BLOOD PRESSURE, CHOLESTEROL CONTENT OF SERUM AND TISSUES, AND ATHEROGENESIS IN THE RAT*

The Effect of Variations in Blood Pressure on the Cholesterol Content of Serum and Tissues and on the Development of Atherosclerosis in Rats on a High Cholesterol Diet

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(Received for publication, October 17, 1957)

Hypertensive patients are subject to a greater degree of atherosclerosis than are comparable normotensive individuals (1). Studies by Moss, Kelly, Neville, Bourque, and Wakerlin in dogs (2) and by Bronte-Stewart and Heptinstall in rabbits (3) indicate that induced arterial hypertension intensifies the atherosclerosis produced by dietary methods.

Most observers (1) attribute this correlation between hypertension and the severity of atherosclerosis to local damage to the vessels produced by the pressure or turbulence of the blood. This concept is supported by the fact that the sites of predilection for atherosclerotic lesions are frequently those of high pressure or turbulence.

In man, no significant difference has been demonstrated between the serum cholesterol concentrations of comparable groups of normotensive and hypertensive individuals. In a cooperative study of a normotensive population reported by Lewis (4) very slight differences in serum lipide level were found to be associated with differences in blood pressure. Anselme (5) suggested (without interpretable experimental support) that hypertension was accompanied by elevated serum cholesterol concentrations in the rat. In the dog (6) Wakerlin, Moss, and Kiely, while showing a correlation between blood pressure and the severity of atherosclerosis produced by dietary means, failed to show a relationship between blood pressure and serum lipide concentrations.

* This investigation was supported in part by research grants (H-1397, H-52, A-597) from the National Institutes of Health, United States Public Health Service, Bethesda, and by a gift from the Albert and Mary Lasker Foundation.

The desoxycorticosterone acetate used was made available as "percorten" through the courtesy of Dr. John Saunders and Dr. C. N. Sullivan of Ciba Pharmaceutical Products, Inc., Summit, New Jersey. The cholic acid used was provided through the courtesy of the Ames Company, Inc. Elkhart, Indiana.

Fillios (7) has shown that atherosclerosis can be produced in rats by dietary means. The present study was designed to determine whether the presence of hypertension modifies the rate of development of this type of atherosclerosis. During the course of the experiment it became apparent that the sera of hypertensive rats differed from the sera of normotensive rats in respect to the concentrations of some lipides. The experiment was therefore modified to permit detailed examination of this difference.

The results of the present study indicate that induced hypertension intensifies the effectiveness of an "atherogenic" diet in producing not only atherosclerotic lesions but also hypercholesterolemia, hyperlipemia, and an increased content of cholesterol in the liver and the carcass of the animals. These results occur in spite of the fact that endogenous production of cholesterol appears to stop when this diet is employed.

Methods

Animals.—Male rats, from the Wistar Institute, weighing 100 to 200 gm. were used.

Caging.—In Experiment 1 all the rats of each subgroup were caged together. In Experiments 2 and 3 two rats were kept in each cage. In Experiment 4 the animals were caged singly.

Diet.—Stock diet was Purina laboratory chow. The "atherogenic" diet was prepared by adding 4 per cent cholesterol, 1 per cent cholic acid, and 0.5 per cent thiouracil to the ground Purina chow. A solution of cholesterol in ether was mixed with the diet and the ether removed by evaporation. The cholic acid and thiouracil were blended in powder form with the dry diet in a power mixer.

Induction of Hypertension.—. In Experiments 1 and 2, hypertension was induced by the subcutaneous implantation of one 25 mg. pellet of desoxycorticosterone acetate. The implantation site was massaged once a week. The drinking water, which was provided *ad libitum*, contained NaCl 140 m.eq./liter and KCl 35 m.eq./liter.

In Experiment 4, hypertension was induced by the method of Wilson and Byrom (8). Two weeks after the removal of the right kidney, the left renal artery was constricted by the application of a silver clip 0.23 mm. in internal diameter.

Measurement of Blood Pressure.—Systolic blood pressure was measured in the foot of the unheated, unanesthetized rat by the method of Kersten (9) with a "photoelectric tensometer."

Blood Samples.—Under light ether anesthesia, rats were bled by clipping the end of the tail. For determination of whole blood cholesterol, the blood was allowed to drop directly into test tubes containing 0.1 cc. of heparin solution (10,000 u./cc.). For all other determinations, the blood was drawn into melting point capillary tubes (1.5 to 2.0 mm. in diameter); one end of the tube was sealed in a flame and, after clotting had occurred, the tubes were centrifuged.

Cholesterol.—In Experiment 1, cholesterol was measured in whole heparinized blood by the method of Abell (10). In all subsequent experiments, cholesterol was measured in serum. The colorimetric method of Hanel and Dam (11) was applied to the lipide extract prepared by the method of Abell. (It was found that the addition of 2 per cent absolute alcohol to the chloroform prevented the formation of a precipitate in the final step.)

Tissue Cholesterol Content.—The animals were sacrificed by ether anesthesia and liver, heart, aorta, and one kidney were removed. The liver was refluxed with 10 per cent alcoholic KOH for 3 hours. The residual carcass was stored at −15°C. and was worked up at a later date by refluxing with 25 per cent KOH for 4 hours. The non-saponifiable material was extracted and the cholesterol was precipitated as the digitonide by standard procedures.

Rate of Cholesterol Synthesis.—Rats, fed *ad libitum*, were injected intraperitoneally, early in the morning, with 1,750,000 C.P.M. of carboxyl-labelled sodium acetate-C^{14} (specific activity 1 mc./mM). The rats were sacrificed with ether exactly 30 minutes later and the extraction procedure described above was followed. After weighing of the cholesterol digitonide, it was counted in a gas flow counter.

Total lipides in serum were determined by the method of Swahn (12). 10 λ of serum from each capillary tube was transferred to Whatmann 3 MM filter paper and allowed to dry. An equal amount of standard human serum was similarly applied. (The standard serum had been frozen in aliquots and was thawed once immediately before use). The papers were stained in Sudan black B, then eluted with 20 per cent acetic acid in absolute ethanol, and the optical density of eluate was determined at 590 mμ. Results were expressed as the ratio of unknown to standard.

Electrophoresis was performed in paper by a modification of the method of Kunkel (13), developed by Spaet, and reported by Payne and Deming (14).

Sudan black B was used for staining of lipides and bromphenol blue for proteins.

Pathology.—The hearts and aortas were removed and examined immediately after ether sacrifice of the animals. A drawing was made representing the size, appearance, and location of every atherosclerotic lesion visible on gross inspection of specimens in the fresh, unstained state. The comparative extent of atherosclerotic involvement occurring in different rats was estimated from the drawings without knowledge of the experimental group to which the animal belonged. The usual sites of involvement were the leaflets of the mitral valve; the aortic valves and sinuses of Valsalva and coronary orifices; the valves of the right heart; and the aorta itself. Each of these sites was rated 0 to 4 plus on a scale in which 4 plus represented the maximal involvement encountered. The number of pluses at each of the 4 sites was added, so that a rating of 16 plus was theoretically possible. The number, the size, and the thickness of the lesions at a given site were all considered. A 1 plus rating represented a pin point–sized thickening and opacification of a transparent surface; 4 plus represented the presence of more than one lesion, 1 mm. or more in width, visibly raised from the intimal surface and differing from it in color.

The atherosclerotic nature of the lesions was confirmed by histologic techniques in representative specimens (*vide infra*).

RESULTS

Histologic Evaluation of the Intimal Lesions Produced

The atherosclerotic lesions encountered in this study were similar to those described by Fillios (7) although the diets used in the two studies were somewhat different.

The earliest lesions appeared in the bases of the mitral and aortic valves and along the line of closure of the leaflets. When observed unstained, they appeared as minute yellow streaks or pin-point dots. Similar lesions were frequently observed in the sinuses of Valsalva, about the coronary ostia, and, less frequently, about the origins of the great vessels of the aortic arch and the orifices of the intercostal arteries in the thoracic aorta. Fig. 1 shows black staining lipide in the leaflets of an aortic valve and at the mouth of a coronary artery. No predilection for the iliac arteries or the lower abdominal aorta, such as prevails in the dog, was found.

Microscopically, these early lesions were characterized by a lack of cellular response to the lipide which was demonstrable within the walls of the valves and the aorta. In the aorta, endothelial nuclei appeared large, hyperchromic and often distorted, being surrounded by masses of sudanophilic material, so that cell outlines were lost. Fine lipide droplets were deposited along the elastic fibres and extended into the adjacent media where stainable lipide appeared to lie within the smooth muscle cells as well as free in the interstitial substance. Lipide was more abundant in the inner than in the outer layers of the media. These characteristic localizations of lipide are illustrated in Fig. 2, which represents an early lesion without cellular response.

When the lesions were more advanced, plaque formation began, as evidenced by heaped-up nuclei surrounded by masses of sudanophilic material projecting above the surface. Cell borders were indistinct because of the intense staining of the sudanophilic material. Presumably the nuclei were derived from multiplication of endothelial cells, from phagocytic cells, and from infiltrating lymphocytes. The intracardiac branches of the coronary arteries often showed greater proliferative changes than those seen in the aorta or valves. Fig. 3 shows a low power view of an intracardiac branch of a coronary artery with extensive lipide infiltration (stained black) and with plaque formation. Fig. 4 shows a higher magnification of the plaque, with cells heaped up on the intimal surface.

The atheromatous lesions described were readily distinguishable from the necrotizing visceral arteritis seen in some rats made hypertensive with desoxycorticosterone and from the trachea-like lesions of "medial disease" of the aorta. Atheromatous lesions are primarily intimal, whereas the others are primarily medial. Visceral arterial lesions are characterized by an intense inflammatory response of polymorphonuclear cells and often have lumina occluded by thrombi. Trachea-like lesions are characterized by degeneration of muscle fibers and condensation of the elastic fibres with subsequent fragmentation and loss of continuity. Cellular infiltration is notably lacking; as a result, the wall is thinned and calcium deposits are often found in the media.

Experiment 1:

Fifty rats were used, 25 of which were treated with the subcutaneous implantation of a 25 mg. pellet of desoxycorticosterone acetate and the addition of sodium and potassium chloride to the drinking water. One month was allowed to elapse, during which hypertension developed in the treated animals. The groups were then further divided as follows: 1(*a*) 15 desoxycorticosterone-treated rats were placed on the "atherogenic" diet. 1(*b*) 15 normotensive rats were placed on the "atherogenic" diet. 1(*c*) 10 desoxycorticosterone-treated rats were kept on stock diet. 1(*d*) 10 normotensive rats were kept on stock diet.

All rats were weighed weekly. The rats of groups 1(*a*) and 1(*b*) were bled in equally distributed groups at intervals, so that all rats were bled once between the 6th and 11th week on the diet and again between the 14th and 16th week on the diet. Blood pressures were determined within a few days preceding each bleeding. At each bleeding, serum was taken for electrophoresis for both protein and lipide staining and for determination of total stainable lipide. Whole blood was taken for determination of cholesterol concentration. After 6 weeks on the "atherogenic" diet, two rats from group 1(*a*) and two rats from group 1(*b*) were removed for measurement of their rate of cholesterol synthesis. The measurement was completed in only three. Thirteen rats from group 1(*a*) and 10 rats from group 1(*b*) were sacrificed between the 17th and 20th week on the diet. The intimal lesions were graded and the cholesterol content of the livers and carcasses were determined. Three rats selected at

random from group 1(*b*) were kept for prolonged observation. Groups 1(*c*) and 1(*d*) were used only for weight and growth controls and for controls in the electrophoretic studies.

Weights.—There was a normal gain in weight in the rats of groups 1 (*c*) and 1 (*d*) throughout the experimental period. The rats of groups 1 (*a*) and 1 (*b*) began to lose weight slowly from the time they were placed on the "atherogenic" diet. Their weights stabilized at about the 15th week on the diet. The averages of the weights of the rats of these two groups remained almost identical at all times. The individual weights and group means just prior to sacrifice are indicated in Text-fig. 1, column 1.

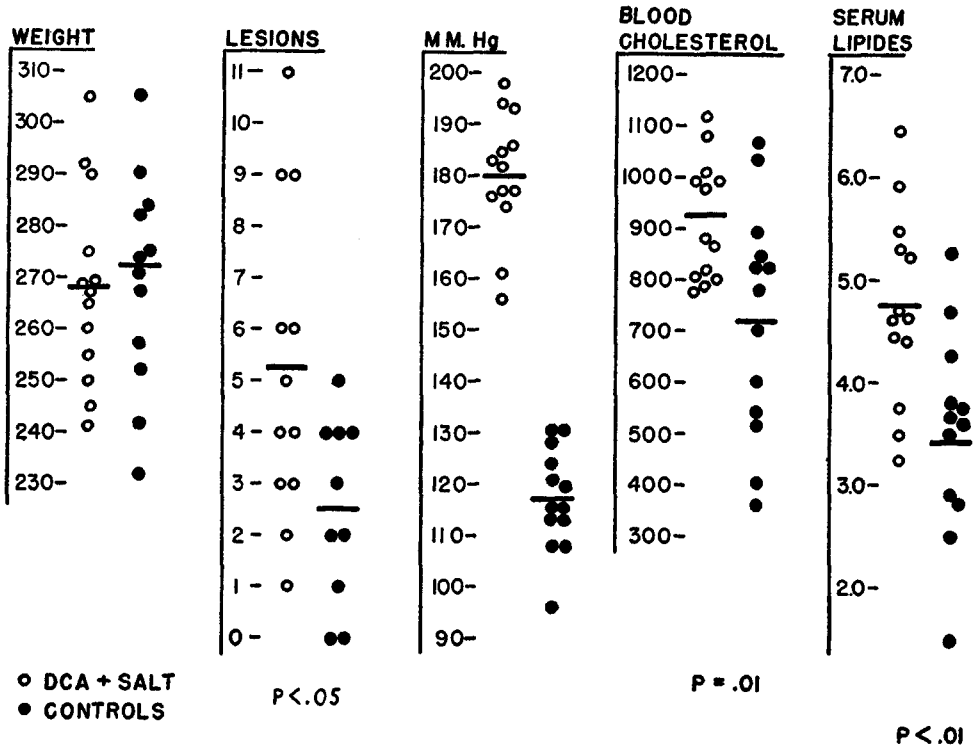
Cholesterol Synthesis.—After the rats had been on the "atherogenic" diet for 6 weeks, there was no detectable label in the cholesterol isolated from either their livers or the remaining carcasses one-half hour after the injection of labelled acetate. Under these conditions, experience in this laboratory has shown that cholesterol in the livers of rats on a stock diet has a specific activity averaging 75C.P.M./mg. and that cholesterol in the carcasses has a specific activity averaging 8 C.P.M./mg. It was concluded that 6 weeks on the "atherogenic" diet had resulted in a virtual cessation of endogenous cholesterol synthesis in both the normotensive and the hypertensive rats.

Electrophoresis.—At no time was any systematic difference demonstrated between the serum protein patterns of any of the 4 subgroups. The lipide stain demonstrated a consecutive increase in the lipide concentrations of the sera of the rats on the "atherogenic" diet as compared to those on the stock diet. There was no visible increase in the stainable lipide which moved with the speed of albumin or α-1 globulin, all the increased lipide showing a mobility equal to or less than that of β-globulin. Throughout the first series of bleedings, gross inspection showed the increase in density in these slow areas to be greater in the serum from the hypertensive rats on the "atherogenic" diet than in the serum from the simultaneously bled normotensive rats on the "atherogenic" diet. In the second series of bleedings, the lipide density of the sera of both groups was so high that differentiation by inspection was difficult.

Whole Blood Cholesterol Concentrations and Total Stainable Lipides of the Serum.—The data on cholesterol and lipide concentrations from groups 1 (*a*) and 1 (*b*) are presented in Text-fig. 1, columns 4 and 5. Each spot represents the average of two determinations made on one rat between the 6th and 16th week on the diet. The range of whole blood cholesterol concentrations in three hypertensive and three normotensive stock-fed animals (1 (*c*) and 1 (*d*)) was from 50 to 150 mg. per cent, and the range of total serum lipide in four hypertensive and four normotensive stock-fed animals was from 0.24 to 0.54 times that of the standard, with an average of 0.41. The mean of the whole blood cholesterol concentrations of the normotensive rats on the "atherogenic" diet (group 1 (*b*)) was 720 mg. per cent.[1] The mean of the whole blood cholesterol

[1] If the 3 rats which were not sacrificed and hence did not enter into the evaluation of pathology are excluded, the mean is unchanged, 722 mg. per cent.

concentrations of the 13 hypertensive rats on the "atherogenic" diet (group 1 (*a*)) was 922 mg. per cent. On the basis of the t test, the probability that this difference occurred as a result of chance is <0.01.



TEXT-FIG. 1. Experiment 1. The open circles "DCA + salt" = group 1(*a*). The closed circles "controls" = group 1(*b*). *Weight* is in grams and is the final weight after 17 weeks on the "atherogenic" diet. *Lesions*, each spot represents the number of pluses assigned to the lesions of one rat, 17 to 20 weeks on the "atherogenic" diet.

The systolic blood pressure in millimeters Hg, whole *blood cholesterol concentration in milligrams per cent* and *serum lipides* as a ratio to a fixed standard, represent the averages of two determinations on each rat, between the 6th and 16th week on the "atherogenic" diet. The horizontal lines represent group means.

The data indicate that the hypertensive animals of group 1(*a*) developed higher concentrations of cholesterol and total stainable lipide in the blood and more extensive atherosclerotic lesions than did the normotensive animals of group 1(*b*) after the same length of time on the same diet.

The mean of the total stainable lipides of group 1 (*b*) was 3.41 times that of the standard. The mean of the total stainable serum lipides of group 1 (*a*) was 4.75. Again, the probability that this difference between the two means occurred as a result of chance is <0.01.

Thus, the mean whole blood cholesterol concentration and the mean serum

total lipide concentration of the hypertensive rats on the "atherogenic" diet was significantly higher than that of the normotensive rats on the "atherogenic" diet.

Atherosclerotic Changes.—The results of the gross grading of the pathologic lesions are shown in Text-fig. 1, column 2. The mean number of pluses assigned to the 10 rats of group 1 (*b*) (the normotensive rats on the "atherogenic" diet) was 2.5. The mean number of pluses assigned to group 1 (*a*) (the hypertensive rats) was 5.25. The probability that this difference was due to chance is <0.05. The results suggest that the incidence of atherosclerosis was greater in the hypertensive group than in the normotensive group.

Actually, the visual difference in intensity of lesions was more striking than the results of the grading might suggest. The grading was based on an arbitrary system of scoring applied to 4 areas, and the presence of a number of small lesions in all 4 areas could outweigh a large lesion in one area. In spite of the overlap of scores seen in Text-fig. 1, column 2, after the first 6 rats had been examined and the appearance correlated with the treatment group, it was possible for the pathologist to determine with only one error which rats had been hypertensive and which had been normotensive by the intensity of the intimal involvement. One of the hypertensive rats with extensive involvement, showed a large scar at the apex of the left ventricle—presumably a healed cardiac infarct.

Tissue Cholesterol Content.—The results of the determination of the total content of cholesterol in the livers and carcasses of these animals are illustrated in Text-fig. 2. Only 12 hypertensive animals are represented since the material from one was lost.

The average cholesterol content of livers from control rats on stock diet was 22 mg. The average cholesterol content of their carcasses was 520 mg.

The average cholesterol content of the livers from group 1 (*b*) (the normotensive animals on the "atherogenic" diet) was 951 mg.; that of the livers from group 1 (*a*) (the hypertensive animals on the diet) was 1356 mg. The standard error of the difference between the means is 86, $t = 4.7$. The probability that the difference was due to chance is <0.01.

The average cholesterol content of the carcasses (exclusive of liver, heart, aorta, and one kidney) of group 1 (*b*) was 726 mg.; that of group 1 (*a*) was 971 mg. The standard error of the difference between these means is 52, $t = 4.7$. The probability that this difference was due to chance is <0.01.

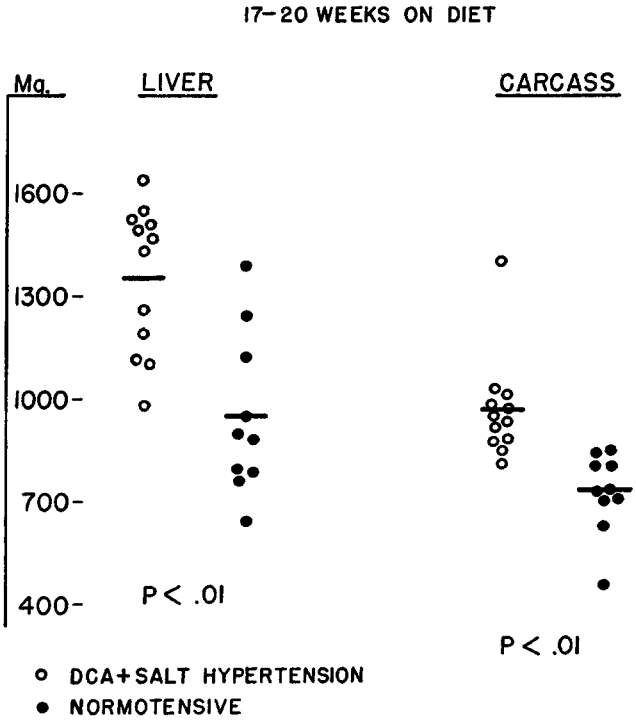
Thus, under the conditions of this experiment, the cholesterol content of the whole hypertensive animal on an "atherogenic" diet was significantly greater than that of the whole normotensive animal on the same diet.

Experiment 2:

Experiment 2 was a simplified repetition of Experiment 1, undertaken for purposes of confirmation.

Thirteen normotensive rats and 10 DCA hypertensive rats were maintained on the "atherogenic" diet for only 6 weeks. The rats were weighed weekly. At the end of 53 days on

the diet, the blood pressure, serum cholesterol concentration, and the total stainable serum lipides were determined. All rats were sacrificed on the 63rd day for gross estimation of atherosclerotic involvement. Determination of cholesterol synthesis was not made and the tissue cholesterol content was not measured.



TEXT-FIG. 2. Experiment 1. Each spot represents the total cholesterol content in milligrams of the whole liver or whole carcass (exclusive of liver, heart, aorta, and one kidney) of one rat. Horizontal lines are group means.

The data indicate that the cholesterol content of the whole hypertensive animal on an "atherogenic" diet is significantly higher than that of the whole normotensive animal on the same diet.

The data are presented in Text-fig. 3. The weights of the rats in the 2 groups remained comparable, the final mean being 296 gm. for the normotensives and 302 gm. for the hypertensives. The mean of the blood pressures of the normotensive animals was 117 mm. Hg, that of the hypertensive animals was 181 mm. Hg. The mean concentration of cholesterol in the serum of the normotensive animals was 1029 mg. per cent and of the hypertensive animals was 1450 mg. per cent. The probability that this difference between the means was due to chance alone is <0.05. The concentrations appear higher than in Experiment 1 because they were measured in serum rather than in whole blood. The mean

total stainable lipide in the serum of the normotensive animals was 1.77 times that of the standard. The mean total stainable lipide in the serum of the hypertensive animals was 2.23 times that of the standard. The probability that this difference between the means was due to chance alone is <0.05.

The intimal lesions in these rats, after only 6 weeks on the "atherogenic" diet, were less extensive than those of the rats of Experiment 1, which had been on the diet for 17 to 20 weeks, but a difference between the normotensive and the hypertensive rats was again apparent. The mean number of pluses assigned to the lesions of the normotensive animals was 1.3. The mean number assigned to the lesions of the hypertensive animals was 2.3.

In Experiment 2, as in Expriment 1, the hypertensive animals on the "atherogenic" diet had higher concentrations of cholesterol and lipide in their sera and more atherosclerosis than did the normotensive animals on the same diet.

Experiment 3:

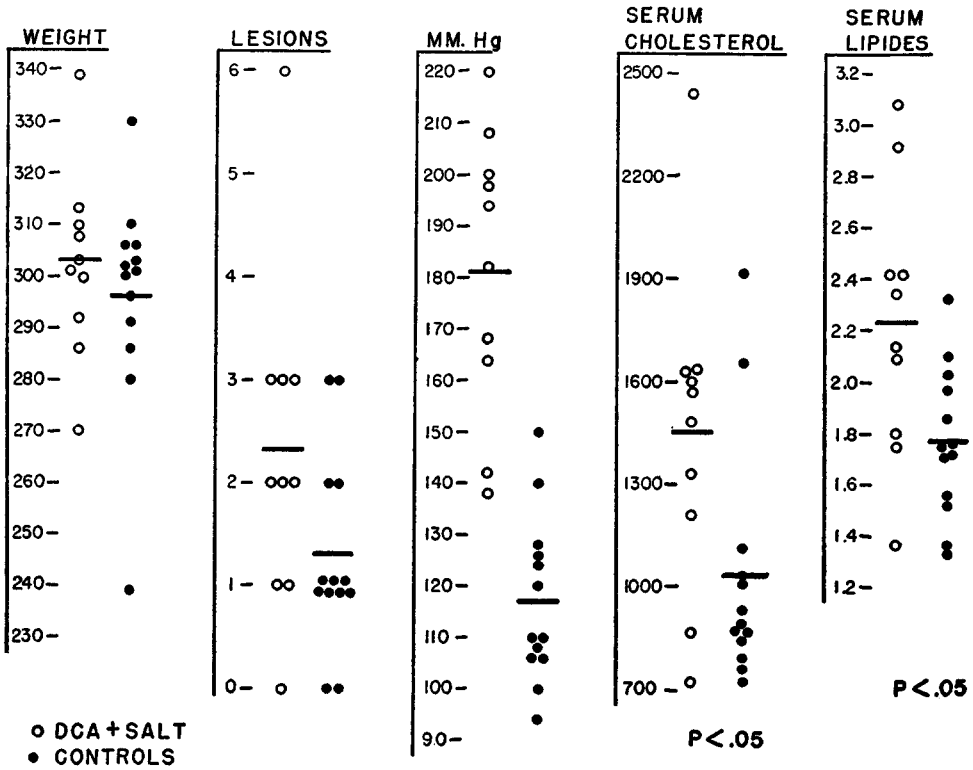
The purpose of Experiment 3 was to determine whether the differences shown between the hypertensive and normotensive animals were dependent on the difference in blood pressure or whether they could be accounted for by the presence of either the desoxycorticosterone acetate or the saline drinking water.

The rats of group 3 were divided into three subgroups: 3(*a*) was a control group of 12 rats which received the "atherogenic" diet for 6 weeks; 3(*b*) consisted of 11 rats, each of which had a 25 mg. pellet of desoxycorticosterone acetate implanted subcutaneously 1 month before the start of the diet but had no salt added to the drinking water; 3(*c*) consisted of 11 rats which were not treated with desoxycorticosterone but which had saline drinking water for 1 month prior to the start of the "atherogenic" diet and throughout the dietary period. The rats were weighed weekly. After 40 days on the special diet, blood pressure, serum cholesterol concentration, and serum total lipide concentration were determined, as in Experiment 2 and on the 53rd day the rats were sacrificed for grading of their atherosclerotic lesions.

The data are presented in Text-fig. 4. The weights of the three subgroups were comparable, averaging 294, 283, and 287 gm. The blood pressures of groups 3 (*a*) and 3 (*b*) were similar, averaging 126 and 130 mm. Hg respectively, while the blood pressure of salt-treated animals in group 3 (*c*) averaged 152 mm. Hg. The serum cholesterol concentrations of the three groups averaged 716, 786, and 792 mg. per cent respectively. The means of the total stainable lipide in the sera of the groups were 1.55, 1.66, and 1.65 times that of the standard. The means for the number of pluses assigned to the intimal lesions of the groups were 1.3, 1.2, and 1.7.

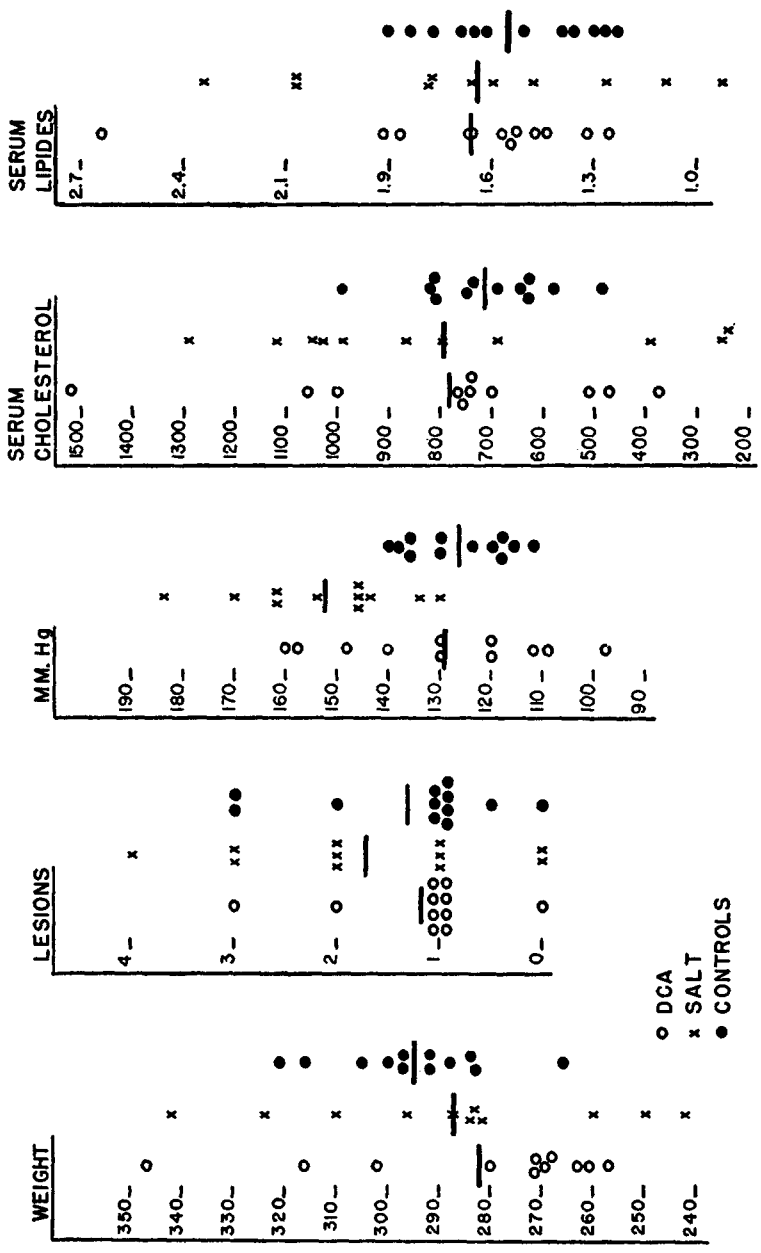
The results indicate that the presence of desoxycorticosterone in the absence of added salt and in the abence of hypertension does not increase either the concentration of cholesterol in the serum or the extent of intimal atherosclerosis. The results with group 3 (*c*), which received salt but no desoxycorticosterone, are not clear cut. There was some elevation of the blood pressure, which was to

be expected (14). While there was not a significant increase in atherosclerosis, the number of pluses assigned was somewhat higher than for the other two groups. The mean concentration of cholesterol in the serum was almost exactly



TEXT-FIG. 3. Experiment 2. Each spot represents a single determination on one rat, made at the end of a 53 day period on the "atherogenic" diet, except for the lesions which were measured at the end of a 63 day period on the diet. Horizontal lines are group means. The data confirm those presented in Text-fig. 2. Hypertensive animals on an "atherogenic" diet develop higher concentrations of cholesterol and total stainable lipides in the serum and more extensive atherosclerotic lesions than do normotensive animals on the same diet for the same length of time.

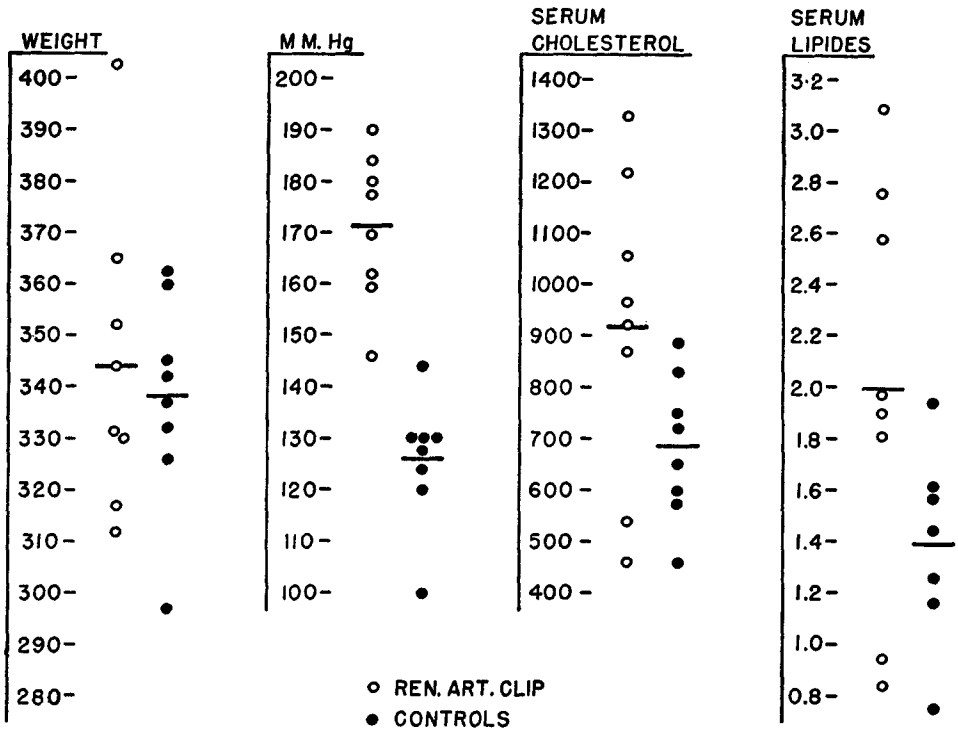
the same as that of the control group but inspection of Text-fig. 4 reveals that this result depended on two rats with unusually low concentrations. These two rats were in the same cage. It is not possible to conclude from these results that salt alone has no effect on cholesterol concentration or on lesions. However, the effects, if any, were not of a magnitude comparable to that seen in the hypertensive rats which received both desoxycorticosterone and salt in Experiments 1 and 2.



TEXT-FIG. 4. Experiment 3. Each spot represents a single determination on one rat forty days after the start of the "atherogenic" diet, except for the lesions which were measured 53 days after the start of the diet. The data indicate that neither desoxycorticosterone without salt nor salt without desoxycorticosterone produced a significant change in the concentration of cholesterol or lipide in the serum or in the degree of atherosclerotic involvement.

Experiment 4:

The purpose of Experiment 4 was to determine whether the differences in lipide response previously demonstrated between normotensive animals and animals made hypertensive with desoxycorticosterone and salt could also be



TEXT-FIG. 5. Experiment 4. Renal hypertension. Each spot represents a single determination made on one rat 43 days after the start of the "atherogenic" diet. The data indicate that animals made hypertensive by compression of one renal artery and removal of the other kidney develop higher concentrations of cholesterol and total stainable lipides in the serum than do control animals on the same "atherogenic" diet.

demonstrated if the hypertension were induced by renal manipulation in the absence of both desoxycorticosterone and salt.

Hypertension was induced in 8 rats by the method of Wilson and Byrom (8), one kidney being removed and a silver clip (0.23 mm. internal diameter) being applied to the renal artery of the remaining kidney. The 8 control rats had one kidney removed. One month later the atherogenic diet was started. For 3 weeks the animals were pair-fed, one normotensive with one hypertensive. For the second 3 weeks, they were fed *ad libitum*. At the end of the 6 week period (43 days) the blood pressures were measured, the rats were weighed, and blood was obtained for measurement of cholesterol and lipides.

The data are presented in Text-fig. 5. The weights of the two groups were comparable—the normotensive group averaging 338 gm. and the hypertensive group averaging 344 gm. The blood pressure means were 126 and 171. The cholesterol concentrations in the serum averaged 686 mg. per cent for the normotensives and 915 mg. per cent for the hypertensives. The probability that this difference between the means could have been the result of chance alone is <0.1. The means for the total stainable lipide were 1.4 and 2.0 times that of

TABLE I

The Correlations between Blood Pressure and Cholesterol, Blood Pressure and Lesions, and Cholesterol and Lesions Occurring in the Subgroups and Total Combined Subgroups of Experiments 1, 2, and 3

Exp.	Subgroup	Blood pressure-cholesterol	Blood pressure-lesions	Cholesterol-lesions
1	DCA + NaCl	−0.38	−0.07	+0.24
	Control	+0.75 < 0.01	−0.04	+0.49 < 0.1
	Total.....	+0.51 < 0.02	+0.44 < 0.05	+0.52 < 0.02
2	DCA + NaCl	+0.64 < 0.05	+0.64 < 0.05	+0.74 < 0.01
	Control	−0.3	−0.25	+0.09
	Total.....	+0.54 < 0.02	+0.49 < 0.02	+0.58 < 0.01
3	DCA	+0.35	−0.22	+0.59 < 0.05
	NaCl	+0.23	+0.01	+0.54 < 0.1
	Control	+0.58 < 0.05	−0.15	+0.52 < 0.1
	Total.....	+0.3 < 0.1	+0.06	+0.42 < 0.01

Probabilities determined by the "t" test are presented when they are less than 0.1.

A positive correlation is shown between blood pressure and serum cholesterol concentration not only when hypertensives are compared to normotensives (total, groups 1 and 2) but within the normotensive groups in 1 and 3. A positive correlation is usually shown between serum cholesterol concentration and the extent of atherosclerosis (lesions). The correlations between blood pressure and lesions are positive only when the range of blood pressure is wide (1 and 2, total and 2, DCA and NaCl.)

the standard. The probability that this difference between the means could have been the result of chance alone is <0.1. These rats were not sacrificed for evaluation of intimal lesions.

Calculation of Correlation in Experiments 1, 2, and 3.—In every group and every subgroup the correlation between the concentration of cholesterol in the serum or blood and the concentration of total stainable lipides in the serum was very close. The lowest correlation coefficient found was 0.67 and the usual coefficient was about 0.9. Calculations of other correlations are therefore presented only for the concentration of cholesterol. The correlation with the concentration of total lipide would be roughly similar.

Table I presents correlations between blood pressure and cholesterol concentration, between blood pressure and lesions, and between cholesterol concentration and lesions, for the various subgroups of Experiments 1, 2, and 3, as well as for the total combined subgroups of each experiment. The "p" values, derived by the "t" test, are indicated when they are less than 0.1.

The correlation between blood pressure and cholesterol concentration is significantly positive in all three experiments when the subgroups are combined and in three of the individual subgroups.

The correlation between cholesterol concentration and the degree of atherosclerotic involvement is significantly positive in all three experiments when the subgroups are combined and in five of the individual subgroups.

These two correlations are more consistently positive than is the correlation between blood pressure and the degree of atherosclerotic involvement.

Although the correlations between blood pressure and lesions are not significant in the subgroups of Experiment 1 and 2, they are significantly positive for the total combined subgroups of these experiments. This confirms what has already been demonstrated by the evaluation of the significance of the differences between the means. The correlations between blood pressure and cholesterol concentration and between cholesterol concentration and lesions are always more positive than are those between blood pressure and lesions.

DISCUSSION

The data which have been presented in this paper indicate that the presence of hypertension, whether induced by desoxycorticosterone and salt or by compression of one renal artery and removal of the other kidney, resulted in a greater degree of hypercholesterolemia and hyperlipemia than occurred in normotensive rats on the same "atherogenic" diet in the same period of time. The fact that there was a positive correlation between the blood pressure and the serum cholesterol concentration in the normotensive "control" rats of Experiments 1 and 3 may be interpreted as additional evidence that the differences between the cholesterol concentrations of the hypertensive and the normotensive rats of the various experiments were the result of the differences in blood pressure rather than the direct result of the different regimens to which the animals had been subjected. Spontaneous differences in blood pressure of "normotensive" rats on this diet also were associated with different degrees of hypercholesterolemia.

For the group made hypertensive with desoxycorticosterone and salt, the data indicate that the increase in cholesterol concentration was not limited to the serum but was present in the liver and carcass as well (Experiment 1), and that it was accompanied by a greater degree of atherosclerosis than occurred in the normotensive animals (Experiments 1 and 2).

The data have not answered the question, "Why do the hypertensive animals get more atherosclerosis than the control animals?" The two general theories which have been offered to explain the correlation between hypertension and an increased severity of atherosclerosis are: (*a*) that there is a local effect on the vessel walls produced by the increased pressure or turbulence of the blood which promotes the deposition of lipide and the development of lesions; and/or (*b*) that the higher concentration of one or more of the lipide elements in the blood promotes a higher rate of lipide deposition in the intima and secondarily of lesions. If there is an alteration in lipide metabolism in the presence of hypertension, it is conceivable that this alteration may be present in the arteries themselves and that the lipide deposited in them may be produced in them at an increased rate, or that the altered vessels may be less efficient at eliminating lipide.

In these experiments the correlations between blood pressure and cholesterol concentration, and between cholesterol concentration and lesions, were closer than the correlation between blood pressure and lesions. This might suggest that the latter correlation was a consequence of the first two; *i.e.*, that the effect of blood pressure on the severity of lesions was mediated through its effect on cholesterol or lipide concentration. However, the evidence presented cannot establish or disprove this causal relationship.

The high correlation between blood pressure and serum cholesterol concentration makes it difficult to clarify the situation experimentally. It has been shown that a sufficient elevation of the serum cholesterol concentration can induce atherosclerosis in the rat in the absence of hypertension (7), and the data presented here indicate the correlation between the concentration of cholesterol and the degree of atherosclerosis is a positive one. It is general experience that hypertension in the absence of elevation of the serum cholesterol concentration does not induce atherosclerosis in the rat. In order to demonstrate whether elevation of the blood pressure has any effect on the rate of atherogenesis not mediated through its effect on the metabolism of cholesterol or lipide, it will be necessary to design an experiment in which rats can be maintained with identical cholesterol concentrations and different blood pressures.

SUMMARY

Rats on a stock diet with added cholesterol, cholic acid, and thiouracil developed increased concentrations of cholesterol, total lipide, and beta lipoprotein in the serum, and an increased content of cholesterol in the liver and carcass, despite the fact that the diet produced a cessation of endogenous cholesterol synthesis. Rats with high serum lipide concentrations developed intimal lesions similar to those of human atherosclerosis.

The induction of hypertension by desoxycorticosterone and salt accelerated

the development of hypercholesterolemia, hyperlipemia, increase in tissue cholesterol content, and atherosclerotic changes in the intima. Hypertension induced by renal artery constriction also intensified the hypercholesterolemia and hyperlipemia. On the other hand, rats receiving desoxycorticosterone acetate without salt or salt without desoxycorticosterone acetate did not show any intensification of hypercholesterolemia or hyperlipemia.

The extent of the atherosclerotic lesions was correlated with the concentration of cholesterol in the serum. There was also a positive correlation between blood pressure and the degree of hypercholesterolemia. It remained uncertain whether the increase in atherosclerosis in the hypertensive animals was dependent on the increased lipide content of serum and tissues or on a local effect of the elevated blood pressure.

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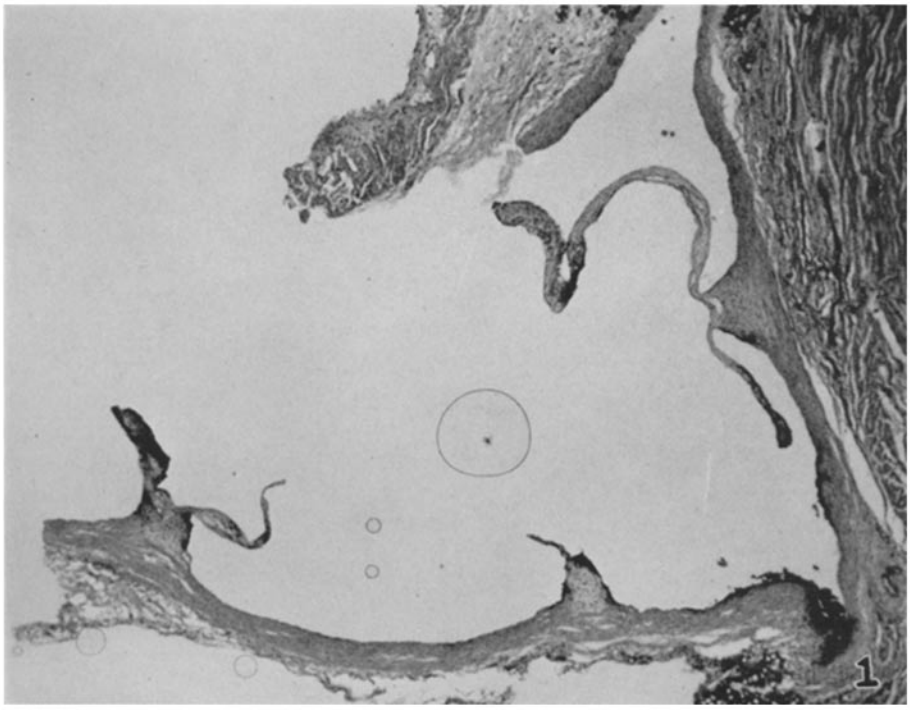
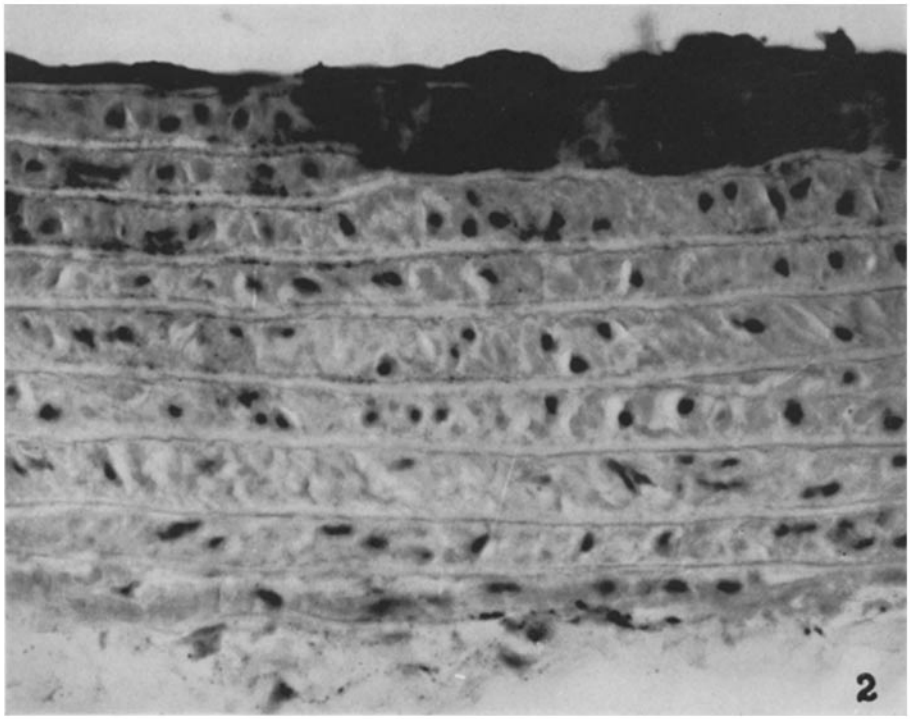
EXPLANATION OF PLATES

PLATE 45

FIG. 1. Section through sinus of Valsalva showing distribution of lipide in the valve commissures and leaflets. Lipide appears black. The coronary ostia are visible at the top and bottom on the right. A small plaque is seen at the mouth of the coronary artery at the lower right. Oil red O stain. × 25.

FIG. 2. Thoracic aorta. Lipide appears black. Endothelial nuclei are obscured by heavy deposits which penetrate the inner layers of the media. Stainable lipide visible as dots along the elastic fibres and within the smooth muscle cells at upper left. Note lack of cellular infiltration despite the presence of lipide. Oil red O stain. × 600.



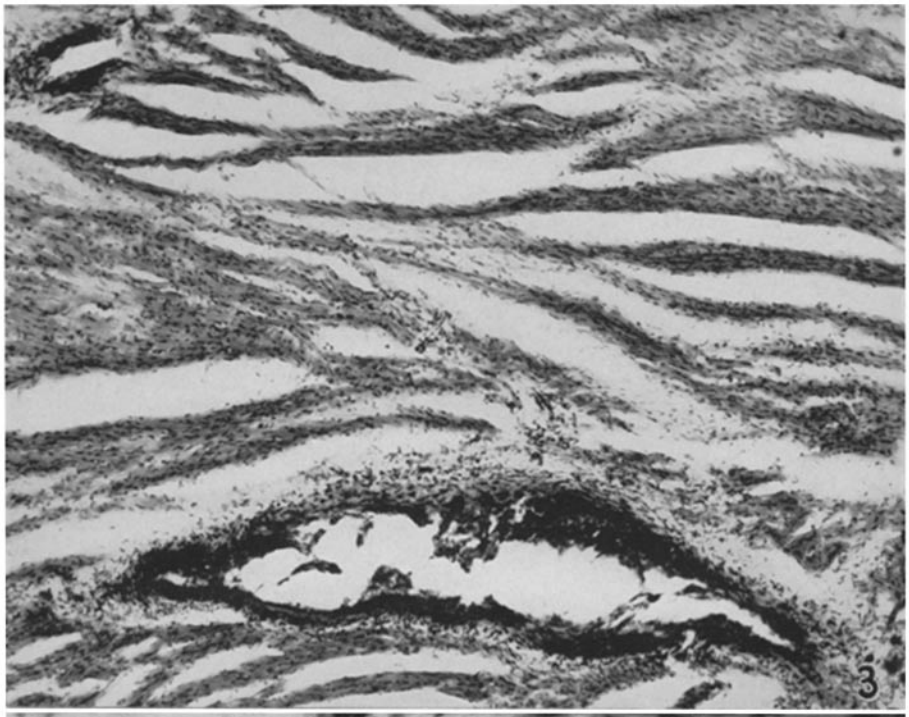


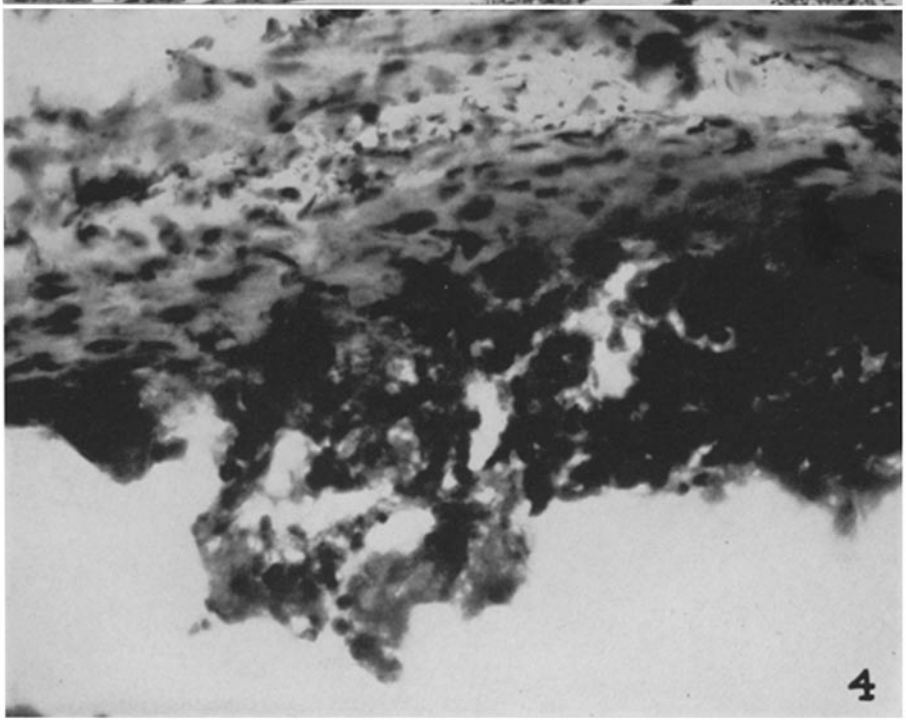
(Deming *et al.*: Blood pressure, cholesterol, and atherogenesis)

PLATE 46

FIG. 3. Plaque formation and abundant lipide throughout the wall of an intracardiac branch of a coronary artery. Another small branch of the artery is present at the upper left. Oil red O stain. × 82.

FIG. 4. Higher magnification of the plaque seen in Fig. 3 showing cells heaped up on the intimal surface. Lipide is also present on the underlying media. Oil red O stain. × 410.





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