EXPERIMENTAL ATHEROSCLEROSIS AND CARDIAC INFARCTS IN RATS*, ‡

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PLATES 33 TO 35

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Atherosclerosis has been induced experimentally in several species (1, 2). However, only a few investigators have reported that life-threatening sequelae of atherosclerosis, *e.g.* cardiac infarcts, have been reproduced experimentally in some individual laboratory animals (3–7). We do not know why experimental atherosclerosis in laboratory animals has been so rarely accompanied by severe consequences, but many laboratories are now investigating possible differences in the clotting mechanism and other factors as causal. To aid in understanding this discrepancy between the high incidence of cardiac infarcts in man and the low incidence in laboratory animals, we developed a method by which atherosclerosis *and* its sequelae could be produced in rats. The rat was believed to be resistant to the induction of atherosclerosis (1, 7–22), but recently these older concepts have been revised (23–27). The rat can be induced to reproduce the equivalents of almost all the major arteriopathies that occur in man (28). It does not respond to atherogenic regimes so readily as do the rabbit or the chicken, which is an advantage in that a more clear cut elaboration of the etiological factors is possible. This report is a confirmation and extensive enlargement of a preliminary publication in which the methods for the production of cardiac infarcts in rats have been described in detail (4, 5).

Materials and Methods

A total of 135 male Wistar rats (80 gm. initial weight) was employed in this long term study. With the exception of group 1, which was fed Purina fox chow, all groups were fed the basal diet WGF-1 before they were transferred to their new diets at a weight specifically indicated for each group (see Table I). The experiment was performed in three sets:—

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‡ A preliminary description of this work was reported at the Federation of American Societies for Experimental Biology, Chicago, April, 1957.

TABLE I

Atherosclerosis, Coronary Occlusion, and Myocardial Infarction in Rats

Experimental Outline and Results

Group	Diet	Dietary* supplement	Experimental procedure	Weight at transfer and change in weight‡	Initial No. of rats	No. of rats§ autopsied	No. of deaths and survivals	Coronary lesions		
								Pathogenesis	Type‖	Incidence
				gm.						
1	Chow	None	Control	80 Gain	10	10	4 deaths; 6 survivors killed after 17 mos.	Aging	Lipomatous, spontaneous (minimal)	2 out of 10
2	WGF-1	None	Obesity	80 Very marked gain	15	14	1 death; 13 survivors killed after 17 mos.	Mild hyperlipemia¶	Lipomatous	5 out of 13
3	WGF-2	2% cholesterol	High cholesterol intake	215 Good gain	10	9	3 deaths; 6 survivors killed after 14 mos.	Mild hypercholesterolemia	Lipomatous	5 out of 9
4	WGF-1	None	Exposure to cold	215 Moderate	15	13	7 deaths; 6 survivors killed after 12 mos.	Stress	Lipomatous	8 out of 13
5	WGF-14	1% cholesterol Choleate*	Exposure to cold; high cholesterol intake	350 Moderate gain	15	15	11 deaths; 4 survivors killed after 13 mos.	Stress; mild hyperlipemia¶	Lipomatous, atheromatous, fibrinous	15 out of 15
6	WGF-13	1% cholesterol Choleate*	*Adrenalectomy*; high cholesterol intake	315 Good gain	15	14	6 deaths; 8 survivors killed after 13 mos.	hypocorticalism; mild hyperlipemia¶	Lipomatous and atheromatous	10 out of 14
7	WGF-4	Viosterol*	Hypervitaminosis D	425 Initial gain, final loss	10	10	6 deaths; 4 survivors killed after 12 mos.	vascular injury	Moenckeberg	8 out of 10
8	WGF-11	Cholesterol Choleate* Thiouracil	Hypothyroidism	400 No change	15	15	10 deaths; 5 survivors killed after 12 mos.	Severe¶ hyperlipemia	Lipomatous and atheromatous	11 out of 15
9	WGF-12	Cholesterol Choleate Thiouracil* Viosterol	Hypothyroidism and hypervitaminosis D	450 Loss	30	26	All died; life span 8–12 mos.	Severe hyperlipemia¶ with vascular injury	Lipomatous Atheromatous Atherosclerotic Coronary occlusion Myocardial infarct	90% 78% 64% 30% 13%

* See Tables II *a* and II *b*.

‡ See Text-figs. 1 to 3.

§ Animals dying within the first 2 months on the experimental diets were discarded.

‖ See text, Results, Terminology.

¶ See Table III.

Set I consisted of 50 rats divided into 4 groups. The rats in groups 3 and 4 were transferred to their new regimens when they reached a weight of 215 gm. (Text-fig. 1).

Group 1: 10 control rats fed Purina fox chow

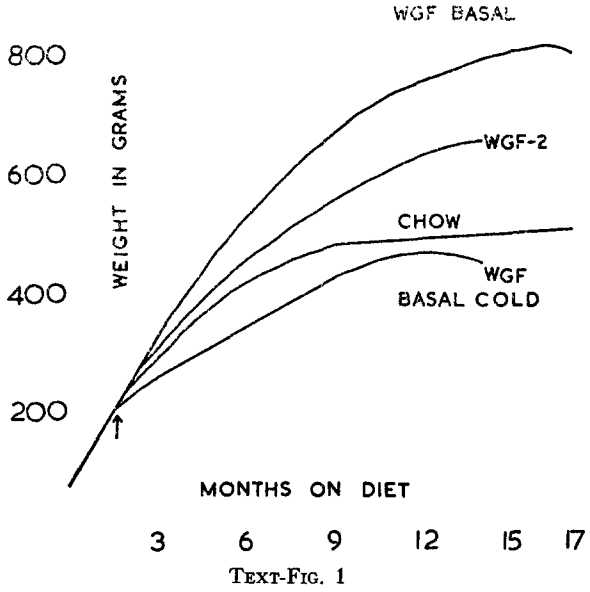
Group 2: 15 rats fed the basal diet (WGF-1, Table II *a*) throughout the whole experiment

Group 3: 10 rats fed the basal diet supplemented with 2 per cent cholesterol (WGF-2)

Group 4: 15 rats fed the basal diet WGF-1 and exposed to cold

Set II consisted of 2 groups of rats weighing 315 to 350 gm. at the time of transfer from the basal diet to their experimental regimens (Table I and Text-fig. 2).

This experimental set (II) was arranged to see whether the absence of the adrenals, or their activation by the stress of cold, would affect the development of coronary lesions asso-



TEXT-FIG. 1

ciated with mild hyperlipemia and hypercholesterolemia. Such an increase in blood lipides occurs in rats given a high fat, egg yolk–rich diet supplemented with cholesterol and sodium choleate (29–31). Thiouracil was omitted in this diet (3, 32, 33) because neither cold-exposed nor adrenalectomized rats can withstand hypothyroidism.

Group 5: 15 rats exposed to cold and fed diet WGF-14. In this diet 1 per cent cholesterol and 0.2 per cent choleate were added to the basal diet WGF-1.

Group 6: 15 rats adrenalectomized under ether anesthesia, and after recovery for 14 days, fed a diet composed of the basal mix slightly modified to suit the metabolic condition of these animals. The fat content of the basal diet was reduced from 25 per cent to 10 per cent with sucrose making up for the difference. This modified basal diet was then supplemented with 1 per cent cholesterol and 0.2 per cent sodium choleate (WGF-13). The adrenalectomized rats had a choice of water and 0.9 per cent saline to drink.

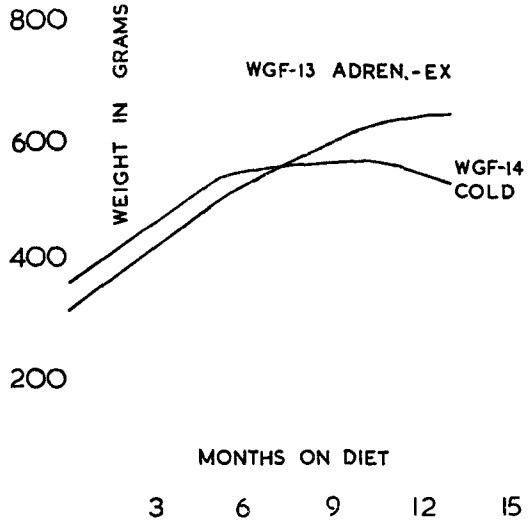
Set III was designed to produce cardiac infarcts in rats. The weight of the animals at the time of transfer to their experimental diets was between 400 and 450 gm. (Table I, Text-fig. 3).

Group 7: 10 rats fed diet WGF-4. In this diet a supplement of 0.35 per cent viosterol

(vitamin D concentrate) was added to the basal diet to induce vascular injury by hypervitaminosis D (34–37).

Group 8: 15 rats given 1 per cent cholesterol, 0.2 per cent sodium choleate, and 0.2 to 0.3 per cent thiouracil as supplements to the basal diet WGF. This diet (WGF-13) was designed to produce marked hyperlipemia and hypercholesterolemia (3, 29–33).

Group 9: 26 rats consuming diet WGF-12. In this diet the agents that induce hyper-



TEXT-FIG. 2

TABLE II *a*

Composition of Basal Diet WGF-1

	per cent
Dried egg yolk powder, "Borden"	35
Whole wheat flour	10
Soya bean flour	10
Sucrose	10
Bran	6
Lard	25
Salt mixture*	2
Vitamin sucrose mixture*,‡	1
Fat-soluble vitamins§	1

* For details see *Canad. J. Med. Sc.*, 1953, **31,** 135.

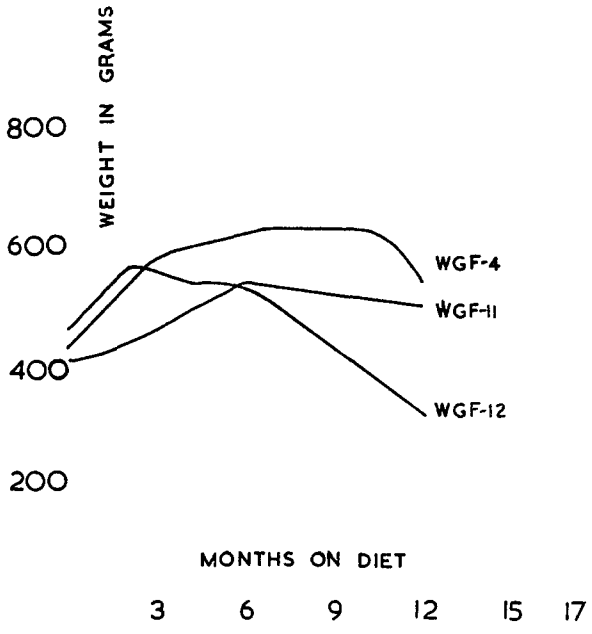
‡ This vitamin mixture was supplemented with 3 micrograms vitamin B_{12} per 100 gm. of food.

§ Fat-soluble vitamins dissolved in cornoil so that 1 per cent of dietary corn oil will supply the desired amount of fat-soluble vitamins. These were obtained from Ayerst, McKenna, and Harrison, Montreal: the content is 200,000 I.U. vitamin A and 50,000 I.U. vitamin D/gm. concentrate. The level of vitamin A per 100 gm. of basal food is 2000 I.U. vitamin A and 500 I.U. vitamin D.

lipemia (same as in group 8) and high doses of vitamin D (same as in group 7) were incorporated into the basal diet (WGF-1) with the intent to produce elevated blood fats in combination with vascular injury.

All animals were in an air-conditioned room in *individual* cages. They ate food *ad libitum* from round glass jars which were cleaned twice weekly. The food intake of cold-exposed rats was roughly twice that of rats kept at room temperature.

Serum total lipides were determined on tail blood by the method of Lucas *et al.* (38); phospholipides by the method of King (39); and serum total cholesterol by a modification of Schoenheimer's technique (40).



TEXT-FIG. 3

Animals were autopsied as soon as possible after death (12 hours post mortem at the most). The survivors in each group were killed by decapitation after a period that is indicated in Table I, column 8. Heart, aorta, lungs, kidneys, and liver specimens were washed in tap water after removal at autopsy and fixed in 10 per cent formalin calcium for the preparation of frozen sections. The slides were stained with a 0.4 per cent solution of oil red O in triethyl phosphate. Selected pieces of tissue were embedded in paraffin and prepared for staining with Lendrum's method (41) after mordanting the deparaffinized sections in Zenker's fixative for 12 hours.

Terminology

Spontaneous Coronary Lesions.—Several types of spontaneously occurring lesions have been described in old male rats. A diffuse fibrosis with hyperplasia has been noted by Wilens and Sproul (42) and a medial type of vascular damage by Hueper (43) and by Hummel and Barnes (44). Malinow, in a painstaking study, proved the occurrence of occasional lipomatous lesions (45) while Humphreys (27) noted connective tissue plaques with a deposition

of a fibrin-like material in the subintimal space (see (*b*) below "fibrinous type of atheroma"). There is little doubt that the rat is subject to a variety of spontaneously occurring coronary lesions which appear during the 2nd year of life, and sometimes are observed at an earlier age.

Lipomatous Coronary Lesions.—Appearance of stainable lipide in intima and/or media without signs of proliferation (Figs. 2 and 5).

Atheromatous Coronary Changes.—Two types of atheromatous lesions could be discerned:—

(*a*) Atheromatous changes of the lipomatous type are characterized by rather large amounts of subintimal fat deposited in a matrix of increased connective tissue ground substance, fibroblasts, and increased amounts of endothelial cells. This proliferation of mesenchymal elements is an essential criterion for the diagnosis of atheroma. The fat appears mostly extracellularly, both above and below the internal elastic membrane (Fig. 6).

(*b*) The fibrinous type of atheroma is a lesion in which fibrin-like material predominates over stainable lipide (27). The endothelial cells are usually not increased in number but are lifted from the underlying thin subintimal connective tissue layer. A narrow width of this layer is characteristic for the normal vascular anatomy of the rat. When fibrinous atheromata are present, the space between this single layer of endothelial cells and the internal elastic membrane becomes enlarged and is filled with a proteinaceous fibrin-like mass that has some fat. The vessel wall is usually edematous (Figs. 3 and 4).

Although both types of atheromatous lesions lead to plaque formation, the histological features of these two types of plaques are different. While the lipomatous type of atheroma is usually the product of experimental hyperlipemia, the fibrinous type of atheroma occurs spontaneously in older rats. It may also appear at a somewhat earlier stage of life when the animals have been exposed to cold.

Moenckeberg Type of Medial Sclerosis.—This lesion has necrosis and calcification of the elements of the media as its outstanding characteristic. The vessel wall becomes thinned and the lumen widens. On occasion, intimal areas overlying the medial injury show signs of reactive proliferation, both of connective tissue ground substance and endothelial and subintimal cells (Fig. 9).

Atherosclerotic Coronary Changes.—Calcification, splitting of the internal elastic membrane, and pronounced degrees of proliferation and lipide accumulation in the subintimal space are the features of atherosclerosis in the rat. Frequently these changes were accompanied by thrombosis and occlusion of the vascular lumen (Figs. 7, 8, and 10).

Cardiac Infarcts versus Focal Cardiac Necrosis.—A clear distinction is made here between cardiac infarcts and focal cardiac necrosis. The former is characterized by either diffuse or well circumscribed larger areas of necrotic tissue and cellular debris that is studded with stainable lipide. These areas of infarction were frequently walled off by leucocytic infiltrates (Figs. 11 and 12). This never occurred in the areas of focal necrosis, owing to hypervitaminosis D. These focal necroses were multiple and rather small; inflammatory cells did not abound in these areas, which, instead, had more connective tissue.

Stress.—Stress is defined as a harmful state of disturbed homeostasis within the organism (Ingle, 46).

Aging and Vascular Injury.—These terms are used in their usual "colloquial" sense, for no clear definition seems to satisfy scientific criteria.

RESULTS

The results will be discussed for each group separately, the main emphasis being on the coronary arteries. Findings in other parts of the vascular system as well as changes in organs other than the heart will be mentioned only if they deserve special discussion.

Set I, Groups 1 to 4.—The animals in this group were controls for the other experimental groups. Only mild lesions in their coronary arteries were observed. This point is emphasized because it proves that the more severe lesions observed in groups 5 to 9 were experimentally induced and were not occurring spontaneously. Spontaneous lesions in the animals fed chow (group 1) and in those given diet WGF-1 (group 2) were mostly mildly lipomatous. Occasionally mild hypertrophy of the vessel walls with intimal thickening and plaque formation was found. The fibrinous type of atheromatous lesion was observed in only 3 of these 25 control rats.

The extensive Moenckeberg type of medial injury observed by Ingle and Baker to occur in old female breeder rats (46) was not seen in this series of experiments involving male rats. Likewise, panarteritis nodosa-like changes, first observed by Wilens and Sproul (42), did not occur spontaneously in this study. In addition to the above mentioned coronary changes, there was usually some widening of the vessel diameter. Also frequently observed was an irregularity of the contour of the vessel wall which was commonly hyperplastic and edematous. Because the rats used in this experiment were either younger than 2 years at the time of sacrifice or died before reaching this age, spontaneously occurring changes were rarely seen.

The most significant finding in this group was the striking degree of obesity exhibited by the group 2 animals on the WGF-1 diet (Fig. 1). This type of obesity was induced by spontaneous overeating alone without recourse to drastic procedures other than feeding the high fat diet. The rats were allowed to move around freely in large cages. They had blood lipide values that were about twice as high as those of the chow-fed animals (Table III), and their incidence of lipomatous coronary changes was twice as great.

The rats in group 3 given 2 per cent extra dietary cholesterol ate well and gained more weight than the chow-fed rats, but their weight gain was distinctly less than that of the animals consuming the basal diet without cholesterol (group 2). Their livers were, in the gross, very fatty, and on examination with a polarizing microscope showed a large amount of optically active material (most of it presumably cholesterol esters). Possibly this material interfered with liver function and so limited the weight gain. Despite the large amount of cholesterol in the diet, only lipomatous lesions were observed in the coronary arteries of these rats. This result agrees with the long known fact that rats are resistant to the induction of atheromatous lesions with cholesterol feeding, alone (1, 8–23), apparently because they metabolize exogenous cholesterol very efficiently. More drastic measures such as were applied in groups 5 to 9 are necessary to elevate the blood cholesterol levels of rats to such a degree that atheromatous lesions develop.

Group 4 was exposed to cold, while control group 2 ate the same diet at room temperature. The cold-exposed animals grew at a rate about half that of the

rats at room temperature (RT) despite a doubled food intake. The incidence of lipomatous lesions was greater in the cold-exposed animals than in the controls, but no severe coronary lesions were observed. The increased incidence of lipomatous lesions in the cold-exposed rats (47) may be due to the doubled food intake. The cause of death in groups 1 to 4 was either a respiratory infection or obstructive nephropathy (48).

Set II, Groups 5 and 6.—The results in group 4 seemed to show that cold stress does not cause any particular vascular lesions in rats that are given a normal salt intake. Again, more severe lesions could be induced only by more drastic measures (*cf.* references 49–51). Such measures were put on trial in groups 5 and 6 as a pilot study. Atheromatous lesions occurred in the presence and in the absence of the adrenal gland, which was slightly enlarged in group 5. The incidence of atheromatous lesions of the lipomatous type was greater in the rats exposed to cold than in the rats that were adrenalectomized and kept at room temperature and which ate normal amounts of food. The fibrinous type of atheroma was observed in 5 out of 15 rats exposed to cold, the highest incidence of this type of lesion observed in any of the groups. In most cases the cause of death in the cold-exposed animals was respiratory infection. However, bleeding, bitten tails frequently caused slow exsanguination; while in other cases, bladder concrements led to urinary obstruction and a fairly severe type of obstructive nephropathy. The livers of the rats given cholesterol and sodium choleate (groups 5 and 6) were moderately fatty. The aorta showed lipomatous and atheromatous changes of the fatty type comparable to those found in the coronary arteries but did not exhibit fibrinous lesions.

Set III, Groups 7 to 9.—This set represented the most important part of the experiment. Again, the control was group 2, fed the basal diet WGF-1. Addition of viosterol to the diet produced a typical Moenckeberg type of medial necrosis (group 7), both in aorta and coronary arteries. The cause of death in this group was usually nephrocalcinosis. The animals gained initially, but later lost weight (see Text-fig. 3).

Group 8 was fed the basal diet supplemented with cholesterol, choleate, and thiouracil (Table II *b*). The rats of this group did indeed exhibit atheromatous coronary and aortic changes as described first by Fillios (3). Three of the fifteen rats had a portal type of cirrhosis associated with deposits of cholesterol in their livers (3). It is worthy of note that these 3 cirrhotic animals had no vascular lesions. All animals had extensive obstructive nephropathy and very fatty kidneys. They were myxedematous, and tended to have loose stools.

Group 9, supplemented with viosterol in addition to the agents used in group 8, showed both vascular injury and hyperlipemia. These rats lost weight in the early stages of their feeding period and most of them died before the 12th month. The lesions of the vascular system confirm results obtained in the pilot study published previously (5). Almost all rats had lipomatous coronary changes, about 78 per cent had atheromatous lesions of the fatty type, and

64 per cent had severe atherosclerosis. This incidence and degree of atherosclerosis was not observed in any other group, nor did the fibrotic and sclerotic component of these lesions occur in any other group. One-third of the rats in

TABLE II *b*

*Supplements to Basal Diet WGF-1**

	per cent
Cholesterol	1
Thiouracil‡	0.2–0.3
Sodium choleate	0.2
Viosterol§	0.35

* These supplements were added to the basal diet as indicated in Table I, column 3.

‡ The thiouracil content of the diet was continually adjusted between these two values, so that the ensuing hypothyroidism did not become too severe. The guide for the adjustments was the weight curve.

§ Obtained from Nutritional Biochemicals, Ltd., Cleveland. Contains 400,000 I.U. vitamin D per cc. of concentrate. A level of 0.35 per cent provides 140,000 I.U. vitamin D per 100 gm. of food.

TABLE II *c*

Nutritional Data of Basal Diet WGF-1

Compounded from Nutritional Data, Pittsburg, Heinz Nutritional Research Division, H. J. Heinz Co., Publisher, 1953.

	% of diet WGF	Per 100 gm. of diet—WGF					
		Calories*	Water	Protein	Fat	Ash	Carbohydrate
Dried egg yolk	35	242.6	1.0	10.9	21.5	1.1	0.5
Soya bean flour	10	26.4	1.0	4.3	0.6	0.5	3.7
Whole wheat flour	10	36.4	1.2	1.0	0.1	0.0	7.6
Sucrose	10	38.5	0.0				10.0
Bran	6	17.5	0.2	0.7	0.1	0.3	4.7
Lard	25	225.5			25.0		
Corn oil	1	8.8			1.0		
Minerals	2					2.0	
Vitamins in sucrose	1	3.8	0.01				1.0
Total	100	600*	3.41%	16.9%	48.3%	3.9%	27.5%
Calories/100 gm. WGF		620*		69.3	439.5		110.
% of total calories				11.2%	71.1%		17.7%

* The caloric value for 100 gm. food is 600 calories on the basis of measurements obtained by direct calorimetry in a calorimetric bomb. The value 620 is computed by multiplying the percentage figures for protein, fat, and carbohydrate by the conventional factors 4.1, 9.1, and 4.0, respectively.

this group had coronary occlusion, total or subtotal, caused by severe atherosclerosis. Half the animals with detectable coronary occlusion had myocardial infarcts, which could be well differentiated from areas of focal cardiac necrosis

due to hypervitaminosis D. We do not know why only half the rats with coronary occlusion had myocardial infarcts.

The livers of these animals were very fatty, and the kidneys frequently showed severe degree of nephrocalcinosis. Respiratory infections were common. Urinary concrements were frequent. The blood lipides of these rats were as high as those of group 8, illustrating the efficacy of the dietary ingredients used to induce hyperlipemia (Table III).

No attempt is made here to explain the mechanism of induction of the hyperlipemia by dietary choleate, cholesterol, and thiouracil (52). Likewise, no

TABLE III

*Blood Lipide Values**

All 6 groups were fed *ad lib.*

Diet	Mos. on diet‡	No. of rats‡	Serum (38) total lipides	Serum (39) total phospho-lipides	Serum (40) total cholesterol
Control chow	17	7	580 ± 48	135 ± 30	80 ± 16
Basal WGF-1	17	12	948 ± 260	240 ± 74	175 ± 48
WGF-13 Adrex.	9	6	—	282 ± 79	205 ± 64
WGF-14 cold	9	14	—	254 ± 68	174 ± 53
Experimental WGF-11	9	13	3905 ± 682	692 ± 278	1007 ± 301
Experimental WGF-12	9	9	3273 ± 590	685 ± 253	986 ± 306

* All values show statistically a significant difference from the control group fed chow ($p < 0.01$).

‡ The determinations were done on animals surviving an experimental period as indicated in column 2.

interpretation is offered to explain the induction of vascular injury by high oral doses of vitamin D (53–56).

DISCUSSION

Vascular lesions similar to or identical with those seen in the coronaries were frequently discerned in the aorta and in the vessels of lung and kidneys. However, this report is limited to the coronary arteries.

Spontaneous and experimentally induced coronary lesions were observed in male rats in this long term study in which an attempt was made to imitate in as "normal" a fasion as possible pathologic processes as seen in man. While many of the experimental procedures were drastic, they allowed an average life span of at least 8 months, which compares favorably with the longevity of rats subjected to other experimental procedures (3, 6). Despite the fact that the rat was

believed to be resistant to the induction of atherosclerosis (1, 8, 23), a great variety of spontaneously developing lesions can be observed in the coronary arteries (41–45). However, in this study the age of the rats never exceeded 2 years and, therefore, some of the changes described by other investigators in older rats were not seen.

Lipomatous deposits, mostly extracellular, were seen in intima and media. Hypertrophy of the media with thickening of the subintima, increase in width of the internal elastic lamella, and tiny plaque formation within the endothelial layer were common. The most interesting spontaneous lesion was the fibrinous type of atheroma, which occurred sporadically in the control rats. The etiology and pathogenesis of this type of lesion are unknown but are being studied. We cannot decide at present whether the fibrin-like material is deposited from the bloodstream in the vessel lumen or is "exuded" from an injured vessel wall. The Lendrum stain for fibrin is positive in these plaques, confirming the original observation by Humphreys (27), who found that these plaques take a stain for fibrin. Although there is no specific stain for fibrin, circumstantial evidence strongly suggests that this material is present in these naturally occurring plaques.

Several observations in the experimental groups are worthy of note. Cholesterol feeding alone did not produce atheromatous changes in the rat. Apparently this species is well equipped to dispose of a high dietary cholesterol load. This agrees with established views (1, 8–23). The basal diet used in this study led to voluntary overeating and marked obesity. The animals on this diet were roughly twice as heavy as their chow-fed controls. Their blood lipides were about twice normal. The incidence of lipomatous coronary lesions was also doubled. Obesity has been produced in rats by several procedures, most of which can be considered unphysiologic (57–61). Mickelsen (62) used diet alone, as we did. His diet contained up to 60 per cent fat; in our experiment, the dietary fat was kept at 48 per cent, with the protein content being adequate for mature animals (see Table II *c*). We therefore feel that this type of obesity was induced without undue recourse to drastic procedures. The cause of spontaneous overeating is unknown to us.

The experimental results obtained in intact stressed rats (exposed to cold on a normal salt intake, group 5) and in the adrenalectomized animals at room temperature (group 6) require further study. The preliminary data seem to indicate that the fatty type of experimental vascular lesions does not relate to adrenal function—at least in the rat. The increased incidence of lipomatous lesions in these cold-exposed animals on the basal diet (group 4) is probably more related to the increased food intake rather than to aberrations in adrenal cortical function. Of great interest was the severity of both lipomatous and fibrinous lesions in cold-exposed animals fed the basal diet supplemented with cholesterol and cholic acid (group 6). The lipomatous lesions in the coronary

arteries of these cold-exposed rats can be related to the high intake of these dietary agents. The relatively great incidence of fibrinous lesions, however, cannot be easily explained. Whether this is a sign of accelerated aging in the cold, due to cold injury *per se*, or a sign of vascular injury caused by the obstructive nephropathy that is so pronounced in cold-exposed animals remains to be determined. Since this type of fibrinous lesion does occur spontaneously in older rats, as has been shown by Humphreys and now confirmed by us, we are led to believe that some process connected with aging of rats is accelerated in the cold in preference to the notion that this lesion is due to a pathologic process specific for cold-exposed rats. As stated above, this part of the study is being repeated and extended.

Our most significant results were obtained in groups 7 to 9. *Severe* degrees of hyperlipemia and hypercholesterolemia (group 8) were consistently associated with atheromatous coronary, renal, and pulmonary changes. Such striking increases in blood lipides arise in the rat only after rather drastic dietary manipulations. But the fact remains that high blood lipides cause atheromata. Vascular injury without hyperlipemia, as produced in group 7, leads to a Moenckeberg type of medial necrosis with very little intimal affliction. However, when vascular injury was combined with hyperlipemia and hypercholesterolemia, very severe lesions were obtained (group 9). It was *only in this group* that a marked degree of atherosclerosis caused coronary occlusion and cardiac infarction (13 per cent incidence). Although cardiac infarction can be associated with grossly elevated blood lipides alone, (5, 6) it is obvious that combined vascular injury and elevated blood lipides produce cardiac infarcts much more effectively than either mechanism alone.

The observation that the incidence of myocardial infarction was only half that of coronary occlusion deserves further comment. It is known that the occurrence of myocardial infarcts in man depends not only on the extent of coronary atherosclerosis, but also on changes in the clotting mechanisms and on variations in hemodynamics. Apart from the fact that we might have missed some infarcts, it is possible that the collateral circulation in the myocardium of the rat is such that a blockage in one segment of the coronary artery is not necessarily followed by necrosis of that part of the heart muscle which depends normally on the blood supply from this arterial segment. This hypothesis implies that some occlusive processes might have occurred rather slowly and depended on a clotting mechanism quite different from the processes involved in human thrombus formation. It remains to be studied whether man is more prone to develop thrombi, whether animals have a protective mechanism (such as fibrinolytic activity) that prevents the massive formation of thrombi in an atherosclerotic blood vessel, or whether other, unknown, factors play the decisive role.

Direct application of our vascular findings in the rat to human pathology

is not yet indicated. Not only are the species differences great, but some of the experimental methods used were so drastic as to have no direct clinical counterpart. Still, some general conclusions appear to be justified.

A variety of spontaneous intimal and subintimal lesions develop in aging rats, and it is conceivable that comparable processes occur in human blood vessels. The experimentally produced lesions tend to strengthen the theory that lipide infiltration is an important part of the atherogenic process (68). The rat is resistant to the induction of atherosclerosis and cardiac infarcts by comparably mild methods. But the fact that this resistance may be overcome indicates that we can study this pathogenetic process in the rat without fear of spontaneous atherosclerosis, although a variety of other vascular lesions may occur in aging rats. Our studies show that atherosclerosis and cardiac infarcts can be produced experimentally in this species by a combination of vascular injury and hyperlipemia. One can speculate that these two factors are the critical ones in the etiology of atherosclerosis. While a great deal of work is devoted to elucidate the etiological significance of disturbances in fat metabolism—both dietary and endogenous—little attention is paid to the problem of vascular injury. Research into what is loosely termed "vascular injury" might prove fruitful. We might also wonder whether fibrin deposition in the arterial wall may be more important than is generally believed at present (63–65). Our data do not support the vascularization theory of atherosclerosis, for cardiac infarcts and atherosclerosis were induced in a species such as the rat, which has no demonstrable vasa vasorum in its blood vessels (42, 66). Our results may then be interpreted in the following way: Atherosclerosis has a combination of causes (Aschoff, 1933, (67), Anitschkow, 1933, (68), Page, 1954 (69)). Different etiological factors may contribute to its development in varying degrees at different times. Some features of atherosclerosis may depend on as yet unknown processes of aging. In some cases fibrin deposits may conceivably be important. However, it is our belief that the outstanding factors in the etiology of atherosclerosis are disturbances in lipide metabolism superimposed on what is loosely referred to as "vascular injury" (67, 68).

SUMMARY

Marked obesity was induced in rats by feeding a high fat, egg yolk–rich diet. The obese rats were hyperlipemic and showed an increased incidence of lipomatous coronary lesions, but did not develop severe atheromatous lesions.

Spontaneous vascular lesions of several kinds have been observed in aging rats. Among them, plaques containing a fibrin-like material seem to be conspicuous. However, these lesions differ from the experimentally induced changes, which were more fatty. Atherosclerosis, as it is defined in human pathology, has *not* been observed to develop *spontaneously* in rats.

Experimental induction of marked hyperlipemia and hypercholesterolemia

by feeding a high fat egg yolk–rich diet (supplemented with cholesterol, choleate, and thiouracil), and use of viosterol to cause vascular injury, led to severe atherosclerosis, coronary occlusion, and myocardial infarction.

A consideration of all the findings reported here leads to renewed support of the concept that atherosclerosis has a combination of causes (Aschoff, Anitschkow, Page). Of all the etiological factors considered here, elevation of blood lipides and vascular injury are thought to be the most important ones.

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EXPLANATION OF PLATES

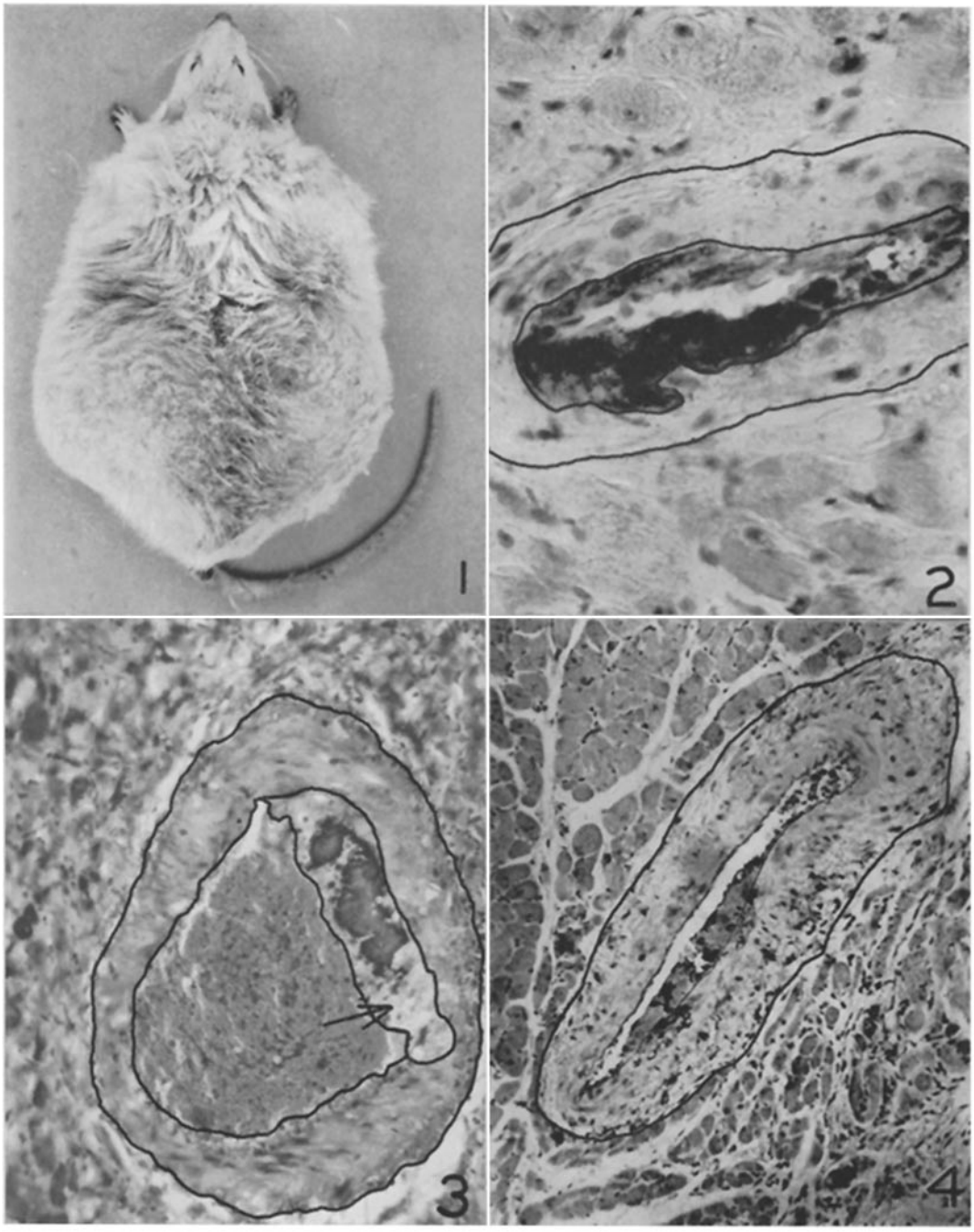
PLATE 33

FIG. 1. Obese rat on basal diet WGF. This rat weighed around 1200 gm. at sacrifice after 17 experimental months. Obesity was induced by spontaneous overfeeding without recourse to unphysiological procedures. Lipomatous coronary lesions were detected in these obese rats, but atheromata could not be discerned (see Fig. 2). ⅓ normal size.

FIG. 2. Lipomatous coronary lesion. High power, frozen section. Oil red O stain. Fat appears almost black. Lipide has infiltrated the subintimal space and also part of the media. The endothelial lining is "lifted" from the underlying mass of lipide. There is, however, no increase in connective tissue ground substance or cellular proliferation. The media and adventitia are outlined in ink. The lesion illustrated was observed in the obese animal shown in Fig. 1. × 450.

FIG. 3. Fibrinous type of atheroma. High power, Lendrum stain (picro acid–Mallory method) for the detection of fibrin. This stain is not truly selective for fibrin, although its specificity is claimed to be fairly great. Fibrin appears bright purplish in the stained section. Here in this photograph the original color is represented by a blackish grey. A larger coronary artery is demonstrated, the lumen of which is filled with a postmortem aggregate of erythrocytes. A plaque protrudes into the lumen from the right side of the vessel wall, which is slightly edematous. The greater part of the plaque is filled with the fibrin-positive material, while in the lower portion of the plaque a light reticulated area is indicative of fat. The plaque is outlined by ink for better demonstration. The arrow indicates the spot of lipide accumulation. × 400.

FIG. 4. Fibrinous type of atheroma. Medium power, frozen section. Oil red O stain. Fat appears black. The proteinaceous material in the plaque on the right wall of the vessel appears greyish. Note the edema of the vessel wall and the slight increase in cellularity in that part of the adventitia which underlies the plaque. This plaque is covered by a single layer of endothelial cells, which are separated from the underlying, distinctly visible internal elastic lamina by a mass of fibrin-like material. Lipides are visible as black spots above and below the internal membrane. This fat is mostly extracellular. The vessel wall is outlined in ink. × 180.



(Wilgram: Atherosclerosis and cardiac infarcts)

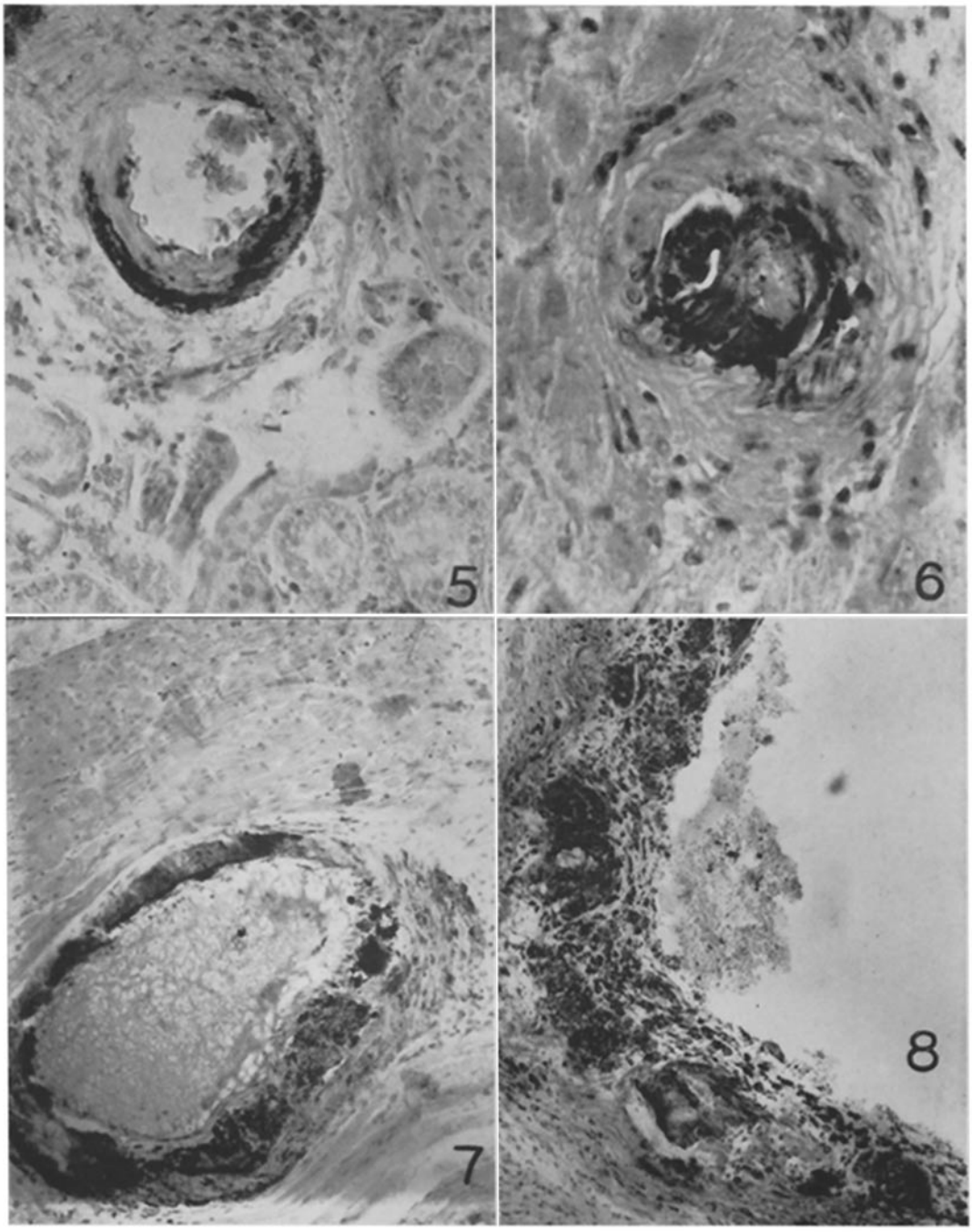
PLATE 34

FIG. 5. Lipomatous lesion in a renal artery. Frozen section, oil red O stain. Fat, as black masses, has infiltrated the entire wall of this renal artery. Again no sign of proliferation is discernible. × 200.

FIG. 6. Fatty type of atheroma in a coronary artery. High power, frozen section, oil red O stain. Lipide infiltration into the intima is accompanied by proliferation of connective tissue ground substance and endothelial cells, leading to an almost complete obstruction of the lumen. Fat has also penetrated deeper into the media. This lesion was induced by severe degrees of hyperlipemia and hypercholesterolemia. × 400.

FIG. 7. Atherosclerosis in the rat. Medium power, frozen section, oil red O stain. The diameter of the vessel is widened, but large plaques protrude towards the lumen. The whole vessel wall is calcified and rigid. The right part of the vessel wall shows large amounts of lipide in a mass of increased connective tissue and debris, separated from the calcified media by a hypertrophic internal elastic membrane. This lesion was induced by an elevation of blood lipides which was superimposed on vascular injury. × 150.

FIG. 8. Aortic atherosclerosis. High power, frozen section, oil red O stain. The normal structure of the aortic arch is completely distorted by masses of lipide, calcified debris, and increased connective tissue. Intima and media are inseparable. The few remaining elastic lamellae are condensed towards the adventitia and are to be seen as remnants in the left upper and lower part of the photograph. The aortic "wall" is about three times the normal width. This lesion was produced by a combination of vascular injury with elevation of blood lipides. The animal died from a coronary infarct. × 380.



(Wilgram: Atherosclerosis and cardiac infarcts)

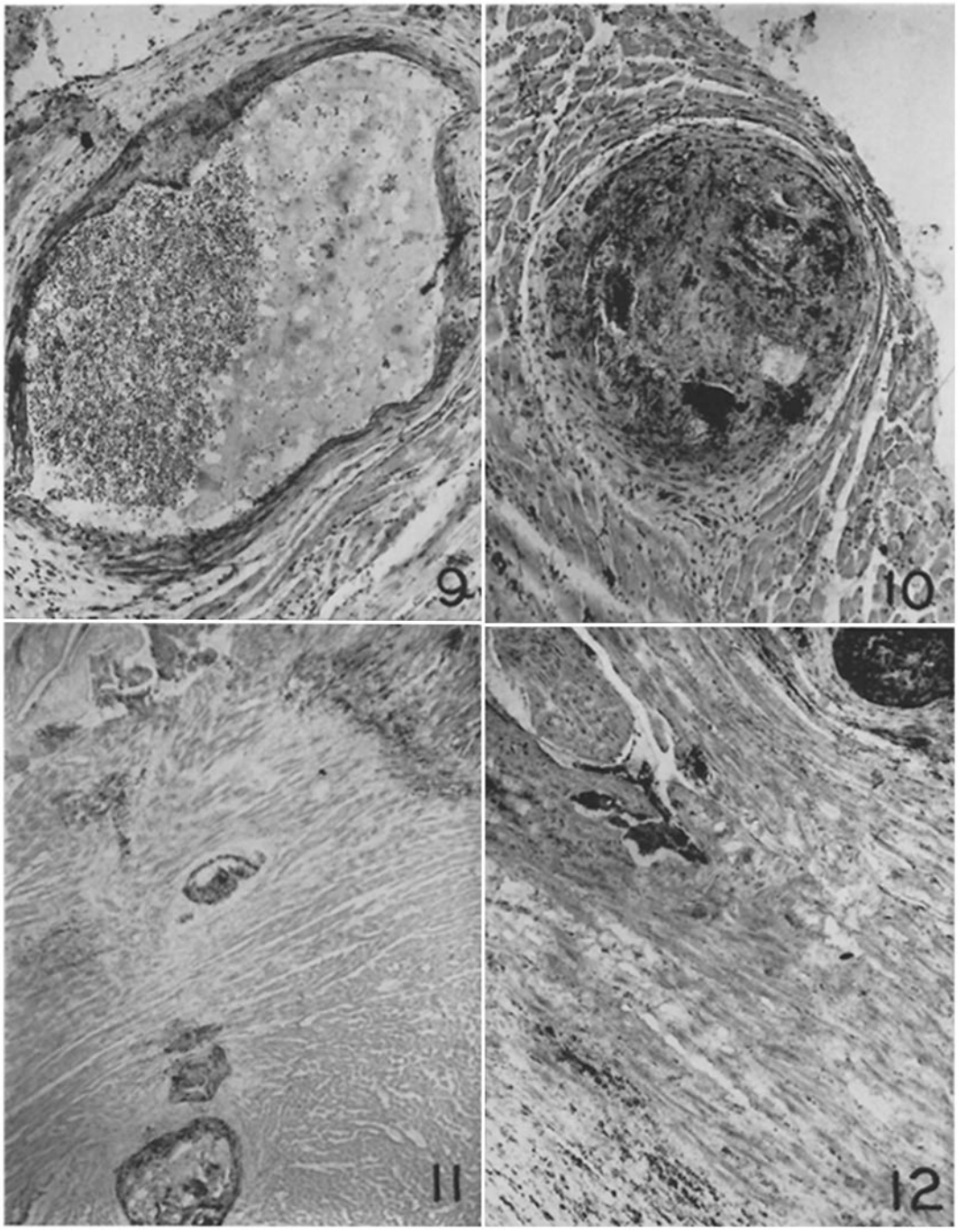
PLATE 35

FIG. 9. Moenckeberg-type of vascular injury. Medium power, frozen section, oil red O stain. Vascular injury in this coronary artery is characterized by a thinning of the vessel wall, which becomes dense and rigid. Calcification is visible in the original section by a bluish hue of the vessel wall, indicative of calcium salts taking on the chromalum-hematoxylin counterstain. The media in the left upper portion of the picture is completely replaced by calcified debris. The overlying intima has proliferated as response to the underlying injury. These two processes give this area of the vessel wall the appearance of a protrusion. In reality this "protrusion" is a location of repair and increased fragility. This lesion was produced by large oral doses of vitamin D without accompanying elevation of blood fats. × 200.

FIG. 10. Coronary occlusion in rats. Medium power, frozen section. Oil red O stain. The lumen of a subepicardial artery is completely obliterated by atheromatous masses composed of lipide, cholesterol, debris, connective tissue ground substance, and granulation tissue. The latter may be observed in the upper part of the lumen in an area, where nuclei abound. Calcification is demonstrated by large clumps of darkish staining masses. This complete occlusion was produced in a rat, in which vascular injury was combined with hyperlipemia and hypercholesterolemia. × 220.

FIG. 11. Cardiac infarct in rats (animal WGF-12 No. 28). A major trunk of the left coronary artery shows severe atherosclerotic involvement and is subtotally occluded. One of its branches is completely thrombosed. A well circumscribed necrotic area is seen in the right upper corner of the photograph. This necrotic area undoubtedly represents infarction of the part of the cardiac muscle that depended in its blood supply upon the segment of the coronary artery that is shown as being occluded in this picture. Low power, frozen section, oil red O stain. This infarct was brought about by a simultaneous combination of vascular injury and elevation of blood lipides. × 50.

FIG. 12. Cardiac infarct in rats (animal WGF-12, No. 15). The features of infarction are essentially the same as shown in Fig. 11. A larger branch of the coronary tree (right upper corner) is completely occluded. The infarcted cardiac muscle is detectable in the left lower corner of the photograph and is characterized by debris that is lipide-laden and infiltrated with polymorphonuclear leucocytes. Medium power, frozen section, oil red O stain. × 75.



(Wilgram: Atherosclerosis and cardiac infarcts)