EXPERIMENTAL PRODUCTION OF GROSS ATHEROSCLEROSIS IN THE RAT*

By LOUIS C. FILLIOS,‡ Sc.D., STEPHEN B. ANDRUS,§ M.D., GEORGE V. MANN,‖ M.D., AND FREDERICK J. STARE, M.D.

(From the Department of Nutrition, Harvard School of Public Health and the Department of Pathology, Harvard Medical School, Boston)

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(Received for publication, June 12, 1956)

In the past it has been generally conceded that the rat is resistant to atherosclerosis, either spontaneous (1) or induced (2). Many attempts to produce this disease experimentally, based for the most part on cholesterol feeding with or without supplemental procedures, have resulted in no discernible vascular changes (3–12). Several investigators, however, have produced lesions consisting solely of lipide infiltration of various layers of the arterial wall without other histologic changes (13, 14). A third group of experiments has been characterized by lipide-bearing arterial lesions with some degree of proliferative intimal change (15–20). These latter changes, however, have been of such a minimal, inconstant, or atypical nature as to make this group appear relatively far removed from human atherosclerosis. It is difficult to evaluate the lesions produced by Pfleiderer (21) and by Schmidtmann (22) in the absence of more detailed descriptions and illustrations. These authors combined cholesterol and vitamin D feeding with forced exercise and produced lipide cushions in the coronary and renal arteries. In some instances these lesions all but occluded the arterial lumina (Schmidtmann). Aortic changes included, in addition to medial swelling and calcification, some degree of lipide accumulation and elastic tissue changes.

In contrast to this last group of lesions (15–20) are those produced by Wissler and his associates (23–25) and by Malinow *et al.* (26, 27). Here lipide accumulation is accompanied by proliferative changes of an extent that approaches that seen in the human counterpart. The former workers fed a synthetic diet containing cholesterol, lard, choline, and thiouracil with or without a hypertensive regimen. Nearly one-

Supported in part by grants-in-aid from the National Heart Institute (No. 136), National Institutes of Health, Public Health Service, Bethesda; the Albert and Mary Lasker Foundation, New York; J. M. Kaplan Fund, Inc., New York; and the John A. Hartford Memorial Fund.

* A preliminary report was presented at the Federation Meetings, Atlantic City, N. J. April 16–20, 1956.

‡ A portion of this work was carried out while the senior author was a Fellow of the National Heart Institute, Public Health Service.

§ Part of this work was done during the tenure of a Research Fellowship from the National Cancer Institute, Public Health Service.

‖ Surgeon (R), United States Public Health Service. Present Address: Heart Disease Epidemiology Study, Framingham, Mass.

third of the animals so treated for 26 to 40 weeks showed arterial lesions, most extensive in the coronary arteries but with minimal involvement of the aorta and renal arteries. In a recent study (25), these authors found an incidence of coronary artery lesions of 50 per cent in sexually normal rats after 90 to 143 experimental days. Malinow *et al.* combined the feeding of cholesterol or sunflower oil with thiouracil and/or cellophane-induced perinephritis for periods of time up to 110 days. A number of these animals developed lesions in the aorta and the coronary, renal, and carotid arteries. Comparable lesions had been produced by these latter workers by injecting cholesterol in oil into the lumina of doubly ligated femoral arteries (28). Proliferative lesions quite comparable to those of these two groups of workers have been produced in mice by Löwenthal (29). Cholesterol derived from gallstones was administered by intubation over a period of 5 months, the animals being fed bread supplemented by oats. Lesions were commonly found in the aorta and heart valves and occasionally in other arteries including the coronary arteries.

In the present study rats were fed purified diets which contained cholesterol, sodium cholate, and thiouracil. Gross and microscopic arterial lesions were seen in all the animals examined. Induced hypothyroidism and cholesterol feeding have not in the past resulted in arterial lesions (8, 9, 12). The supplementation of these factors with dietary cholic acid by Page and Brown has produced hypercholesteremia and lesions of the reactionless, fat-infiltrative variety mentioned above (14).

Since it has been shown that the protein composition of the diet may influence cholesteremia in the rat (30), a study of the effect of dietary protein on hypercholesteremia and atherogenesis was carried out. The level of protein in the diet was found to influence hypercholesteremia but did not appear to effect the rate of atherogenesis under the experimental conditions described below.

EXPERIMENTAL

The animals used in these studies were albino rats of the Charles River strain. They were housed in individual mesh-bottomed cages in a temperature-controlled room and were weighed weekly. Daily food intake measurements were carried out. The diets used are described in Table I. These were prepared at intervals of 2 weeks and stored at 0–5°C. in closed containers. The cystalline cholesterol[1] used in these diets was dissolved in hot corn oil before addition to the diet.

Experiment A was designed to investigate the influence of age, body weight, and sex on the extent of hypercholesteremia and atherogenesis. Thirty-eight rats of both sexes and varying ages and body weights were maintained on diet I for periods of time up to 363 days (Table II).

In experiment B, the influence of the quantity and quality of the dietary protein on hypercholesteremia and atherogenesis was studied. Three matched groups (II, III, and IV, Table II) of eight male rats per group, were placed on experimental dietary regimens as described in Table I. These animals were so maintained for varying periods up to 193 days.

[1] Cholesterol for these studies was generously supplied by the Armour and Company Laboratories, Chicago. Sodium cholate was supplied through the generosity of the Miles Ames Research Division, Elkhart, through the courtesy of Mr. Paul deHaen.

Sufficient blood for individual serum total cholesterol determinations was withdrawn from the tail vein of each rat at intervals of 4 weeks. At these same intervals selected animals were sacrificed by etherization and exsanguination *via* cardiac puncture. Both serum total cholesterol and beta lipoprotein analyses were carried out on these terminal samples. The serum cholesterol determinations were carried out by the method of Abell *et al.* (33). The serum

TABLE I

Composition of Diets

Composition	I	II	III	IV
	gm.	*gm.*	*gm.*	*gm.*
Casein	20.0	10.0	60.0	—
α-Protein*	—	—	—	20.0
Corn oil	8.0	8.0	8.0	8.0
Cholesterol	5.0	5.0	5.0	5.0
Sodium cholate	2.0	2.0	2.0	2.0
Cod liver oil	2.0	2.0	2.0	2.0
Sucrose	58.3	68.3	18.3	58.3
Salt mixture‡	4.0	4.0	4.0	4.0
p-Aminobenzoic acid	0.1	0.1	0.1	0.1
Inositol	0.1	0.1	0.1	0.1
Choline chloride	0.2	0.2	0.2	0.2
Thiouracil	0.3	0.3	0.3	0.3
Total	100.00	100.00	100.00	100.00

To each kilogram of diet the following vitamins were added:—

Thiamine	5.0 mg.	Folic acid	0.25 mg.
Riboflavin	5.0 mg.	Biotin	0.2 mg.
Pyridoxine	2.5 mg.	Menadione	5.0 mg.
Calcium pantothenate	50.0 mg.	α-Tocopherol	100.0 mg.
Niacin	80.0 mg.		

In addition, 60 gm. of cellu-flour were added to each kilogram of diet.

* The soybean protein (α-protein) has been shown to be deficient in sulfur amino acids when used as a source of dietary protein for chicks (31), rats, mice (30), and *Cebus* monkeys (32).

‡ Hegsted *et al.*, *J. Biol. Chem.*, 1941, **138**, 459.

beta lipoproteins were determined by the ultracentrifuge procedure described by Gofman *et al.* (34).

Complete autopsies were performed on the animals sacrificed as described above. A small percentage (∽12 per cent) of the rats died at various intervals during the experimental period, and only those animals which were autopsied within an hour of death, have been considered in this report. Liver analyses were carried out as described in an earlier publication (30) in which a modification of the Schoenheimer and Sperry method was used for total cholesterol and the methods of Youngberg and Youngberg and of Fiske and SubbaRow were employed for the determination of phospholipides. The heart, aorta, and major aortic branches were opened *in situ*, fixed in 10 per cent neutral formalin and stained in the gross with Sudan IV. These specimens were graded as to the extent of intimal Sudanophilia, photographed in

color, and prepared for microscopic examinations. In grading the gross lesions, designations of 1+ to 4+ were recorded for each artery and for various subdivisions of the aorta and endocardium. Comparable tissues from 17 control rats which were fed only Purina laboratory chow were examined by the same techniques. These latter animals included males and females and ranged in age from 14 days (24 gm.) to 1½ years (450 gm.). Selected viscera were also examined microscopically. The tissue techniques included survey staining methods as well as differential methods for lipides, elastic tissue, ground substance, and collagen. A more detailed account of the histologic techniques will be presented elsewhere (35).

TABLE II

*Distribution of Rats According to Age, Body Weight, and Sex Prior to Dietary Treatment**

Exp.	Rat group	Sex	No. of rats	Approximate age	Body weight‡	Serum total§ cholesterol	Experimental diet (Table I)
				wks.	*gm.*	*mg. per cent*	
A	I	M	8	15	301 ± 18	61 ± 9	I
	IA	M	19	40	461 ± 40	65 ± 10	I
	IB	M	6	10	224 ± 6	64 ± 6	I
	IC	F	5	10	228 ± 8	86 ± 8	I
B	II	M	8	15	311 ± 16	59 ± 4	II
	III	M	8	15	322 ± 18	57 ± 5	III
	IV	M	8	15	324 ± 19	61 ± 6	IV

* Seventeen additional rats were used as histologic controls. (See procedures.)

‡ Mean ± one standard deviation.

§ Mean ± one standard deviation.

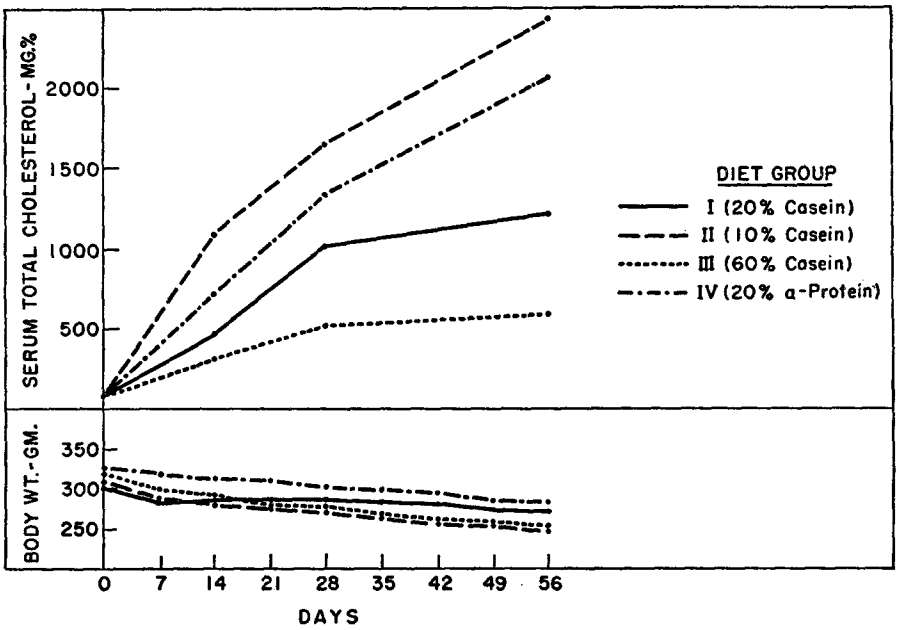
RESULTS

The Effect of a Diet Containing Cholesterol and Sodium Cholate on the Serum and Liver Lipide Components of Rats.—The five female rats (group IC, Table II) had a pretreatment serum total cholesterol of 86 ± 8 mg. per cent compared to a value of 64 ± 6 mg. per cent for the six male rats of comparable age and body weight (group IB). However, there was no apparent sex difference in hypercholesteremic response after diet I was introduced. Similarly, age and initial body weight did not appear to influence significantly the hypercholesteremia.

All the rats fed diet I demonstrated sharply elevated serum cholesterol levels which averaged 1100 mg. per cent after 28 days of dietary treatment with relatively little elevation thereafter. As there were no significant differences in the response of these rats under the present conditions, regardless of age or sex, the response of the rats in group I is shown as representative of all animals fed diet I (Text-fig. 1). Similarly, there were marked elevations of the concentrations of beta lipoproteins in all ranges (S_f0–11, S_f12–20, S_f21–35, S_f35–100, and S_f100–400). The sera were generally lipemic. Interestingly, a close

correlation (r = 0.9) was found to exist between the concentration of beta lipoproteins of the S_f21–100 range and the serum cholesterol levels in all the rats studied including those described in Experiment B (Text-fig. 2). There was no apparent quantitative correlation between beta lipoproteins other than of the S_f21–100 range and the total serum cholesterol.

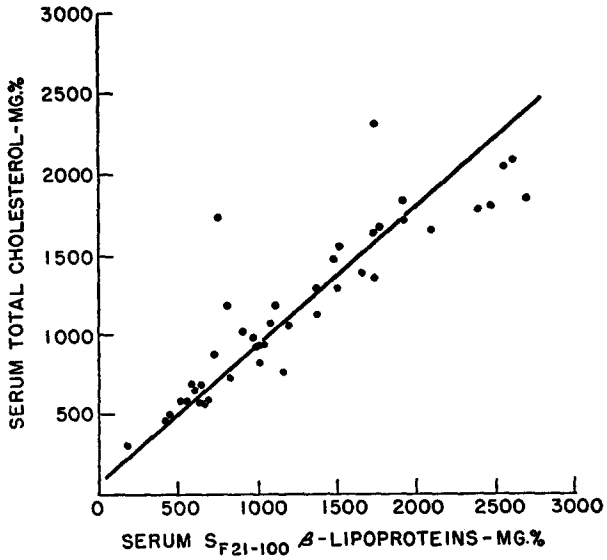
Rats maintained on this dietary regimen for 34 days or more had exceptionally high levels of liver cholesterol as shown in Text-fig. 3. The liver total



TEXT-FIG. 1. The effect of dietary protein composition on cholesteremia in hypothyroid rats fed cholesterol and sodium cholate. Each point represents the mean value of several rats (at least 5; not more than 8).

cholesterol levels reached a maximum average of about 18,000 mg. per cent with time, though levels in excess of 20,000 mg. per cent were not uncommon (normal range: 150–300 mg. per cent). The greatest increase in lipidosis took place in the first 56 days. Thereafter the increase in total lipides and cholesterol concentrations was less marked. It appeared that the liver cholesterol concentration reflected the concentration of total lipides whereas concentrations of liver phospholipides and the total liver lipides appeared to be inversely correlated. The liver weights of these rats showed an increase with time. During this same interval, body weights were decreasing even among immature animals. Although the concentration of cholesterol in the liver increased only slightly beyond the 60th day, the liver weights had approximately doubled by the

end of 282 days. Thus, the total amount of liver cholesterol increased twofold over this period. On the other hand, the total amount of phospholipides per liver did not change appreciably since the concentration of the phospholipides decreased with time as the liver weight increased. The increment in liver weight gain can be specifically correlated in part with the development of cirrhosis in the older experimental animals. Thus, of the 14 rats on the experiment for 195 days or longer, 7 showed cirrhosis while in 7 the alterations were only focal. The mean liver weight of the cirrhotic animals was 30.4 (±9.6)[2] and of the non-cirrhotic group 20.9 (±5.7). The difference between these two



TEXT-FIG. 2. A diagram relating serum total cholesterol to the S_f20–100 levels of rats fed the atherogenic diets (correlation coefficient = 0.9).

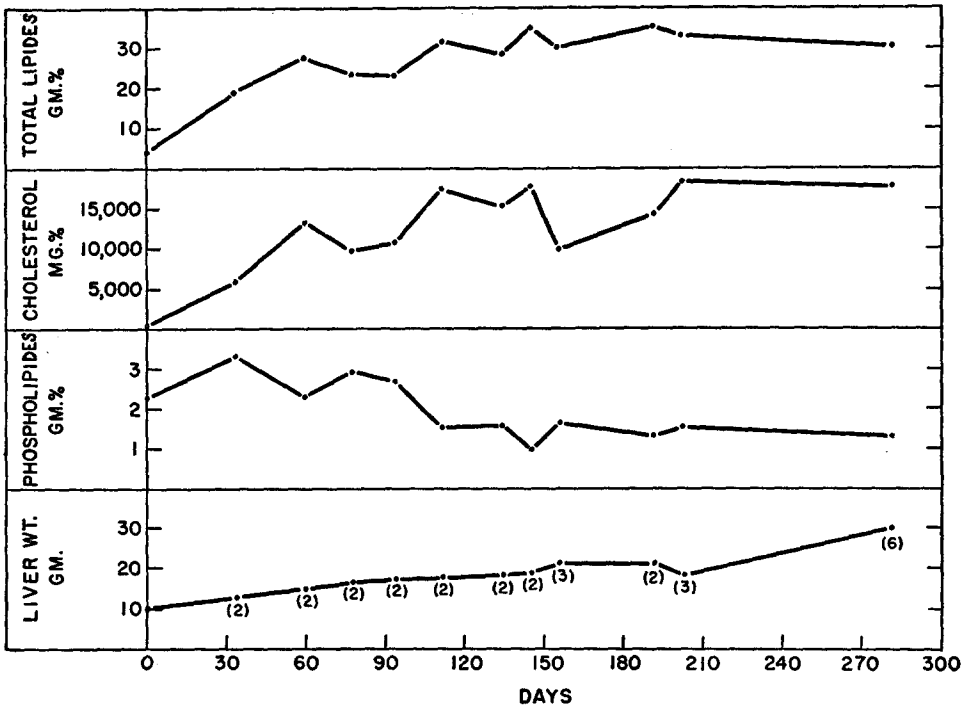
groups of liver weights appears significant ($p < 0.05$). In comparing the above 14 animals, there were no significant differences in the hepatic concentrations of total lipides, total cholesterol, or phospholipides. It is of interest that there were many individual discrepancies in the general positive correlations between the liver and serum cholesterol levels and between the concentrations of the liver lipide components and the various classes of serum beta lipoproteins.

Some loss of weight was noted in all animals during the first 56 experimental days; thereafter such weight loss became negligible. These dietary treatments caused a mild anorexia in all the rats, especially during the first 28 days. The stools were loose and of a pale yellow color.

[2] Mean ± standard deviation.

Pathological Findings in Rats Maintained on Diet I.—Autopsies were performed on 30 rats maintained on Diet I. Of these animals 5 were selected from group I, 17 from group IA, 3 from group IB, and 5 from group IC (Table II). Thus, these animals were predominantly adult males of 40 weeks of age initially, though younger males and females were included. The animals were sacrificed at various periods ranging from 34 to 363 experimental days.

Sudanophilic arterial lesions were present in the gross in all 30 animals. The sites of most severe involvement were the pulmonary artery, the aortic and mitral valves, and the ascending aorta and arch (Fig. 1). Less severe involvement was seen in the tricuspid and pulmonary valves, the lower thoracic and abdominal aorta, and the major arteries arising from the aorta. The extent of the lesions did not appear related to the age or sex of the animals. Fairly elaborate attempts were made to correlate the extent of the gross lesions with the degree and duration of the serum cholesterol elevation. These time and intensity factors were combined by charting serum cholesterol levels with time and measuring the areas beneath the curves by planimetry. A positive correlation existed only in the broadest sense, in that there was a rough progression of the lesions with duration of serum cholesterol elevation. When this relationship was examined in detail there were many discrepancies which could not be related to such additional factors as age and sex of the animals or the presence or extent of liver disease. No centrifugal extension of the lesions was noted with increasing age.



TEXT-FIG. 3. The effect of diet I on the liver lipide components. The numbers of individual animals observed are indicated in parenthesis.

One animal was taken off the dietary regimen after 180 days, returned to the control diet (Purina laboratory chow) and sacrificed after an additional 86 days. There appeared to be

in the gross some regression of the extent of the Sudanophilia. In one animal which died after 282 days of the dietary regimen, the heart showed thinning and discoloration of the left ventricular wall and a large adherent thrombus in the left auricle. Generally, the liver was the only organ to show lipide accumulation of any extent in the gross.

Microscopically, the aortic lesions were characterized at first by extracellular fat and cholesterol deposition in the ground substance of the media and intima. In older lesions the lipides were seen to be predominantly intracellular within foam cells and other mesenchymal cells. Discrete cellular intimal plaques were common, with proliferation of spindle-shaped mesenchymal cells, ground substance, elastic tissue, and collagen (Fig. 2). Medial fat was accompanied by increased ground substance and changes in concentration, form and orientation of muscle cells. Microscopic lesions were found in the coronary arteries, consisting of focal intimal accumulations of ground substance, rich in lipide both extracellular and within foam cells (Fig. 3). Lesions of this nature were seen in 40 per cent of the animals. Frequently, the lumina of such arteries were markedly narrowed. The thinning of the ventricular wall in the animal noted above was found to represent a large healing myocardial infarct. The coronary arteries of this animal showed the intimal lesions described and one small branch was occluded by what appeared to be a recanalized thrombus.

The liver cells contained massive amounts of fat and cholesterol and after an initial lag the Kupffer cells began to accumulate fat in focal areas. The cirrhosis referred to above as developing in 7 of the 14 older experimental animals (on experiment for 195 days or longer) was of central origin but eventually became generalized. Intracapillary accumulations of foam cells were seen in the older animals in lungs and kidneys. The oldest experimental animal (sacrificed at 363 days) showed xanthomatous lesions of lungs, testes, and skin.

The Effect of the Protein Composition of the Diet on Hypercholesteremia and Atherogenesis.—The effect of dietary protein was determined with three additional groups of rats (Table II). One group of eight males received a diet which was low in casein (10 per cent); another group received a high casein diet (60 per cent), and a third group received a diet containing 20 per cent alpha protein as the only protein source. The three groups were comparable in age and size with the eight male rats in group I which received a 20 per cent casein diet. Variations in the protein level were made at the expense of sucrose. Otherwise the diets were similar (Table I).

It was found that these dietary protein variations significantly affected the serum cholesterol response during the first 56 days (Text-fig. 1). There was no significant difference in cholesteremic response between the 10% casein group and those fed the 20 per cent alpha protein diet ($p > 0.1$). However, the group fed 20 per cent casein showed a lower serum total cholesterol response after 56 days than did either the 10 per cent casein or 20 per cent alpha protein groups ($p < 0.01$). Moreover, the 60 per cent casein group showed a cholesteremic response that was significantly lower than that of any of the other three experimental groups, $p < 0.01$ when comparing the response of this group and that of the 20 per cent casein group. Apparently the higher the protein level in these diets, the lower the cholesteremic response. The differences in cholesteremic response between the high, low and normo-protein diets cannot be attributed to differences in food intake. There was no statistical difference in

body weight response among these four groups of rats following these dietary treatments.

Pathological Findings in Rats Maintained on Diets II, III, and IV.—Autopsies were performed on 16 animals, selected as follows: 5 animals from group II, 4 from group III, and 7 from group IV. These animals were sacrificed at 31 to 193 experimental days. Sudanophilic lesions were present in the gross in all of these animals. No significant differences could be detected in the severity or extent of these lesions as compared with those in animals maintained for comparable periods of time on diet I, though the numbers of animals in the present groups were obviously small. The vascular and hepatic lesions of all groups of animals were histologically identical, though some of the late manifestations seen in animals of group I, such as cirrhosis, were not found in the present groups of rats.

DISCUSSION

Gross atherosclerosis was produced in the rat by feeding a synthetic diet containing sodium cholate, cholesterol, and thiouracil. This treatment produced extensive lesions in the aorta and its major branches, the pulmonary artery, and the heart valves. The technique of staining gross organs with Sudan was invaluable in detecting incipient lesions in the aorta and larger arteries, seen as early as 31 days. The technique also greatly facilitated demonstration of the extent of involvement. To our knowledge there have been no vascular lesions demonstrable in the gross heretofore described in rats, with the exception of the aortic medial calcific lesions of Hartroft *et al.* (17–19). It was only in those animals that had been on the experimental regimen for 8 months or more that aortic lesion became visible in the gross without the aid of Sudan staining.

Microscopically, intimal plaques were characterized by lipophagocytosis and proliferation of various stromal elements. This degree of intimal reactivity exceeds that previously reported in experimental vascular lesions in rats. Nor have the structural changes in the smooth muscle accompanying medial lipide infiltration been described in this species. Both Wissler and Malinow and their coworkers described Sudanophilic and Liebermann-Burchardt–positive lipide in the intima and media (23–27). Their intimal plaques contained lipophages and increased amounts of fibrous tissue. Wissler *et al.* mention fatty changes in the ground substance of intima and media as well as myxomatous and hyaline degeneration of the fibrous tissue in these locations. Malinow *et al.* specify the following intimal alterations, proliferation of endothelial cells, mesenchymal cells, and of ground substance, and degeneration, hyalinization, and probable calcification. The latter authors also mention fragmentation of the internal elastic membrane and necrobiotic medial changes. In mice Löwenthal has described many of these features, postulating that the more protuberant intimal plaques in his animals represent thrombi secondary to intimal ulceration (29). In the material herein reported endothelial cells did not appear to play an active role in the histologic process. Coronary artery involvement in

one animal had apparently given rise to thrombosis and myocardial infarction. Both Wissler and Malinow and their associates found instances of myocardial infarction accompanying coronary artery lesions (24, 27).

The severity of the induced metabolic abnormality in the present animals is apparent. The vascular changes developed rapidly and certain of their histologic features suggest an acute tissue response. The significantly higher serum total cholesterol levels of normal female rats as compared with normal males, demonstrated in the present animals, has been fully documented elsewhere (36). With the rigorous dietary conditions herein used, however, no differences between the sexes in hypercholesteremic response could be demonstrated. With less strenuous dietary measures such sex-determined differences in hypercholesteremic response are apparent, female rats showing significantly higher serum total cholesterol levels (36). In the present animals no sex-determined differences could be detected in the incidence or severity of either the aortic or coronary artery lesions. In view of the small numbers of female animals, this finding has little significance. It may well be that the present severe metabolic alterations have masked differences between the sexes both as to serum and tissue responses. On the other hand, Moskowitz *et al.* have recently demonstrated in experimental coronary artery lesions in rats the same preponderance among males that has been extensively documented in man (25).

It will be of interest to modify the dietary regimen with the intent of attenuating the serum cholesterol elevation and perhaps decelerating the tissue response. It is of interest that fairly extensive lesions were present in the rats receiving 60 per cent casein whose serum total cholesterol levels were least elevated—in the range of 500 mg. per cent. From other experiments conducted in this laboratory, it is apparent that less extensive, yet demonstrable in the gross, aortic lesions and microscopic coronary artery lesions can be produced without hypothyroidism and in the face of only moderate serum cholesterol elevations (37). Similarly, it has been found that the mild anorexia, diarrhea, and initial weight loss noted in the present animals, can in large measure be eliminated by lowering the dietary cholate content in normothyroid animals (36). In the experiments of Malinow and Wissler and their coworkers (27, 24), serum cholesterol levels far lower than those here reported were accompanied by vascular lesions (Malinow, 100 to 200 mg. per cent). Certainly the older concepts of a species resistance in the rat to experimentally induced atherosclerosis (2, 4, 12, 14) must be distinctly qualified.

Similarly, older views concerning the lack of spontaneous atherosclerotic lesions in rats have been challenged by Malinow *et al.* (38). These authors in a painstaking study have demonstrated lipide infiltrates in the arteries of normal rats as well as occasional fibroblastic intimal plaques. The minute size and scanty distribution of these lesions, requiring enormous numbers of sections

for their demonstration, would not appear to invalidate the significance of the lesions described above.

It is difficult to reconcile the results of the present work with those of Page and Brown (14). These authors maintained elevated levels of serum total cholesterol (averaging 1500 mg. per cent) as well as beta lipoproteins for periods up to 8 months. These changes were effected by means of dietary cholesterol (4 per cent) and cholic acid (2 per cent), combined with hypothyroidism induced by radioactive iodine. To attribute the absence of reactive lesions in their material to less selective sampling of tissue than can be achieved through gross Sudan staining, does not appear to be an adequate explanation. In the present material, even in those early lesions without intimal plaque formation, reaction on the part of the medial smooth muscle was conspicuous.

It is of note that the present experimental dietary regimen included adequate levels of choline. On this ground as well as others including histologic criteria, the present lesions would appear quite distinct from those reported by Hartroft and coworkers (17–19). In so far that choline deficiency does not appear a factor, the present animals resemble these reported by Wissler *et al.* (24) as well as *Cebus* monkeys with induced atherosclerosis (32).

Although the mechanisms involved in the atherogenic process are still subject to speculation, the bulk of experimental evidence favors the theory that the occurrence of atherosclerotic lesions is associated with impaired cholesterol metabolism. In the present study, the role of sodium cholate in the atherogenic process may be manifold. The dietary cholate by a mass action effect, may interfere with cholesterol-to-cholic acid conversion in the liver and hypercholesteremia may result (39). In addition, sodium cholate feeding may also facilitate the absorption of the dietary cholesterol and this in turn contribute to an increased blood cholesterol level. Friedman and Byers (40) believe that bile acids interfere with the removal of cholesterol from serum because of surface tension effects. On the other hand, Frederickson *et al.* (41), have pointed out that cholic acid greatly accelerates the synthesis of cholesterol in the rat liver although this later mechanism may be of minor importance in the present trials since exogenous cholesterol was made available.

Other studies carried out in this laboratory suggest another possible consideration. The inclusion of cholate in these diets may facilitate the depletion of body organic sulfur stores since the sulfur-containing compound, taurine, conjugates with cholic acid. White (42) has shown that an organic sulfur deficiency can be produced in the rat by cholate feeding. The efficacy of sulfur amino acid-deficient protein diets in facilitating or enhancing induced hypercholesteremia has been described in *Cebus* monkeys (32) and in rats (30). This phenomenon appears to be seen in the present data. Thus, the dietary cholate by draining the body sulfur reserves may further aggravate the hypercholesteremia.

Of particular interest was the demonstration of the effect of the protein levels of the casein diets on the degree of hypercholesteremia. The partial inhibition of hypercholesteremia with a high protein diet may be related to bile acid metabolism. It has been shown (43, 44) that the production of bile is correlated with the levels of essential amino acids in the diet. If bile acid production is increased by a high protein diet, cholesterol to cholic acid conversion in the liver may be accelerated leading to a more rapid excretion of hepatic cholesterol. No effect of the dietary protein on the extent of the gross vascular lesions was apparent. Such an effect might well be masked by the rigorous dietary conditions and the severe metabolic alterations. In any case, in view of the variations in individual tissue response and the relatively small numbers of animals in diet groups II, III, and IV, this question cannot be considered answered.

Wissler has discussed the advantages of the rat as an experimental animal in the study of atherosclerosis (24). It is of obvious value to expand the spectrum of susceptible species in attempts to approach the human counterpart. In this species it is of importance to consider both the comparative metabolic mechanisms involved in the individual experimental procedures as well as the comparative tissue changes. There would appear to be a distinct need for standardization of experimental techniques and an increased reproducibility of tissue response. With the present procedures the latter has been largely achieved in that lesions demonstrable in the gross were produced in all animals with a rough quantitative correlation between duration of hypercholesteremia and tissue response. Whether this degree of reproducibility can be achieved or even approached with less strenuous dietary procedures remains to be proved. Nevertheless, a relatively severe hypercholesteremia and liver lipidosis has been observed with diets containing only 1 per cent cholesterol and 0.25 per cent cholic acid. In only 20 days, serum cholesterol levels of approximately 500 mg. per cent in female rats have been found (45). A more detailed histologic report of the vascular and hepatic changes in the present material will be published elsewhere along with a comparative evaluation in terms of other species (35).

SUMMARY

Gross atherosclerosis was produced in the rat by feeding purified diets containing cholesterol, sodium cholate, and thiouracil for periods up to 363 days. In a few weeks a marked increase of the serum cholesterol and beta lipoproteins as well as of the liver lipides was observed. Lesions visible in the gross were found on the intimal surfaces of the vessels of all 46 animals examined. These were most prominent in the heart valves and aortic arch. The earliest lesions, which were seen at 31 days, required Sudan staining for gross demonstration. Older lesions were visible without staining. Microscopic coronary

artery lesions were present and in one instance were accompanied by massive myocardial infarction. Vascular lesions were characterized by medial and intimal lipide infiltration and cellular intimal plaque formation.

In a part of this study the protein level of the diet was altered at the expense of sucrose. The hypercholesteremic response among the rats varied according to the dietary protein level. The lowest response was observed among those animals receiving the highest level of dietary protein. A difference, however, in the severity and extent of the arterial lesions among these relatively small groups of rats could not be established under these experimental conditions.

In all these experiments a close correlation existed between the serum cholesterol levels and beta lipoproteins of the S_f20–100 range.

We wish to thank Dr. Eleanor Y. Lawry for her cooperation in analyzing the serum beta-lipoproteins, Mr. Thomas Faherty for his microscopic preparations, and Miss Ann McNally for her laboratory assistance.

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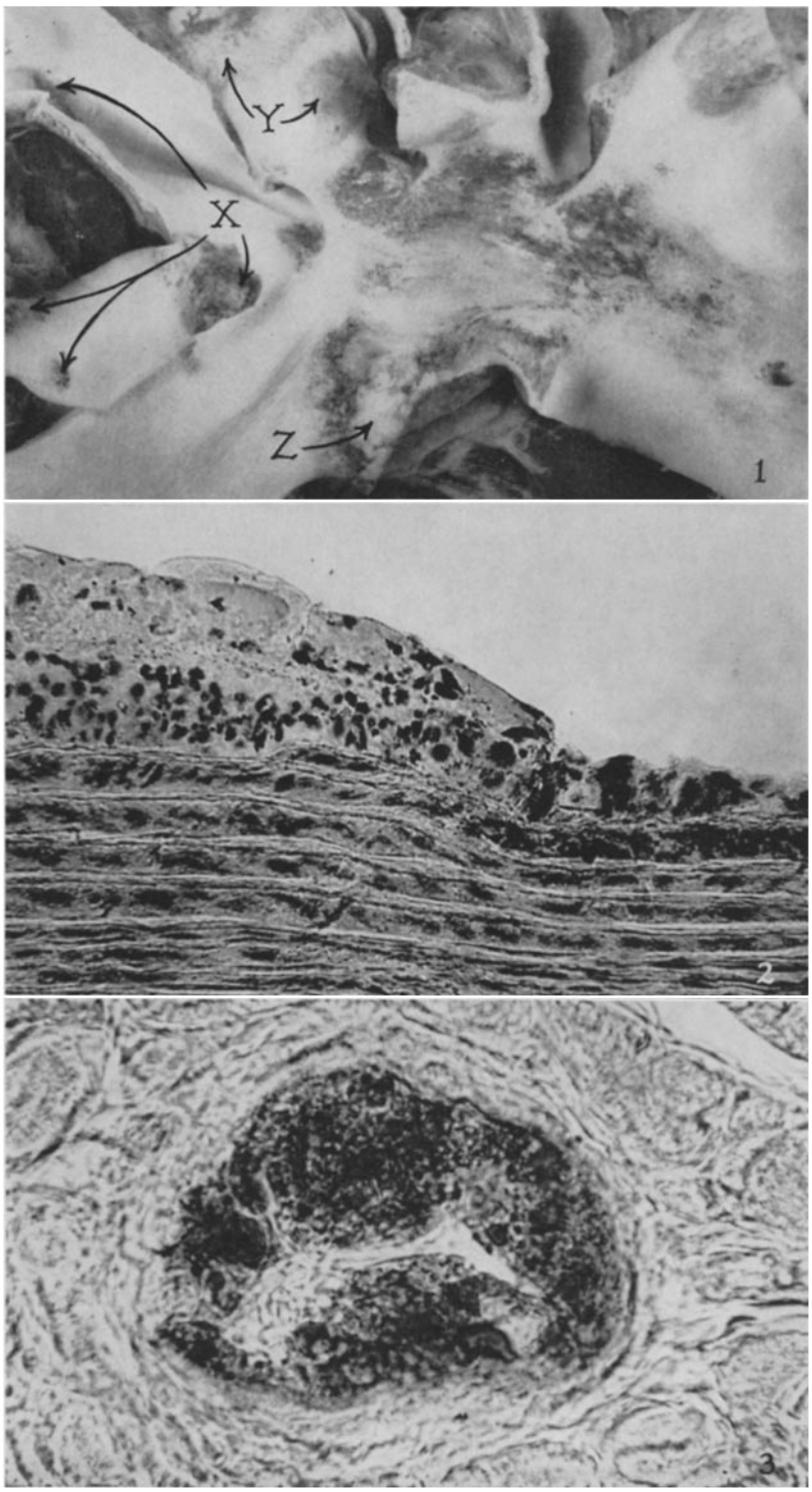
PLATE 46

EXPLANATION OF PLATE 46

FIG. 1. Aortic arch from a rat maintained on diet I for 365 days. The aorta has been opened, fixed in formalin and stained with Sudan IV. Intimal Sudanophilia, here presented as darker areas, is extensive at the apex of the arch, involving almost the entire circumference. The ostia of the great vessels and intercostal arteries are involved, and there is focal staining (*X*) at the bifurcation of the innominate artery and in the right internal carotid and subclavian arteries. Certain areas (*Y*) stain weakly while others (*Z*) are unstained though here the intima is elevated and opaque. ×7.

FIG. 2. Frozen section stained with hematoxylin and Sudan IV from same specimen as in Fig. 1, and selected from the pale elevated area (*Z*). This field represents the junction of a discrete intimal plaque (on the left) and the adjacent thickened intima (on the right). In the latter region there is extensive Sudanophilia in both the intima and the immediately subjacent media. In the plaque proper, lipide and nuclei (both histiocytic and spindle-shaped mesenchymal) are concentrated at the intimal base of the lesion. The overlying relatively acellular ground substance represents the opaque non-Sudanophilic area seen in the gross. ×270.

FIG. 3. Frozen section of a medium sized coronary artery, stained with Sudan IV. This lesion was seen in a rat maintained on diet I for 155 days and is typical of the majority of the coronary artery lesions found. The intima is thickened with Sudanophilic material, with marked reduction of the lumen. Paraffin sections revealed increased amounts of metachromatic ground substance and foam cells. ×420.



(Fillios *et al.*: Production of gross atherosclerosis)