Campbell, H. W., *J. Clin. Invest.*, 1947, v26, 90.
13. Seibert, F. B., Pfaff, M. L., and Seibert, M. V., *Arch. Biochem.*, 1948, v18, 279.
14. Gutman, A. B., *Advances in Protein Chem.*, v4, Academic Press Inc., New York, 1948, 155.
15. Rutstein, D. D., Ingenito, E. F., and Reynolds, W. E., *J. Clin. Invest.*, 1954, v33, 211.

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Effect of Caloric Restriction on Cholesterol Atherogenesis in the Rabbit. (21301)

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The effect of caloric restriction on the genesis of atherosclerosis has received very limited attention. Studies of human obesity(1-3) and surveys of the nutritional and health status in various countries(4,a,b) have resulted in a general impression that low caloric intake exerts a protective action in any atherogenic situation. Scanty information is available concerning the influence of caloric restriction on the development of experimental atherosclerosis(5,6). The present study was undertaken to furnish more detailed findings regarding the effects of severe caloric restriction in rabbits fed cholesterol.*

Our experimental results are significant because they 1) illustrate the fallacy of regarding severe caloric restriction as the opposite of obesity and 2) point out the necessity for paying careful attention to the diet and weight of rabbits subjected to experimental procedures or unusual diets designed to elucidate factors involved in atherogenesis.

Material and methods. Male, adult Swiss rabbits weighing between 3.5 and 5.5 kg were kept in the laboratory for several weeks prior to the experiments and the average daily food intake necessary to maintain weight equilibrium was estimated. A standard food mixture† was used which has the following content of nutrients, minerals and vitamins: fiber 15%, fat 3%, protein 17.50%, calcium 1.66%, P .37%, I .0033%, salt 1.00%; pantothenic acid 13-14 mg/lb., niacin 17.5 mg/lb., thiamin 1.28 mg/lb., riboflavin 3.40 mg/lb., biotin 0.16 mg/lb., vit. C .81 mg/lb., vit. D 450 USP units/lb., vit. E 30 mg/lb., choline 640 mg/lb. This diet was supplemented with 10-20 g/day raw carrots. Two series of experiments were carried out differing in 1) duration, 2) degree of caloric restriction, 3) dose of cholesterol, and 4) mode of cholesterol administration. The control groups in both experiments were fed *ad libitum;* the average consumption approximated 130 g/day of the standard ration. There were 18 animals in Exp. A and 22 in B. Half of this number were on restricted food intake, the rest served as control. *Duration.* Exp. A lasted 12 weeks, Exp. B 8 weeks. *Degree of caloric restriction.* The underfed group in Exp. A received 50 g/day, Exp. B 30 g/day of rabbit pellets plus 20 g raw carrot. This constituted approximately ½ and ⅓ of average control ration. *Dose of cholesterol.* 1 g/day in Exp. A and 2 g/day in Exp. B. Thus the absolute cholesterol content in the groups on adequate and on restricted calories was the same, but the percentile content varied, being considerably higher in the restricted diets. *Mode of Administration.* Measured amounts of crystalline cholesterol were mixed thoroughly with shredded raw carrots without adding any oil. In Exp. A the rabbits consumed the carrot-cholesterol mixture at the same time as their pellets. In Exp. B regular feeding was delayed until 4 to 5 hours after completion of cholesterol ingestion. The uneaten

* While the manuscript was being prepared for publication, an article appeared by McMillan, Whiteside, and Duff (*J. Exp. Med.*, 1954, v99, 261) dealing with the same experimental problem. It is of interest that the findings and conclusions are virtually in agreement with ours.

† B-B Laboratory Rabbit Diet (Maritime Milling Co., Inc., Buffalo, N. Y.)

TABLE I. Average and Range of Values for Total Serum Cholesterol, Phospholipids, Fatty Acids and Lipoproteins in Rabbits Fed Cholesterol on Full and Restricted Rations. Ranges are in parentheses.

Experiment		A		B	
Diet group:		Full	Restricted ½	Full	Restricted ⅔
Dose of cholesterol:		1 g/day	1 g/day	2 g/day	2 g/day
Duration of exp.:		12 wk	12 wk	8 wk	8 wk
Avg wt, kg	I.*	4.3	4.54	3.71	3.85
	F.*	4.28	3.55	3.91	2.85
Avg wt change, kg		−.02	−.99	+.20	−1.00
Cholesterol, mg %	I.	29.7 (20.4–39.2)	31.0 (17.4–38.2)	50.9 (26.9–65.8)	43.0 (30.9–45.7)
	F.	236.0 (121–682)	943.0 (755–1.670)	330.0 (64–432)	786.0 (565–1012)
Phospholipids, mg %	I.	2.9 (2.4–3.7)	3.0 (2.3–3.4)	3.84 (2.2–5.7)	3.48 (2.6–5.6)
	F.	6.7 (5.3–15.2)	15.3 (9.5–25.4)	9.24 (6.0–18.0)	15.28(13.1–18.0)
Fatty acids, mg %	I.	106.0 (79–128.0)	114.0 (82–162)	147.0 (82–220)	128.0 (81–220)
	F.	213.0 (141–590)	480.0 (412–852)	295.0 (164–491)	454.0 (338–685)
Lipoproteins, mg %					
S_f 0– 11	I.	—	—	—	—
	F.	—	—	215 (65–310)	205 (80–530)
11– 21	I.	19 (11– 36)	20 (10– 29)	—	—
	F.	159 (62–427)	561 (215–1030)	350 (93–780)	493 (340–680)
21– 35	I.	14 (6– 29)	16 (9– 21)	—	—
	F.	122 (30–440)	632 (355–1200)	278 (20–665)	509 (308–863)
35–100	I.	16 (4– 32)	27 (5– 46)	—	—
	F.	74 (29–243)	499 (333– 800)	278 (12–520)	482 (250–760)
100–400	I.	12 (2– 31)	20 (5– 41)	—	—
	F.	36 (12–130)	324 (76– 650)	207 (5–470)	246 (85–600)

* I. = Initial; F. = Final.

cholesterol was carefully gathered and weighed at the end of ingestion period. The average unit uneaten was 0.1 g with a variation of .05 to 0.2 g/day and no significant difference between control and underfed or between individual rabbits could be found. In both experiments the following biochemical tests were carried out: 1. Total blood cholesterol, using method of Abell, Levy, Brodie and Kendall (7); 2. Blood fatty acids, using method of Stern and Shapiro(8); 3. Lipid phosphorous using method of Youngburg and Youngburg (9); 4. Blood lipoproteins using method of Gofman and associates(10).‡ Each determination was run in duplicates and agreement within 3% was required. In Exp. A these tests were made at beginning and after 5, 10 and 12 weeks; the lipoproteins were not determined at the 5 week interval. In Exp. B lipoproteins were determined only once, at the end of experiment, while the other tests were done at beginning and after 4 and 8 weeks. Blood samples were taken by heart puncture at beginning and end of experiments and by withdrawal from earveins at intervals.

Animals were weighed weekly. At end of experiments all animals were sacrificed and their aortas examined for degree of atherosclerosis. In Exp. A only a gross examination was done, in Exp. B gross inspection and microscopic examination were performed. A grading system from 1+ to 4+ was employed where 1+ indicated minimal lesions and 4+ extensive lesions with plaque formation involving entire length of vessel. In the present report only the range of values and their averages at beginning and end of experiments are given; individual findings will be reported elsewhere.

Results. Since both experiments gave rather similar results, they are reported together. The data are presented in Table I.

Weight. Control groups in Exp. A and B showed little change in their weight. The underfed group of 9 rabbits in Exp. A lost in 12 weeks an average of 0.99 kg or approximately 20% of initial weight. Only 2 animals lost less than 10% and one more than 30%

‡ The lipoprotein determinations were processed in the Viral and Rickettsial Research Laboratories, Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y. We wish to express our gratitude to Dr. Raymond A. Brown for his cooperation.

(42%). The 11 animals in Exp. B, kept under caloric restriction for 8 weeks, lost an average of 1 kg, or approximately 26% of initial weight with a variation from 19% to 31.2%.

Biochemical findings. Blood cholesterol showed in both experiments, a rise in control group as in the underfed group. In both experiments, however, the rise was much more pronounced and significantly higher in the underfed groups than in adequately fed groups. The larger dose of cholesterol in Exp. B was reflected also in a higher degree of cholesterolemia in animals on full diet and those on subnutrition. The shorter duration of this experiment and the different intervals at which the tests were taken do not permit, however, an exact comparison.

Blood fatty acids. In both experiments there was a marked increase in fatty acids. Again average values of underfed groups were markedly higher than those on full ration. The differences between these 2 groups were found to be statistically significant.

Lipid Phosphorous. There was a steady rise of lipid phosphorous in control as well as underfed groups. Again the values were continuously higher in subnutrition groups.

Lipo-proteins. Serial observations are available only for Exp. A where the S_f fractions 11/21, 21/35, 35/100, and 100/400 were determined at beginning and after 10 and 12 weeks. All fractions showed a gradual increase. In the full nutrition group the increases were relatively small and significant only in S_f 11/21, and 21/35. In the underfed group the differences were significant in all fractions, with the greatest increase in S_f 11/21 and S_f 21/35. In Exp. B, lipoproteins were determined at the end of the observation period only. Here the 0/11 fraction was also estimated. If the initial values of Exp. A can be used as a baseline, then statistically significant increases were observed in both control and underfed groups, with the latter showing still higher values than the former. Differences between both groups were not significant in S_f 0/11 and 100/400, but had statistical significance in S_f 11/21, 21/35 and 35/100.

Vascular changes. Gross inspection of the aortas after termination of experiments showed arteriosclerotic changes in both groups. Although advanced and extended lesions were found in animals on full rations and on restricted rations, the impression was that these were more pronounced in underfed animals. The anatomical differences were, however, less marked than the biochemical changes. In the underfed group of Exp. B, only one aorta had minimal lesions, 3 showed maximal lesions, and the changes in the remaining 7 were 2+ and 3+, while in the group on full nutrition, 5 aortas were practically free of degenerative changes, while 1 had maximal changes and the 5 remaining showed moderate lesions (2+ to 3+). The gross findings were confirmed by microscopic examination. The degree of severity of lesions, and the difference between control and underfed groups, might find special significance in view of the relatively short duration of observation (8 weeks). It is likely that a higher degree of alteration would have developed in the control groups also, had the experiment been permitted to run for 12 weeks or longer.

Discussion. It is evident from the results that cholesterol feeding caused significant biochemical changes and atherogenesis both in animals on full nutrition as well as those on caloric restriction, the changes being even more severe in underfed group of rabbits. It will be noted that in both feeding groups, the rise of the cholesterol level is not as high as that observed by other investigators. This difference is probably explained by the fact that cholesterol was fed in our experiments without the admixture of other lipids. This fact may also be of interest in view of the observed increase of fatty acid levels in both groups of animals, the control as well as underfed groups.

From these experiments it appears that in the presence of high cholesterol intake, caloric restriction not only does not give any protection against degenerative vascular disease, but seems to enhance it. Similar observations were made by Rodbard, Bolene and Katz(6) in cholesterol atheromatosis of the chick, while Firstbrook(5) reported in his rabbit experiments a parallel between degree of cholesterol atherogenesis and caloric intake. In man, Walker(2) found that weight reduction without dietary cholesterol restriction is associated

with significant reduction of the lipoprotein fractions, particularly the larger aggregates, and a fall in total blood cholesterol level. Whether this difference in results represents a species difference or is due to different amounts of cholesterol ingested will have to be explained. The dietary cholesterol in our experiment was proportionally far higher than that in Walker's observations.

Summary. 1. Rabbits on severe caloric restriction were fed cholesterol and showed significantly increased blood cholesterol, fatty acids, lipoproteins and phospholipids. The degree of these biochemical changes and of the atherosclerosis was greater than that of a control group fed similar amounts of cholesterol in addition to a normal diet. 2. Therefore, severe caloric restriction does not protect, but enhances cholesterol induced atherosclerosis in rabbits. This effect of weight loss should be taken into consideration in experiments on atherosclerosis.

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1. Walker, W. J., *Ann. Int. Med.,* 1953, v39, 705.
2. Wilens, S. L., *Arch. Int. Med.,* 1947, v79, 129.
3. Pomeranze, J., Lucarello, R. J., and Goldbloom, A. A., *Circulation,* 1953, v8, 439.
4a. Keys, A., *ibid.,* 1952, v5, 115.
b. Keys, A., Vivanco, F., Minon, J. L. Rodriguez, Keys, M. H., and Mendoza, H. Castro, *Metabolism,* 1954, v3, 195.
5. Firstbrook, J. B., *Circulation,* 1950, v2, 464.
6. Rodbard, S., Bolene, C., and Katz, L. N., *ibid.,* 1952, v4, 43.
7. Abell, L. L., Levy, B. B. Brodie, B. B., and Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.
8. Stern, I., and Shapiro, B., *Brit. J. Clin. Path.,* 1953, v6, 158.
9. Youngburg, G. E., and Youngburg, M. V., *J. Lab. Clin. Med.,* 1930, v16, 158.
10. Gofman, J. W., Jones, H. B., Lindgren, F. T., Lyon, T. P., Elliott, H. A., and Strisower, B., *Circulation,* 1950, v2, 161.

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Effect of Chronic Administration of Glucagon to Rats and Rabbits.* (21302)

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Glucagon, the hyperglycemic-glycogenolytic factor of the pancreas, causes a transient increase in blood sugar when it is administered parenterally. Until Staub(1) developed methods suitable for the preparation of highly purified material of standard potency, it was not possible to study the effect of chronic administration of glucagon. This study was undertaken in an attempt to investigate the question: Will glucagon produce diabetes? Daily administration of large doses of glucagon to rats and rabbits for 6 months did not produce a diabetic state, but it did exert an interesting effect on liver glycogen stores.

* I am indebted to Dr. Paul N. Harris for performing the autopsies and carrying out the studies on pathology of the animals. My thanks are due Messers. Thomas Lenahan, Zane Frank, and Darrel Caldwell for their invaluable assistance in these experiments.

Method. Rats and rabbits were injected with glucagon daily for 6 months. Thirty-eight rats, housed in individual cages in air-conditioned animal quarters, received a commercial rat food and water *ad libitum.* Thirty of the rats were injected intraperitoneally each morning with 1 mg of a highly purified, noncrystalline, preparation of glucagon (lot 208-108B-284) in a volume of 0.25 ml. The hyperglycemic potency of this lot is such that 1 mg corresponds to approximately 0.14 mg of crystalline glucagon. This preparation contains less than 0.005 unit of insulin per mg of glucagon. The remaining 8 rats were maintained as control animals and received no treatment. Sixteen rabbits housed in air-conditioned quarters were fed rabbit pellets and water *ad libitum.* Six of these animals were injected intravenously twice a day (7:30 a.m. and 4:00 p.m.) 7 days a week with 1 mg of