tion, administration of one further desensitizing dose of endotoxin brought about the return of "nearly complete tolerance" to its febrile effect at subsequent challenge. This discrepancy may be due to a fundamental difference in the reactions employed in the two studies, or to a difference in the sensitivity with which they can be observed. The present observations suggest that at least one parameter of endotoxin tolerance may differ from classical immune systems in both its duration and its susceptibility to recall.

While the pyrogenic effect of endotoxin can be prevented by the passive transfer of serum or serum fractions from rabbits given repeated doses of endotoxin it is difficult to explain why epinephrine induced dermal necrosis could not be abolished in a similar way. If protection against the pyrogenic response represents the action of antibody, it is possible that larger amounts of antibody are needed at the local site to protect against hemorrhagic necrosis. The present study points out that resistance to dermal necrosis differs in an unknown manner from resistance to endotoxin-induced fever or the lethal effect of endotoxin, both of which can be prevented by passive transfer of serum.

Summary. Several aspects of hemorrhagic necrosis of rabbit skin following intravenous injection of endotoxin and intradermal administration of epinephrine have been studied. The reaction was consistently reproduced using 100 μg epinephrine and 10 μg endotoxin. It could be abolished by an 8-day course of intravenous endotoxin as previously demonstrated. No resistance to the reaction could be shown to persist 6 days following immunization. No accelerated response to a booster course of endotoxin injections could be demonstrated during the fourth week following the primary injections. Hemorrhagic necrosis could not be prevented by the passive transfer of 10 ml of whole serum, or with serum fractions separated by DEAE cellulose chromatography and rich in the individual classes of immune globulins. The specificity of dermal necrosis induced by epinephrine in endotoxemia remains unclear. It is not possible at this time to define the nature of the temporary protection against epinephrine on an immunological basis.

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Intensification of Experimental Atherosclerosis by Semi-Starvation Diet.* (31940)

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It is well known that addition of 1% cholesterol to the diet of the rabbit produces extensive deposits of atherosclerotic plaques in thoracic aorta within about 12 weeks. It has been shown previously(1,2), that there is little or no regression of the preformed plaques when the animals are returned to normal diets. It was of interest therefore to test the influence of a semi-starvation regime on regression of the plaques. It was found

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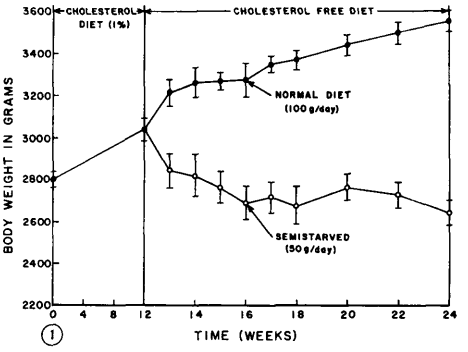
somewhat unexpectedly that a 50% reduction in the normal food intake over a 3-month period brought about a marked increase in the severity of atherosclerotic lesions as compared to the lesions in animals maintained on an adequate food intake. An explanation for this effect is suggested.

Materials and methods. A total of 37 New Zealand white male rabbits 5-6 months in age, were fed a diet of 1% cholesterol mixed with 100 g food pellets for 3 months. Rabbits were bled from an ear vein at 2-3 week intervals and plasma cholesterol was measured on the extracts as described previously (2). After 12 weeks, 10 of the animals were killed and the intensity of atherosclerosis in the thoracic aorta was measured. This was done by slitting the aorta up one side and transferring the pattern of plaques freehand onto a standardized grid stencil on squared paper. All scoring was run "blind" by 2 separate investigators and the plaque grade was expressed as the average percentage of the aorta surface covered with plaques.

The remaining animals were divided into two groups. One group of 14 animals was fed a diet consisting of 100 g plain pellets daily and the other group of 13 animals was fed a ration of only 50 g of food pellets. Animals in both groups were weighed each week, plasma cholesterol levels were determined at 1-2 week intervals and a measure of the visible plasma-lipemia was obtained by recording the optical density of the plasma samples in a 0.5 ml cuvette at a wavelength of 700 mμ. After a further 12 weeks the animals in both groups were killed and the intensity of plaques in the thoracic aorta was measured.

Results. Following 12 weeks on the cholesterol diet the mean serum cholesterol levels increased from 63 ± 5 mg% to 2064 ± 164 mg%. The intensity of atherosclerosis in the 10 animals which were killed at this time was 24 ± 4%. During this initial 12 week period of feeding 1% cholesterol, the average weight of the rabbits increased from 2798 ± 38 g to 3036 ± 58 g. This weight increase was maintained in the 14 animals fed the 100 g of plain pellets daily for the next 12 weeks, the average weight increasing to 3550 ± 55 g. The semi-starvation diet of 50 g daily

BODY WEIGHT CHANGES IN SEMISTARVED VERSUS NORMALLY FED RABBITS



SERUM LIPEMIA LEVELS IN SEMISTARVED VERSUS NORMALLY FED RABBITS FOLLOWING A 12 WEEK PERIOD OF CHOLESTEROL FEEDING

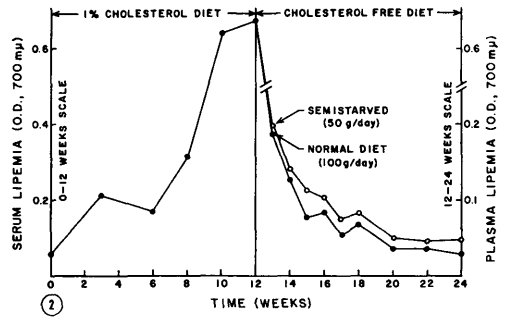


FIG. 1, 2, and 3. A total of 37 New Zealand white male rabbits were fed a daily diet of 100 g commercial rabbit pellets and 1 g of cholesterol for 12 weeks. At 12 weeks 10 of the animals were killed and atherosclerotic plagues in the thoracic aorta were measured. The remainder was divided into 2 groups. One group (14 animals) was fed 100 g plain food pellets daily and the other group (13 animal)s a semi-starvation diet of 50 g pellets daily. Body weight, serum cholesterol and serum lipemia levels were measured weekly. After a further 12 weeks all animals were killed and the severity of atherosclerotic plaques was measured (Table 1).

SERUM CHOLESTEROL LEVELS IN SEMISTARVED VERSUS NORMALLY FED RABBITS FOLLOWING A 12 WEEK PERIOD OF CHOLESTEROL FEEDING

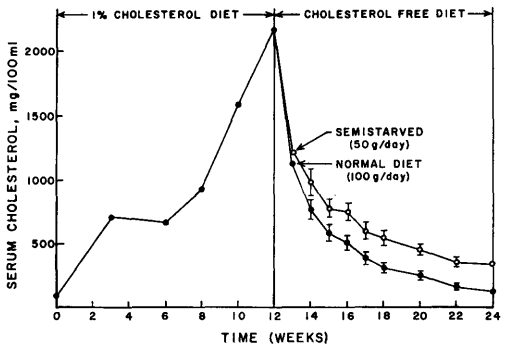


TABLE I. Atherosclerotic Plaque Grades in Semi-Starved *vs* Normally-Fed Rabbits Following a 12-Week Period of Cholesterol Feeding.

Dietary treatment	No. of rabbits	Avg plaque grade, %*
1% cholesterol for 12 weeks	10	24 ± 4
Idem, then normal diet (100 g/day) for 12 weeks	14	36.0 ± 5.9
" , then semi-starvation diet (50 g/day) for 12 weeks	13	58.8 ± 7.6

* % of aorta surface covered with atherosclerotic plaques.

was sufficient to maintain the other group of 13 animals in good health but led to a small but consistent loss in weight of about 33 g per week (Fig. 1). During this final 12 weeks on cholesterol-free diet, plasma lipids and the visible plasma lipemia, although falling sharply in both groups, remained consistently higher in the group maintained on the semi-starvation diet. When the animals were killed after 24 weeks, the group maintained on the semi-starvation diet had an atherosclerotic plaque intensity of 58.8 ± 7.6% as compared to only 36.0 ± 5.9% for the group maintained on an adequate food intake.

Discussion. It is known that the development of atherosclerotic lesions in rabbit aorta in response to addition of cholesterol to the diet, is a function not only of the plasma cholesterol level but also of the duration of the hypercholesterolemia(3).

Although intensification of atherosclerosis in the semi-starved animals may have been due to a specific nutritional deficiency, it seems more probable in the light of these results that it is related to the consistently higher plasma lipid levels during the period of starvation. These higher levels of plasma lipids were being maintained presumably by mobilization of depot fat in response to the inadequate caloric intake. The fact that the severity of atherosclerotic plaques was actually increasing during this period of weight loss and mobilization of depot fats illustrates that the plaque lipids are relatively inert in responding to those influences which deplete other lipid depots.

Conclusion and summary. Following a 12-week period of feeding 1% cholesterol, rabbits developed atherosclerotic plaques in thoracic aorta having an average severity (percent of aorta surface covered with plaques) of 24 ± 4%. Following return to a normal cholesterol-free diet for 12 weeks there was a small increase in plaque intensity to 36 ± 6%. In contrast, in a group of rabbits maintained on a semi-starvation level cholesterol-free diet, there was a striking increase in plaque intensity to 59 ± 8%. These results are interpreted in terms of a more severe hyperlipemia which was found in this latter group of animals in response to the reduced caloric intake rather than to any specific nutritional deficiency.

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