerator, and fed daily *ad libitum* to the rabbits.

Albino rabbits of the New Zealand strain weighing 2-3 kg were kept in temperature-controlled, air-conditioned, separate quarters and fed a standard chow *ad libitum*. All the animals were kept at least 6 weeks in these quarters prior to use, to permit them to adapt to the new, constant environment. Only those rabbits were used which ate well, showed a steady gain in weight and no evidence of disease.

Experimental design. Three groups of normal rabbits (15 per group) were injected intravenously with varying amounts of DNA in isotonic saline; 100 μg, 250 μg, and 1000 μg DNA. One group of controls (15 rabbits) received isotonic saline. Experimental and control animals were fed a standard chow for one year. During this time the serum cholesterol and phospholipids decreased significantly below control levels in the DNA-injected rabbits. At the end of a year, the healthy, surviving animals (for numbers, see Table I) were placed on a powdered, standard feed containing 15% hydrogenated coconut oil. To permit the rabbits to adapt to the powdered feed, one month before starting the fat diet the pellets of standard feed were replaced with the same feed in powdered form. The animals were bled from the ear vein at monthly intervals and serum cholesterol and phospholipids determined. The experiment was stopped after 4 months of the supplementary fat because at this time all the animals, experimental and control, were beginning to appear ill and to lose small amounts of weight.

Results. Three groups of experimental, DNA-injected rabbits and one control group were fed a saturated fat supplement for 4 months. Changes during the fat-feeding period in the total serum cholesterol and phospholipids are demonstrated in Table I and Fig. 1 and 2. All the animals, experimental and control, developed a mild hypercholesterolemia while eating the diet containing the 15% hydrogenated coconut oil. All the groups of rabbits injected one year before with DNA had significantly lower serum lipid levels prior to the start of fat feeding. At the end of one month of fat feed, all 3 DNA-injected groups had significantly lower lipid levels than the controls. However, the continued fat feeding resulted subsequently in differences between the groups injected with varying amounts of DNA. The 1000 μg DNA rabbits maintained a lower serum cholesterol and phospholipid level during the full 4 months of the fat diet. The 250 μg DNA animals maintained lower lipid levels for 2-3 months and then began approaching control values. The 100 μg DNA group had significantly lower serum cholesterol and phospholipids for only one month and levels returned

TABLE I. Mean Serum Lipids of Rabbits During Feeding of Saturated Fat Supplement.

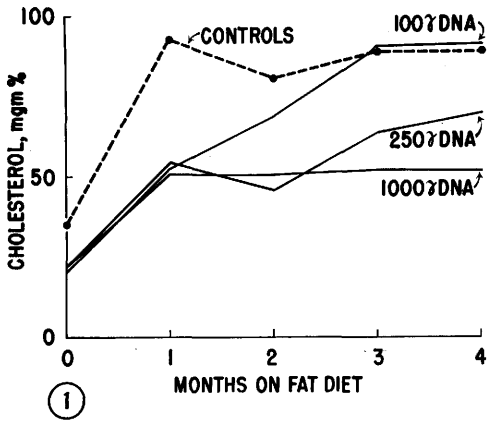
Experimental groups	I 100 μg DNA	II 250 μg DNA	III 1000 μg DNA	Controls
	Mean serum cholesterol (mg % ± S.D.)			
No. of rabbits	11	10	12	11
Pre-fat feed	21 ± 4†	22 ± 3†	22 ± 4†	35 ± 12
1 mo fat feed	52 ± 8†	54 ± 9†	51 ± 9†	93 ± 22
2 " " "	69 ± 18	46 ± 10†	51 ± 9†	81 ± 20
3 " " "	89 ± 29	63 ± 11*	52 ± 9†	89 ± 22
4 " " "	90 ± 23	69 ± 22	52 ± 8†	87 ± 22
	Mean serum phospholipids (mg % ± S.D.)			
Pre-fat feed	70 ± 11*	71 ± 10*	73 ± 13*	92 ± 21
1 mo fat feed	121 ± 32*	129 ± 30*	116 ± 32*	183 ± 42
2 " " "	156 ± 36	126 ± 33*	120 ± 33*	172 ± 41
3 " " "	173 ± 47	139 ± 41	123 ± 26*	181 ± 50
4 " " "	174 ± 37	144 ± 37	130 ± 33*	170 ± 42

* Difference from controls significant, $p < .01$.
† Difference from controls significant, $p < .001$.

to control values by the third month of fat feeding. In short, there was a quantitative, direct relationship between amount of DNA injected and length of time the rabbits maintained lower serum lipids when fed a saturated fat diet.

Discussion. These data indicate that DNA-injected rabbits responded normally, with a mild hypercholesterolemia, to the feeding of a saturated fat. The experimental animals, injected with DNA one year before, maintained lower serum lipid levels both on the normal diet and during the fat feeding. It appears that the "set point" of the serum lipid controls was lower in the DNA-injected animals, but the normal hypercholesterolemic mechanisms of physiologic response to dietary lipid were intact. These results differ from our earlier experiments where feeding a moderate cholesterol supplement to DNA-injected rabbits resulted in lethal effects(4). The toxicity of DNA in severely hypercholesterolemic rats has also been reported by Lamonthezie *et al*(10). Probably, the absence of untoward effects in the experiments reported here is related to the mildness of the lipid stress.

A most interesting finding was that the duration of lower serum lipid levels, while on the fat feed, varied directly with amount of DNA injected. Previous studies of DNA-injected rabbits on normal diets has revealed no return to control values of the lowered



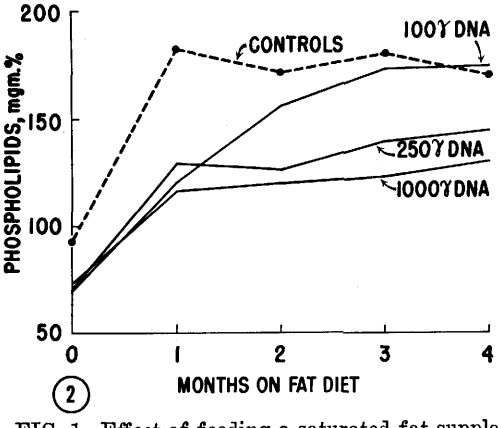


FIG. 1. Effect of feeding a saturated fat supplement on mean serum cholesterol of 3 DNA-injected groups and one control group.

FIG. 2. Effect of feeding a saturated fat supplement on mean serum phospholipids of 3 DNA-injected groups and one control group.

serum cholesterol for at least 2 years(2). This suggests that the injected DNA altering serum lipid regulation may be in some form of a "depot"; utilized slowly (years) under normal dietary conditions and utilized more rapidly (months) when the animal is fed a saturated fat. It appears, for the first time in these studies, that the prolonged, "non-genetic" alteration of serum lipids induced by DNA may not be permanent.

Summary. Three groups of normal rabbits were injected with varying amounts of DNA along with a control group which received saline. One year after the DNA injections, the animals were fed a standard feed supplemented with 15% hydrogenated coconut oil for 4 months. All the animals, experimental and control, developed a mild hypercholesterolemia. At the end of one month of fat feed, all 3 DNA-injected groups had significantly lower serum lipid levels than the controls. However, the continued fat feeding resulted in a quantitative, direct relationship between amount of DNA injected and length of time the rabbits maintained lower serum lipids.

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Iron Deficiency in Monkeys Fed Diets Containing Soybean Protein.* (29180)

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Certain foods reduce iron absorption from the gastrointestinal tract(1). In comparison to many foods the iron of soybeans and of diets containing isolated soybean protein has been found to be well absorbed(2-4). However, in the experiments to be described we observed reduced gastrointestinal absorption of iron and iron deficiency anemia in monkeys receiving a diet containing isolated soybean protein.

Methods. Young Rhesus monkeys *(Macaca mulatta)* of both sexes initially weighing 1.5 to 2.5 kg were used. Different groups received basal diet alone (Table I), basal diet supplemented with 100 mg of choline per 100 g diet, or basal diet supplemented with 100 mg of choline, 630 mg of methionine and 73 mg of cystine per 100 g of diet. Preparation of the diets including heating in an oven to form a cake has been described previously(5). Some of the animals in each group received on their food 80 mg of DL alpha-tocopheryl acetate dissolved in ethanol 3 times weekly. Food and water were given once daily.

Hemoglobin concentrations and reticulocyte counts were obtained by standard hematologic techniques. Bone marrow examinations were done on direct smears of material obtained by tibial aspiration. Serum iron concentration was determined by the method of Trinder(6) and serum iron binding capac-

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