Dietary Method for Induction of Atherosclerosis, Coronary Occlusion and Myocardial Infarcts in Rats. (24396)

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The rat was believed for many decades to be resistant to induction of atherosclerosis. Only recently could the older concept of resistance of this species to atherosclerosis be revised, thru the work of several investigators. Wissler *et al.* stressed the usefulness of the rat in research on cardiovascular problems and pioneered in devising a method for induction of atheromatous lesions in this species (1). The type of lesion produced by different workers using various methods has already been discussed(2). It has been demonstrated that practically every major arteriopathy observed in man can be reproduced, as a morphological equivalent, in the rat.

This report deals only with atherosclerotic lesions in the coronary arteries of rats. The above mentioned review describes arteriopathies other than atherosclerosis(2). Atheromatous lesions, with a marked lipomatous component have until now been produced in rats by inducing hypothyroidism and feeding diets high in fat and cholesterol with and without cholic acid(3). Atheromatous lesions with a marked fibrinous component were observed by Humphreys to occur spontaneously in older rats(4). The work reported here was designed to produce not only mild lesions of a lipomatous or fibrous type but to induce severe atherosclerosis with coronary occlusion and cardiac infarcts. Despite the morphological similarities exhibited by atherosclerosis in man and in the rat, it is obvious that one should not base definite conclusions with respect to the aetiology and pathogenesis of atherosclerosis in man on the findings in rats. The fact must also be recognized that atherosclerosis in the rat can be produced by different methods‡(5). Many observers agree that atherosclerosis in man is a polyaetiological disease in which different mechanisms may lead to apparently similar morphological end-results. Our procedure for inducing atherosclerosis, coronary occlusion and cardiac infarcts in rats depends upon the production of hyperlipaemia and of vascular injury.

Materials and methods. Preliminary trials involving 170 male Wistar rats established that the following regimen was a productive one. In a final experiment these conditions were imposed on 27 white male Wistar rats. This final group was originally composed of 30 animals, but 3 died in the first 4 weeks and were not included. These 3 rats had diarrhea —presumably due to the cholate content of the diet. The remaining 27 rats were the final group in which a state of hyperlipaemia was combined with vascular injury. 1. Basal diet (Table Ia). 2. Hyperlipaemia was induced by supplementing the basal diet with 0.3% thiouracil, 0.2% sodium cholate and 1% cho-

TABLE Ia. Composition of Basal Diet—WGF.

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

* For details see *Can. J. Med. Sci.*, 1953, v31, 135.

† This vitamin mixture was supplemented with 3 μg vit. B_{12}/100 g of food.

‡ Fat-soluble vitamins dissolved in corn oil so that 1% of dietary corn oil will supply the desired amount of fat-soluble vitamins. These were obtained from Ayerst McKenna and Harrison, Montreal, Canada. Content is 200,000 I.U. vit. A and 50,000 I.U. vit. D/g concentrate. Level of vit. A/100 g of basal food is 2000 I.U. vit. A and 500 I.U. vit. D.

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‡ W. S. Hartroft reported recently that myocardial infarction occurred in 10-30% of his rats using a technic somewhat different from ours(6).

TABLE Ib. Experimental Diet WGF-12. Supplements to basal diet WGF.

	%
Cholesterol	1
Thiouracil	.3
Sodium cholate	.2
Viosterol	.35

lesterol (Table Ib). 3. Vascular injury was produced by dietary supplements of irradiated ergosterol (viosterol containing 400,000 units vit. D/g of concentrate, obtained from Nutritional Biochem., Cleveland, O.). 3.5 ml of concentrate was dissolved in 6.5 ml corn oil and added to 1 kg of basal diet. 100 g of food therefore contained 0.35 ml of concentrate or 140,00 I. U. units vit. D. (Table Ib). 4. Average weight of animals at beginning of this experiment was 450 g. One group in the preliminary experiments was fed the basal diet alone. Another pilot group was given the diet with thiouracil, cholesterol and sodium cholate but without vit. D. A third group received the basal diet with vit. D but without the agents that induce hyperlipaemia. Two other groups were given a slightly modified basal diet and exposed to cold (0° ± 2°) for eight months. The animals were housed in individual cages. Their food dishes were filled twice weekly. Food left in the dishes was discarded. Average lifespan of the experimental animals on the dietary regimen WGF-12 was 8 months (Table II). None of the experimental rats were killed. All animals were given the diet *ad lib* until death intervened. The rats in the pilot groups were also fed *ad lib*. Animals in the pilot groups that had survived for a period of one year were killed by decapitation. Heart, aorta, lungs, kidneys and liver specimens were removed as soon as possible after death, fixed in 10% formalin calcium and subjected to the frozen sectioning technic. The slides were stained with a 0.4% solution of oil red O in triethyl phosphate. The findings in the heart only will be discussed here. After fixation for at least 48 hours each heart was rinsed in tepid water overnight, then embedded in warm gelatin. After setting of the gelatin in the ice-box each heart was shelled out of the excess of gelatin and cut like a pine-

TABLE II. Lifespan after Institution of Experimental Diet WGF-12.

No. of months prior to death	No. of animals dying in this month
5	3
6	1
7	5
8	7
9	6
10	4
11	–
12	1
Avg lifespan 8 mo	Total 27 animals

cone on the stage of the freezing microtome at NOT LESS THAN 7 DIFFERENT LEVELS. Blood was withdrawn from the tail vein of 9 experimental rats that had survived 9 months on the dietary regimen and submitted to serum lipid determinations (Table III).

Results. Terminology. The following terms are used in presentation of results with respect to coronary arteries. *A. Lipomatous coronary lesions*: Appearance of stainable lipid in intima and/or media without signs of proliferation. *B. Atheromatous coronary lesions*: Characterized by proliferation or deposition

TABLE III. Blood Lipid Values (mg %).* All 3 groups were fed *ad lib.*

Diet	No. of animals	Serum total lipids†	Serum total phospholipids‡	Serum total cholesterol§
Chow	6	592 ± 47.3	138 ± 35	83 ± 17
Basal WGF	13	947 ± 254.8	242 ± 72	170 ± 50
Exp. WGF-12	9	3396 ± 620	679 ± 269	1072 ± 315.3

* Statistical analyses proved that values obtained showed differences of high significance ($p < .01$).

† Total lipids were extracted by method of Bloor, Pelkan and Allen (*J. Biol. Chem.* 1922, v52, 191).

‡ King's method was used for assay of plasma phospholipids (*Biochem. J.* 1932, v26, 292).

§ Serum cholesterol determined by the method of Sperry and Webb (*J. Biol. Chem.* 1950, v187, 97).

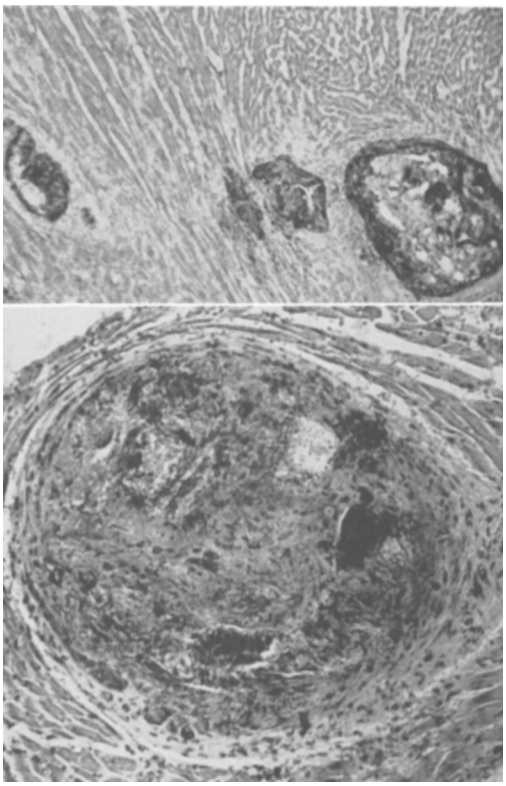

FIG. 1. Cardiac infarct and complete coronary occlusion induced experimentally in rats by means of hyperlipaemia and vascular injury. *Upper photomicrograph.* Low power, frozen section, oil red O stain. Fat appears black. A medium sized coronary artery with 2 of its branches is cut in cross section. Extensive atherosclerosis appears in all 3 lumina, occluding one of them completely. Adjacent cardiac muscle exhibits scattered areas of necrosis in lower half of picture; large area of frank necrosis in upper part. This infarct is well circumscribed, wedge-shaped and has a classical zone of leucocytic infiltration around it. It is histologically distinctly different from areas of focal myocardial necrosis due to hypervitaminosis D. *Lower photomicrograph.* High power, frozen section, oil red O stain. Fat appears black. A subepicardial coronary artery completely occluded by atheromatous and thrombotic masses. Granulation tissue invades lumen towards centre. Thrombotic material is intermingled with fat, debris, cholesterol crystals, proliferating cells and increased connective tissue ground substance.

of a fibrinous material between intima and media accompanied by increase in number of endothelial or subintimal cells. This leads to a narrowing of the lumen by the protruding plaques so formed. Fat also is very conspicuous in the subintimal areas. *C. Atherosclerotic coronary lesions*: Calcification, splitting of internal elastic membrane and pronounced degrees of proliferation and lipid accumulation in the subintimal spaces are features of atherosclerosis in the rat, frequently accompanied by thrombosis and occlusion of the vascular lumen. None of the 170 animals used in the pilot studies exhibited lesions as severe and widespread as those seen in the final experimental group. The rats on the basal diet WGF grew very obese and frequently had lipomatous coronary lesions. When the basal diet was supplemented with cholesterol, thiouracil and sodium cholate, degree of coronary lipomatous lesions was more severe and occasionally atheromatous changes could be observed due to the high degrees of hyperlipaemia(3). The basal diet supplemented with vit. D led to development of vascular injury resembling the Moenckeberg type of medial sclerosis. The rats that were given the basal diet and were exposed to cold ($\pm 2°$) for 8 months, frequently had lipomatous lesions in their coronaries, but severe degrees of vascular change were not seen.

A careful study of all these groups revealed in the pilot experiments that neither hyperlipaemia nor vascular injury alone was able to produce severe atherosclerosis and cardiac infarcts in rats. These severe lesions of the coronary arteries could be discerned only when both hyperlipaemia and vascular injury were induced.

The efficacy of the diet in inducing hyperlipaemia is demonstrable in Table III. Blood lipid values in the experimental animals on diet WGF-12 are considerably elevated as compared with animals that were fed either the basal diet WGF or were given chow.

The efficacy of vit. D supplements in producing vascular injury of a Moenckeberg type (medial sclerosis) was ascertained in the pilot group that received the basal diet with vit. D supplements only.

We will confine ourselves to a presentation of the results produced by vascular injury and hyperlipaemia, in the final experimental group (Table IV). An animal was recorded as positive with respect to any given coronary pathology, if this type of lesion could be discerned *in at least 2 out of the 7 sections* cut from each heart at 7 *different* levels. (See *Methods and Materials.*) It can be seen that

TABLE IV. Pathological Findings in 27 Rats on Diet WGF-12.

Mo on diet WGF-12	Infarct	Coronary occlusion	Coronary arteries: Lipomatous	Atheromatous	Atherosclerotic
6			+	+	+
8		+	+	+	+
7		+	+	+	+
5			+	+	—
8			+	+	+
5			+	+	+
7			+	+	+
7		+	+	+	+
8			+	+	+
8		+	+	+	+
9		—	—	—	—
12*	+		+	+	+
10*	+		+	+	+
10*			+	—	—
10*		+	+	+	+
8	+	+	+	+	+
9*			+	+	—
8		+	+	+	+
9			+	—	—
10*			+	+	—
8		+	+	+	+
7			+	+	+
9*			—	—	—
10*			+	+	—
7	+	+	+	+	+
5			+	+	—
10*		—	—	—	—
	4	9	24	22	17
% of total	14.9	33	88	81	63

* Blood was withdrawn from tail veins of these animals for serum lipid determinations. See Table III.

nearly all animals (88%) had lipomatous lesions in their coronary arteries. The incidence of atheromatous changes was but slightly less (81.5%). The more severe and striking vascular lesions occurred in the following percentages: cardiac infarcts 14%, coronary occlusion 33% and atherosclerotic changes 63%. Thus coronary occlusion was observed in half the animals with advanced degrees of coronary atherosclerosis. Myocardial infarcts occurred in half the number of rats showing signs of complete or nearly total coronary occlusion by thrombotic masses. Incidence of cardiac infarcts was therefore 25% in the rats having atherosclerosis and approximately 14% of all the animals in this final experiment.

A more detailed pathological description of coronary occlusions and cardiac infarcts will be published later. This report presents methods used to secure cardiac infarcts in rats. These cardiac infarcts are quite independent of the occurrence of focal myocardial necrosis, which is caused by hypervitaminosis D in rats.

Summary. Twenty-seven white male Wistar rats weighing 450 g at beginning of the experiment were given a diet that induced a state of hyperlipaemia and produced vascular injury. This diet, arrived at as a result of several pilot experiments, proved to be very effective, and yielded the following incidence of coronary lesions: Lipomatous coronary changes 88%. Atheromatous coronary changes 81%. Atherosclerotic coronary changes 63%. Coronary occlusion 33%. Cardiac infarcts 14%.

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