TABLE I. Potassium Levels in a Patient with Non-Tropical Sprue (A.N.) and 4 Patients with Pernicious Anemia from Relapse to Early Remission.

Name	RBC, $\times 10^6$	Retic., %	K meq/ l RBC	Day of treatment
A.N.	1.69	.5	97	1
	1.92	14.4	104.5	6
	2.62	4.2	95	10
	3.09	1.2	103	17
F.C.	1.30	1.3	99	1
	1.15	2.1	97	2
	1.60	17.8	93	5
	2.11	7.8	96	10
	2.47	2.2	106	13
	2.85	1.3	106	16
T.W.	1.85	.8	99	1
	1.58	1.8	97	4
	1.95	16.6	96.5	7
	2.42	5.1	97.5	12
	2.62	2.7	99	15
C.S.	2.63	1.5	91.5	1
	2.94	1.4	102	3
	2.63	4.8	97.5	7
	2.81	5.8	104	10
	3.15	3.2	103.5	15
	3.40	1	100	21
G.K.	1.82	.6	80.5	1
	2.16	18.6	89.5	8
	2.97	8.3	90.5	12
	3.19	2.2	84.5	16
	3.32	1.8	93	22
A.S.	2.82	1.1	87	1
	2.96	2.4	92	4
	2.95	2.1	97	7
	3.21	3.7	90	11
	3.81	2.2	88	16

factory hematologic and clinical remissions. Reticulocyte responses were in the optimal ranges and regeneration of red blood cells took place rapidly. Table I summarizes the pertinent data and shows that no significant change in erythrocyte potassium level was noted in any stage of red blood cell production. Patient G. K. showed a slightly lower level throughout the study than did the others. This may represent a depletion of total body storage of potassium as suggested by Hutt (4). Values for the other patients fell within the normal values suggested by other investigators(5). In view of these negative results experiments in K^{42} turnover under similar conditions appear warranted.

Summary. Erythrocyte potassium content is unaltered in the relapsed stage of pernicious anemia and non-tropical sprue. No change is noted during the period of reticulocytosis and red blood cell regeneration.

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Atheromatous Changes in Aorta, Carotid and Coronary Arteries of Choline-Deficient Rats.* (19885)

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Evidence suggesting an association of dietary deficiency in rats with the occurrence of vascular damage has not, to our knowledge, been previously reported.† We have recently observed in our laboratory pathological changes, which resemble in some respects those of atheroma, in the major arterial trunks (aortas and carotid arteries) and in the coronary arteries of rats maintained up to 216 days on diets low in choline. A preliminary account of the extent and nature of these lesions is embodied in this paper.

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† We have been informed personally (April, 1952) that in the chronic choline deficiency studies by Salmon, Copeland, and Engel, Agri. Exp. Station, Alabama Polytechnic Institute, lesions similar to those described in this paper have been observed in the aorta and other blood vessels of rats over a period of several years.

Experimental procedure. Male rats of the Wistar strain reared in our own colony were used for this investigation. Since young rats may die from acute renal damage due to choline deficiency(1), animals with initial weights between 180 and 200 g were used in order to reduce the number of deaths from this cause. The rats were housed in individual all-metal cages with a false bottom of coarse wire screen. One group of animals was fed *ad lib* a severely hypolipotropic *diet A*, of the following percentage composition: peanut meal (solvent process) 6; alpha soya protein (Glidden's) 6; hydrogenated vegetable fat (Primex) 20; salts 3; vitamin mixture 1; cellu flour 2; sucrose 61.8; cystine 0.15; alpha tocopheryl acetate 0.035 and cod liver oil concentrate 0.015. The other group of rats was given *diet B* in which the protein moiety was slightly increased at the expense of the sucrose (peanut meal 8, alpha soya protein 8), a mixture of animal and vegetable fat (beef fat 15, corn oil 5) replaced the hydrogenated vegetable fat in diet A and the alpha tocopheryl acetate was increased to 0.100%; the other ingredients were the same as those in diet A. The preparation of the diets, ingredients of the salt mixture and of the vitamin mixture and details concerning the care of the animals have been published previously(2). At the end of 3 to 4 months, food consumption had markedly decreased and a considerable loss in body weight had occurred. Some of the rats died, and examination of the livers revealed the beginning of cirrhosis. In an attempt to prevent further deaths among the animals consuming diet A, a supplement of 2% casein, 1% fibrin and small amounts of choline chloride (0.04 to 0.02%) were included for about two weeks. During this short period, stimulation of appetite promoted gains in body weight of from 30 to 40 g, associated with a marked improvement in their general appearance. Choline chloride was removed from the diet and the protein supplement was continued for a third week. The animals were then transferred to the original hypolipotropic diet A until they were sacrificed. Rats fed diet B ingested the unsupplemented diet B throughout the experiment. One hundred and sixteen animals were studied. Ninety-one received diet A and 49 of these were subjected to both gross and microscopic examination. The remaining 42 were dissected but microsections were not taken, because of extensive post mortem changes in most instances. Twenty-five rats received diet B. Of these, 11 were examined both grossly and microscopically and 14 were autopsied but microsections were not taken, in most cases again because of post mortem changes. All animals in which vascular lesions were grossly apparent were subjected to microscopic examination even in the presence of post mortem changes.

Histological examination included preparation of paraffin sections of all thoracic and abdominal viscera, and of the aorta. Frozen sections of the aorta and of the hearts of 29 animals fed diet A and of 12 fed diet B were stained by Wilson's technic(3). Frozen sections of the hearts of all of the 61 rats subjected to extensive histological examinations were not prepared as in the initial stages of the study it was not appreciated that lipid deposits might be found in the coronary arteries. Paraffin sections were routinely stained with haematoxylin and eosin. A variety of special tinctorial procedures was applied to certain sections including methods for the demonstration of elastic tissue, ceroid (Oil Red O stains of paraffin sections), connective tissue, hemosiderin pigment, and special cytological technics for the demonstration of the cells of the islets of Langerhans and of the anterior lobe of the pituitary. Estimations of liver lipids and of serum cholesterol were carried out in some cases. The biochemical data will be published elsewhere.

Results. Aortas of 9 of the 91 rats fed diet A and of one of the 25 rats fed diet B were grossly thickened, tortuous and studded with calcified plaques (Fig. 1, 2). Definite pathological changes were found in microsections of aortas of an additional 16 rats (14 fed diet A; 2 fed diet B). In 8 of these, the lesions were early or minimal in nature (6 fed diet A; 2 fed diet B). Thus, in a total of 26 animals, some degree of vascular damage was encountered. Lesions of the coronary arteries were found in frozen sections of 8 of the 41 hearts so examined. The results and

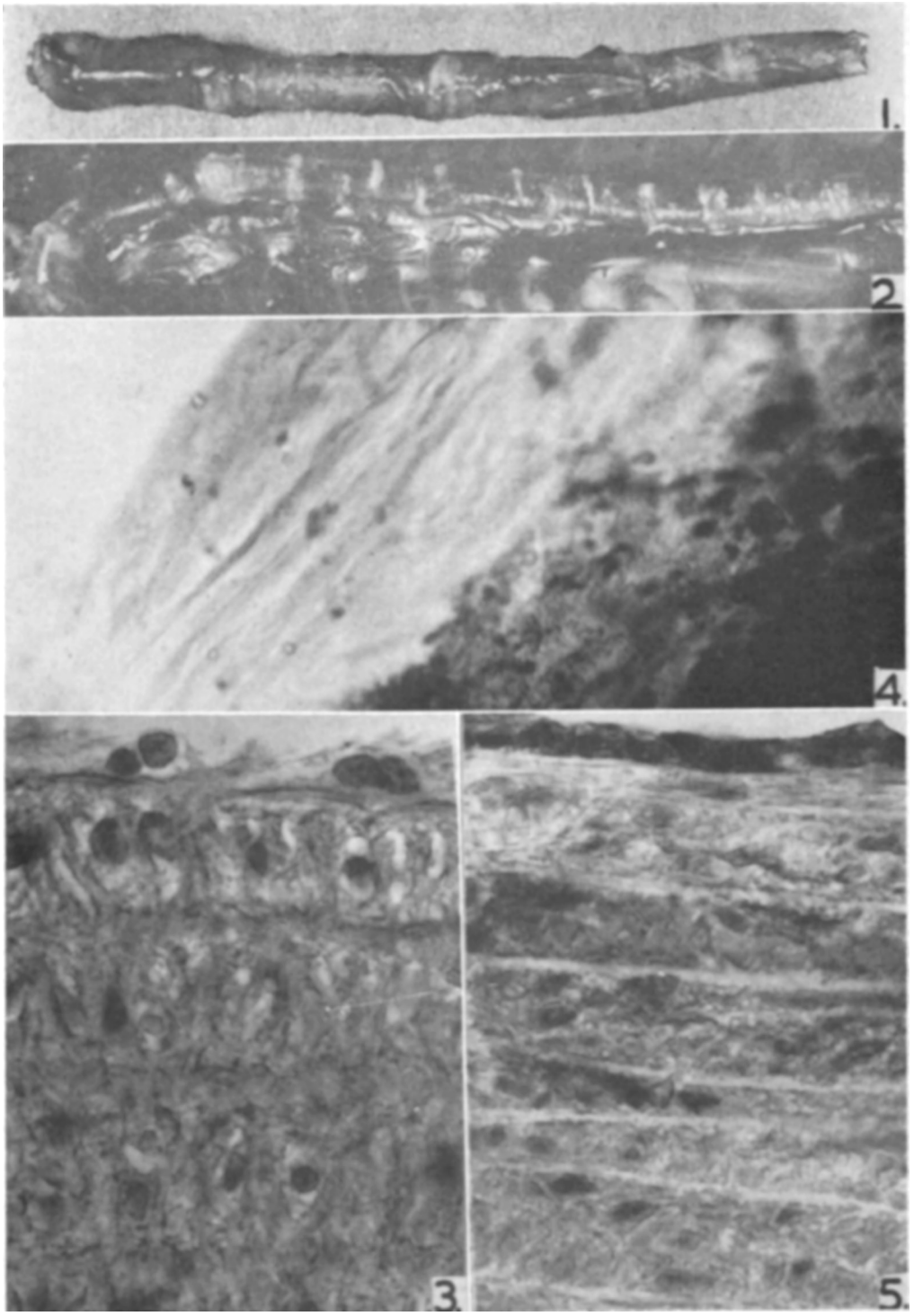


FIG. 1. Gross appearance of thoracic aorta lesions graded as 3+ of a rat fed diet A. The vessel is increased in diameter and a number of plaques and rings (white) are visible along its entire length. × 4½.

FIG. 2. Gross appearance of aorta of a rat (fed diet A) showing lesions graded as 4+.

Aortic arch is at the left and a small portion of the upper abdominal aorta is at right. Annular, calcified bands occur at fairly regular intervals in the thickened wall along the entire length of the vessel shown. The rings are largest and heaviest in the thoracic portion and the arch; they lie opposite the intervertebral junctions, as indicated by the position of the ribs. ×3.

FIG. 3. Microscopic appearance of a frozen section stained for fat (Wilson's technic) of the normal aorta of a rat. Note absence of subintimal tissue. The intima (top) illustrates the *upper limit* of cellularity in control rats. At this magnification, the typical normal rat aorta would exhibit only one or two endothelial cells with scantier cytoplasm than those shown. ×800. (Compare with Fig. 5.)

FIG. 4. The avascularity of the media of the normal aorta of a rat injected intrav. with india ink is demonstrated by this preparation which has been cleared *in toto* with benzyl benzoate and photographed without sectioning. The intimal surface (left) overlies the transparent media (pale grey) which is free of injection media. The adventitia (right) appears almost jet black for it is supplied by a dense network of capillaries which have been filled with ink. The adventitial capillary plexus terminates sharply at the junction between adventitia and media. × 24.

FIG. 5. Preparation and magnification as for Fig. 3 illustrating one of the earliest stages (1+) of the aortic lesion encountered in a choline-deficient (diet A) rat. The intima is hyperplastic and the cells are filled with stainable fat droplets (black) which are so numerous that the nuclei (approximately 10 in the field shown) are almost obscured. In the mid-zonal portion of the underlying media can be seen a focus of early degeneration and prenecrosis (dark grey).

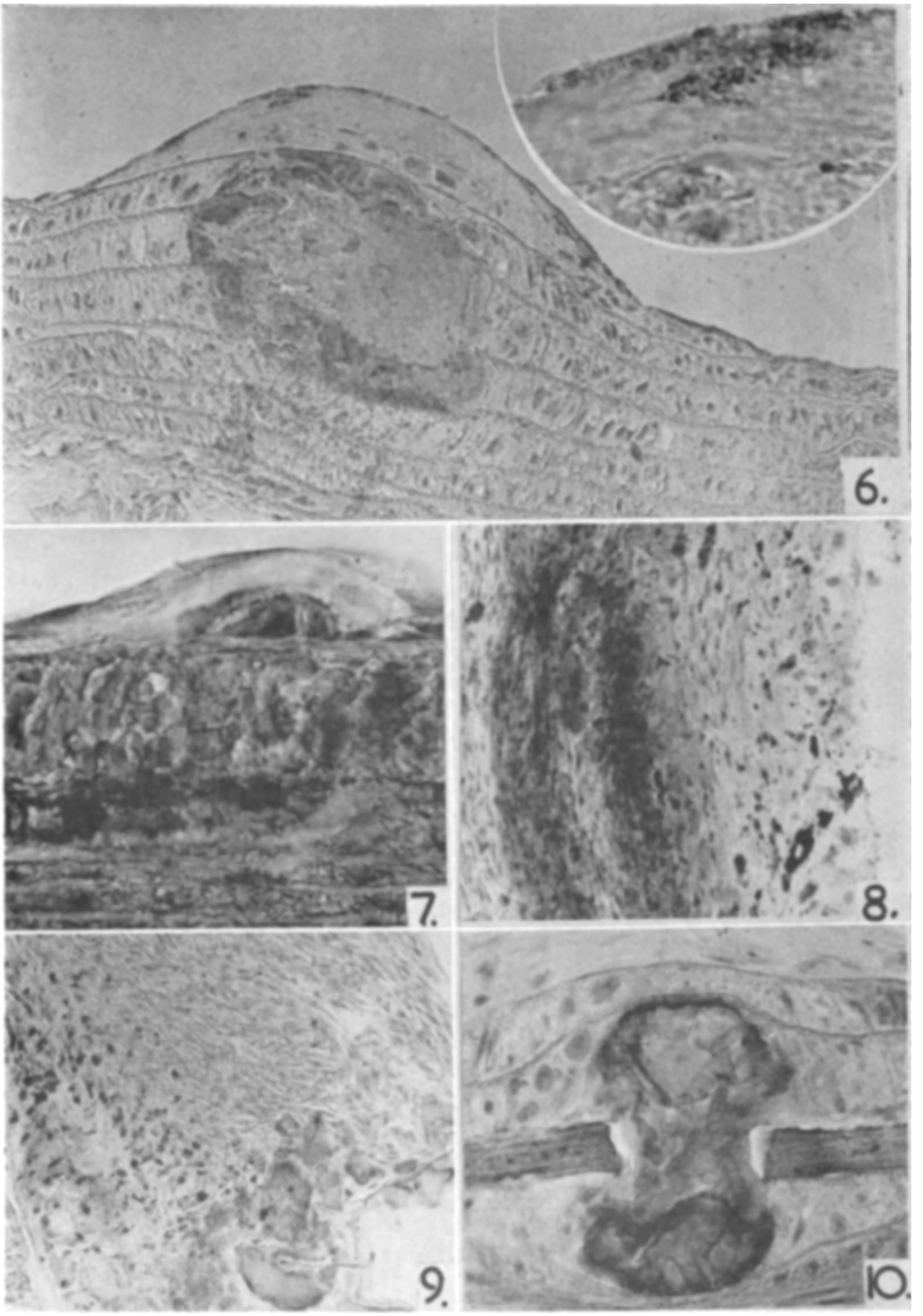
some associated findings are summarized in Table I. The gross and microscopic appearance of the aortas and other vessels are described below. In several animals, blood pressures were measured (direct method) and all were found to fall within normal limits, thus confirming earlier studies of the effect of subjecting rats to continuous and prolonged dietary choline deficiency(4).

The lesions on gross examination (Fig. 1, 2).

TABLE I. Summary of Pathological Findings in 26 Rats Fed Low-Choline Diets, in Which Lesions of the Aorta or Coronary Arteries Were Present.

Rat No.	Diet	Vascular lesions: Aorta	Vascular lesions: Heart	Liver: Fat	Liver: Cirrhosis	Kidney damage	Time on diet, days
5	A	4+	+	1+	3+	3+	210
31	A	4+	?	1+	4+	2+	151
85	A	4+	0	3+	3+	3+	140
106	B	4+	0	2+	3+	2+	170
923	A	4+	?	2+	4+	3+	201
979	A	4+	+	2+	2+	1+	173
859	A	3+	+	2+	3+	2+	219
924	A	3+	+	1+	4+	3+	200
940	A	3+	?	1+	4+	2+	176
988	A	3+	?	3+	3+	1+	167
12	A	2+	?	2+	3+	1+	188
15	A	2+	?	1+	3+	2+	192
16	A	2+	?	2+	3+	2+	195
46	A	2+	?	2+	3+	1+	167
48	A	2+	0	1+	4+	2+	169
92	A	1+	2+	2+	3+	2+	184
934	A	2+	0	2+	2+	1+	195
938	A	2+	+	1+	3+	2+	195
91	A	1+	+	2+	3+	2+	189
110	B	1+	0	2+	2+	1+	170
116	B	1+	0	2+	2+	1+	169
801	A	1+	0	2+	2+	1+	205
869	A	1+	0	2+	3+	2+	216
882	A	1+	+	1+	4+	2+	214
902	A	1+	0	1+	4+	2+	209
997	A	1+	?	1+	2+	2+	180

Grossly visible changes are designated as either 3+ or 4+. Changes indicated by + or 2+ were evident only microscopically. This grading of lesions (+ to 4+) is purely arbitrary and bears no relation to similar classifications that may have been employed by others in studying lesions in other species including man. In those instances where a ? is found, microscopic examination was not carried out for this particular feature (see text).



All sections illustrated in this plate are frozen preparations (stained by Wilson's technic) from aortas of choline-deficient rats fed diet A, with the exception of Fig. 9 which shows an aorta from an animal fed diet B.

FIG. 6. Later stage of an aortic lesion, than that shown in Fig. 5, of an intimal plaque

graded as 2+. The plaque is composed of hypertrophied and hyperplastic endothelial cells, many of which contain stainable fat droplets (black). The subjacent media has undergone more extensive necrosis than that shown in Fig. 5, and small amounts of calcium salts have been deposited in the necrotic tissue. × 250. The inset (upper right, × 800) shows a few of the intimal cells under higher magnification to demonstrate the fat droplets more clearly.

FIG. 7. A single, hypertrophied intimal cell from an early stage of a typical aortic lesion (1+) contains stainable fat droplets in the cytoplasm just at the left of the enlarged nucleus. The cell is swollen and has an hyaline, 'glassy' appearance. The midzonal portion of the media (extreme bottom of field) exhibits degenerative changes indicating early necrosis. × 800.

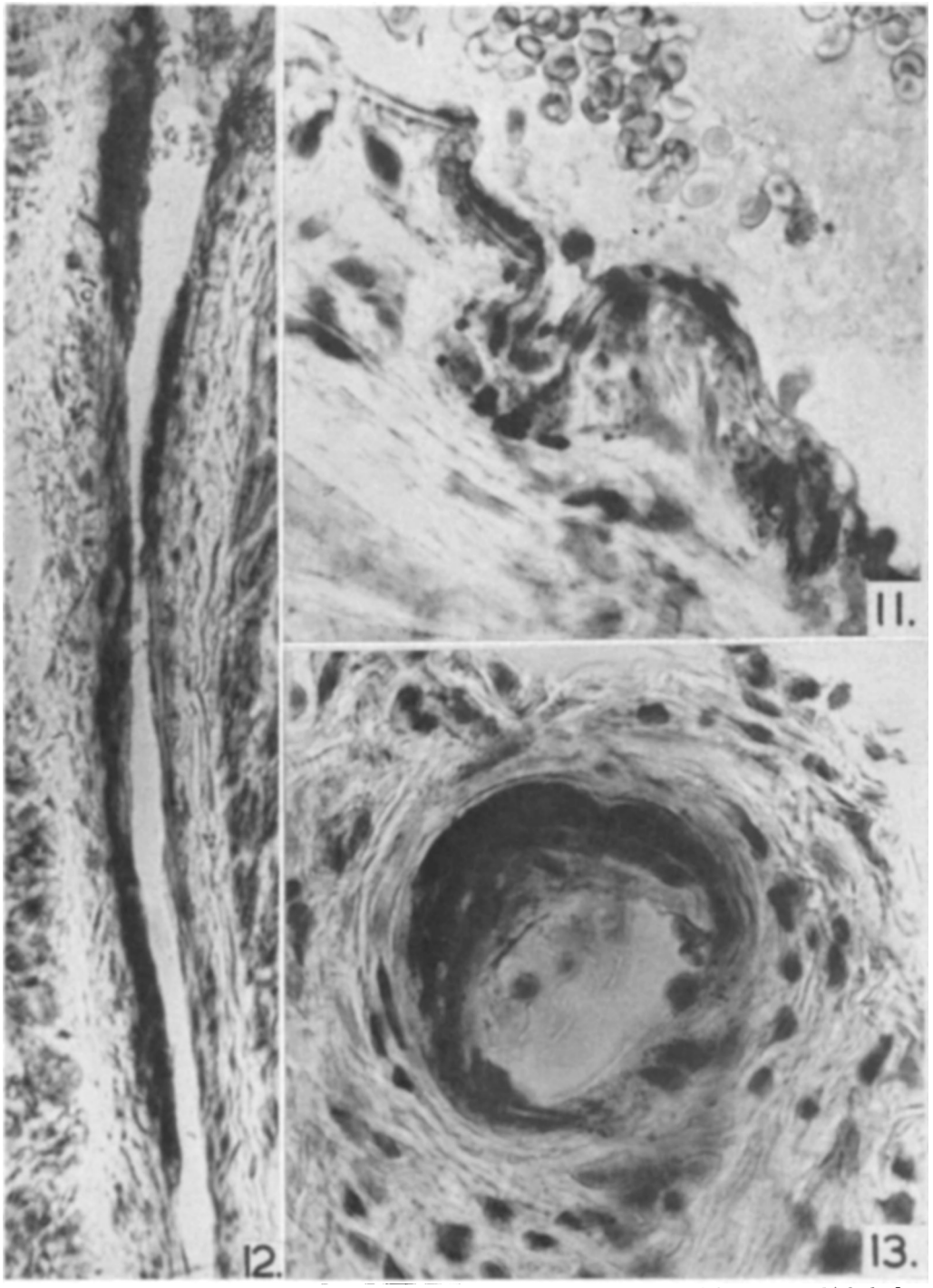
FIG. 8. Appreciable amounts of stainable lipid (black), some of which reacted positively to histochemical tests for cholesterol, are present in the media of this aorta with lesions graded as 3+. The intima is greatly thickened (taking up about one-third of the field at the right) and contains stainable fat (black) within hyperplastic endothelial cells and some macrophages. Intimal lipid was always negative to histochemical tests for cholesterol. × 100.

FIG. 9. A grossly thickened aorta (graded as 4+) is shown in cross section with intima in extreme upper right and a small portion of adventitia at extreme lower left. Calcium deposits appear dark grey; stainable fat, black. The junction between intima and media could not be identified. Note fibrosis. × 100.

FIG. 10. Typical callus ("collar-button lesion") has formed around the site of fracture of the media which has been replaced throughout its mid-zone by a longitudinal bar of calcium salts. The lesions in this aorta were graded 4+. A portion of the greatly thickened intima is included in the upper portion of the field. × 100.

In the most advanced cases (classified as either 4+ or 3+, Table I), the aortas were increased in diameter and frequently deviated a few millimeters to one or the other side of the vertebral column. Scattered throughout could be seen grey to yellow plaques varying from 2 to 4 mm in their greatest dimensions. Sometimes these were in the shape of elongated or oval patches which lacked any apparent constant orientation to the vessel wall (Fig. 1), but most frequently they formed annular bands which often completely encircled the vessel (Fig. 2). Sometimes these bands were most prominent opposite the intervertebral discs and, if numerous, gave the aorta an appearance resembling that of the trachea. Lesions were always most abundant and advanced in any one animal, in the ascending portion and the arch of the thoracic aorta, and decreased in number and size farther away from the heart. The bases of the common carotid arteries at their sites of origin from the aortic arch were affected in a few rats in which the lesions were present in severe degree (4+). In two rats (No. 5 and 859), small yellow plaques were evident at the root of the aorta, and were situated in the wall of the aortic sinus where they partially surrounded the openings of the coronary arteries. They were the only lesions associated with coronary vessels that were detected on gross examination. On palpation, the largest lesions were stony hard and were nearly always grey in color, in contrast to smaller, softer plaques which were frequently quite yellow when examined with the aid of a hand-lens. At the site of the largest plaques, the outer surface (adventitia) of the vessel was elevated, but this was not the case with smaller ones which were most easily visualized from the inner (intimal) surface. Even in the earliest cases, which could be observed grossly, the intimal surface was elevated over the site of a plaque.

Microscopic examination Fig. 3-13. *Normal aorta of the Wistar rat.* Certain characteristics of the rat's aorta are worthy of mention, because they differ from those of the human aorta. The intima of the rat consists of a single row of endothelial cells (Fig. 3) directly overlying the subjacent elasto-muscular media. The nuclei of the intimal lining have not been observed to number more than four per high power field (42X objective with 10X ocular). Areolar connective tissue between the intima and media is lacking in the aorta of the rat, so that there is virtually an absence of subintimal tissue. By injecting India ink into the animal's vascular system (by a technic described previously)(5), it was demonstrated that *vasa vasorum* do not penetrate the media of the aorta (Fig. 4). A fine meshwork of capillaries exists in the adventitial tissue but terminates at the medial-adventitial junction. It would appear then that the nutrition of the media must be wholly dependent on the dispersion of fluid from the lumen of the rat aorta outwards and from the capillaries of the adventitial vascular network



All photomicrographs represent frozen sections of hearts of choline-deficient rats which had been fed diet A. Wilson's stain.

FIG. 11. Stainable fat droplets (black) may be seen on and within the intima of this coronary artery illustrated in longitudinal section. The lumen containing red blood cells is in the upper right half. The media appears normal. × 800.

FIG. 12. Stainable fat (black) fills the thickened intima and a portion of the underlying

media for a considerable distance along the wall of this coronary artery which has been sectioned longitudinally. There is no medial necrosis. × 300.

FIG. 13. Crescentic hypertrophy and hyperplasia has developed in both the intima and media of this coronary artery cut in cross-section. The intima is loaded with masses of stainable fat (black). Note the "onion-skin" layering of the media. × 800.

inwards. The central zone of the media, midway between these two sources, would probably be most susceptible to any interference with the mechanisms responsible for diffusion of fluids into the media.

Initial stages of the aortic lesions. The earliest lesions detected microscopically have been found sometimes in aortas that had appeared normal on gross examination (one-plus, Table I). An increase in the number of endothelial nuclei of the intimal lining had occurred so that the intima appears thickened (Fig. 5). The cells of the hyperplastic intima possess less cytoplasm than normally, but the most striking qualitative change may be observed in the frozen sections stained for fat. In these sections, the hyperplastic intimal cells contain many fine sudanophilic droplets in their cytoplasm. Histochemical tests indicate that these were probably neutral fat, because tests for fatty acids and cholesterol were negative. In some instances the underlying media appears normal, but more frequently there has been interference with the usual intense tinctorial affinity of this tissue for eosin, or for Light Green, indicating early degenerative changes of a prenecrotic nature (Fig. 5). These initial foci of degeneration in the media are always located midway between its inner and outer limits.

Later stages of the aortic lesions. In the less extensive of those lesions which were visible on gross examination (3+), proliferation of intimal endothelium had produced small raised plaques formed by layers of two to five cells (Fig. 6). The amount of stainable fat within these cells had not increased appreciably over that found in earlier lesions. Cells comprising these plaques are often swollen and large, and their cytoplasm possesses a smooth, glassy appearance suggesting a type of hyaline degeneration (Fig. 7). The underlying media in the mid-zone shows varying stages of necrosis and, in a few instances, deposition of small amounts of calcium salts. Fatty change in the media is present in some cases (Fig. 8) and histochemical tests for cholesterol were positive. Cholesterol was not demonstrated, however, in the intima in any of the sections tested.

Advanced stages of the aortic lesions. In the most advanced lesions, in addition to hypertrophy and hyperplasia of the intima, there has been subintimal formation of fibrous tissue in which macrophages containing lipid may be found (Fig. 9). The underlying media is completely necrotic in its mid-zone and is replaced by a bar of calcium salts. In some instances this medial bar had fractured and callus had formed with typical granulation tissue (Fig. 10).

Lesions of the coronary arteries. The earliest change that could be noted consists microscopically of the deposition of stainable lipid droplets *on* and within the cytoplasm of the endothelial lining cells of the largest branches of the coronary arteries (Fig. 11). Sometimes this change has been accompanied by hypertrophy and hyperplasia of the affected cells, but not always. In some cases, for considerable distances the intima and a portion of the underlying media of these vessels are bright red with fat (in frozen sections stained with Oil Red O) which renders the arteries very prominent under the lower magnifications of the microscope (Fig. 12). In more advanced stages of coronary arterial change, the entire intima is swollen with stainable fat and there is evidence that the underlying media has become thickened, for these vessels possess an "onion skin" appearance (Fig. 13). Medial necrosis was not observed in even the most severely affected coronary arteries.

Discussion. The question of spontaneous lesions. It is considered unlikely that the lesions which have been described, were spontaneous in nature. Vascular lesions of this type have not been observed in our colony of rats, including some which were not sacrificed until they had attained an age of 750 days on a stock diet. Spontaneous lesions of both aortas and coronary vessels in Wistar rats

have been reported by others(6,7), but these animals (all more than 600 days old) were twice as old as the choline-deficient rats, and the lesions were not as advanced. None of the animals having arterial lesions associated with dietary deficiency was more than 300 days old.

Dietary factors. Pathological changes in the aorta and coronary vessels were observed only in rats fed the choline-deficient rations. Control animals which had been fed the same basal diets supplemented with choline were not included in these experiments, but in other investigations conducted in this laboratory in which such control groups were studied, evidence of vascular changes has never been noted. It is true, however, that in some of our previous experiments in which similar rats were maintained on less severely hypolipotropic diets for a year or even longer, lesions of the aortas were not observed(2). If present, these changes were so minimal that they escaped notice at routine autopsy. It is possible that secondary factors, in addition to choline deficiency, may have induced, or at least intensified, the vascular lesions so that they were readily detected on gross examination. Such factors might include the nature of the dietary fat (hydrogenated vegetable oil) or the temporary periods of choline supplementation in the early stages of the experiment. It should be noted however that neither of these factors could account for lesions in 3 of the 25 rats fed diet B. The protein content of both diets A and B was extremely low (9 and 12 per cent, respectively) and this should be taken into account when assaying probable factors. Experiments have been planned to investigate these possibilities.

Nature of the lesions and their pathogenesis. Although changes in the coronary arteries of the rats, consisting of intimal lipid deposits and endothelial proliferation, are morphological counterparts of early stages of atheroma in man, this resemblance is not so apparent in the case of the lesions in the aortas of the rats. The latter more closely resemble Mönckeberg's sclerosis of femoral and radial arteries of man, for medial calcification is the cardinal feature of this condition and of the affected aortas of the rats. It is significant in this connection that the aortas of rats resemble these distributing arteries of man more closely than the human aorta. *Vasa vasorum* are limited to the adventitia only in the first two instances, whereas the *vasa vasorum* of the aorta in man penetrate the media(8).

The initial stage of the lesions in the rats' aortas consists of the deposition of intimal lipid. At this period in the development of the disease the microscopic appearance resembles that recently observed by Wilsler *et al.*(9) in the aortas of rats fed diets high in fat and treated with nephrotoxic antiserum, or desoxycorticosterone acetate with and without substitution of 1% NaCl for their drinking water. Bragden and Boyle(10) have described the deposition of sudanophilic material in the intima of rats 24 hours after they had been injected with low-density lipo-proteins obtained from the serum of cholesterol-fed rabbits. The microscopic changes in these rats again resemble the initial changes found in the choline-deficient animals. This type of stainable intimal lipid is reminiscent of that found by Wilens(11) in the artificial production of intimal lipid deposits in excised arteries of man by filtration of normal human blood serum through their walls at normal arterial pressures for 24 hours or longer.

Our studies thus far have suggested that at some stage in the development of the lesions of the liver, kidney and other organs of the choline-deficient rats, factors have operated to favor the deposition of stainable lipid in those portions of the animals' arterial trees where the blood is under highest pressure. These sites were near the heart in the thoracic portions of the aortas, the roots of the common carotids and the stem branches of the coronary arterial trees. Evidence has already been published(2,12) that a state of chronic fat embolism may exist in choline-deficient rats; perhaps it may be a potent factor in the production of vascular changes. In the rat, deposition of intimal lipid in the aorta may be the principal factor directly responsible for medial necrosis. Intimal deposits of lipid may form enough of a barrier that permeation of the mid-zone of the media by nutrient fluids is inhibited to such a degree that at this site the tissue dies and becomes calcified. In man,

intimal lipid in the aorta might not be expected to produce the same result, because medial nutrition is not dependent on fluid permeation alone since *vasa vasorum* penetrate the media. It is hoped that future investigation may elucidate the roles of these factors.

Although there may be some justification for considering that the lesions which have been observed in choline-deficient rats may be related to human atheroma, further discussion is not warranted until the results of more extensive investigations are at hand. It should be of interest however to those concerned with the problem of cardiovascular disease that lesions closely related morphologically to human atheroma can be produced by a dietary deficiency in an animal, the rat, which has previously been considered refractory to aortic disease. It is possible that by following this type of investigation, knowledge of atheromatous conditions in man can be extended.

Summary. Lesions have been observed in the aortas, carotid and coronary arteries in 26 of 116 rats fed a low choline diet for periods up to 216 days. None of these animals exceeded 300 days of age at the time of sacrifice. The initial lesion consists microscopically of the deposition of stainable lipid in the endothelial cells of the intima. In later stages, a proliferation of intimal cells had taken place so that small plaques resulted. In the large vessels (aortas and carotids) the subjacent media had undergone necrosis and eventual calcification. The possible relation of these lesions to atheroma in man is discussed.

The authors are indebted to Mr. William D. Wilson and to Miss E. Berman for their assistance in preparing microsections.

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Use of Normal Rabbit Serum in Production of Hypercholesteremia in Cholesterol Fed Rats.* (19886)

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Recently we have found(1) that the hypercholesteremia following the injection of Triton WR 1339, is due to an alteration in the plasma protein complex induced by the injection of this surface active substance. The hypercholesteremia in the rat following the administration of sodium cholate also has been found (2) by us to be due to an *initial* alteration in the plasma protein complex brought about in some way by cholate. In other words, the injection of either of these substances apparently allows the blood of the treated animal to retain a greater fraction of that cholesterol *normally entering and eventually leaving the blood stream.*

These findings suggested the possibility that other forms of hypercholesteremia also might

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