ATHEROMA AND OTHER LESIONS PRODUCED IN RABBITS BY CHOLESTEROL FEEDING.

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PLATES 15 TO 19.

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Ignatovski (1) in 1908 published the results of his experiments on the effect of animal food on the rabbit. By feeding milk and egg yolk to these animals he produced various lesions, the most noteworthy of which were atheroma of the aorta, cirrhosis of the liver, and enlargement of the adrenals. In the following year Starokadomsky and Ssobolew (2), also by feeding rabbits on milk and egg, confirmed the results of Ignatovski in so far as the production of an atheroma of the aorta was concerned. They also obtained lesions in the innominate artery, carotids, and subclavians. Ignatovski ascribed his results to animal proteids. Several workers had previously described lesions produced in rabbits by animal food (d'Amato (3) lesions of the aorta, and Garnier and Simon (4) lesions of the liver), but these lesions differed essentially from those produced by Ignatovski and the latter's results attracted particular attention on account of the similarity of the aortic lesions as described by him to human atheroma. Stuckey (5) also confirmed Ignatovski's results. He afterward attempted to determine what part of the food given was responsible for the lesions, trying (6) milk, meat juice, egg albumin, and egg yolk, with the result that lesions as described by Ignatovski were produced only by the egg yolk. Later (7) he used different animal and plant fats, fish liver oil, beef fat, and sunflower-seed oil with negative results. He did, however, produce lesions of the aorta, apparently identical with those described by Ignatovski, by feeding rabbits on brain.

Chalatow (8) studied the livers of Stuckey's rabbits and reported characteristic changes in the livers of those which had been fed on egg and brain. In these he found an abundant infiltration of the parenchyma with doubly refracting fat.

Wesselkin (9) fed rabbits on egg and milk and others on lecithin and milk. In the former he obtained an abundant deposit in the liver, aorta, and spleen of lipoid substances which he considered mainly cholesterol esters. In the lecithin-fed rabbits also he found in the same organs a deposit of lipoids which, from their microchemical reactions, he believed to be phosphatids. Because, however, no pathological changes followed the deposit of the latter in the organs, while marked changes were produced by the cholesterol esters, he concluded that the injury was to be ascribed to the cholesterol of the egg yolk.
Anitschkow and Chalatow (10) fed pure cholesterol and produced characteristic lesions, and later also Wacker and Hueck (11). Weltmann and Biach (12), however, though they produced a cirrhosis of the liver, were unsuccessful in obtaining aortic lesions; and van Leersum (13) who fed two rabbits on eggs and two on pure cholesterol found a suspicious lesion in but one of the four. This was an egg-fed rabbit which had been under treatment for a shorter period than any of the other three. These authors are unable to explain their lack of results. Van Leersum states that spontaneous atheroma occurs in about 3 per cent of rabbits, and apparently believes the one lesion observed in his rabbits is to be so explained.

Anitschkow and Chalatow (10, 14) produced similar lesions in guinea pigs by egg feeding, with an abundant deposit of anisotropic fat in the organs, but failed entirely in rats. Adler (15) found aortic lesions in each of two dogs fed on cholesterol, and he also found lesions in two of three dogs receiving cottonseed oil.

Aschoff (16) and Anitschkow (17) believe that other factors, especially an increase in blood pressure, are important in the production of the aortic lesions. The latter believes that the formation of such lesions in these rabbits is facilitated by increasing the blood pressure in the aorta by constricting its lumen in the abdominal portion, by suspending the animals according to the method of Klotz (18), or by the use of adrenalin.

For the purpose of studying the lesions produced by cholesterol-containing food, nine rabbits were fed on egg yolk and six on pure cholesterol. The egg yolk and the cholesterol, the latter dissolved in cottonseed oil by heating, usually in the proportion of 0.5 gm. to 10 cc. of oil, were mixed with the rabbits' daily ration of crushed barley. On an occasional day greens were given with the barley without the addition of egg or cholesterol. One rabbit, as a control, received large doses of cottonseed oil without cholesterol. This rabbit was fed on oil over a much longer period than any of the rabbits receiving cholesterol, and none of the latter received as much oil in the course of their feeding as did the control in a similar period.

Smears and frozen sections of the fresh tissue were examined for doubly refracting fat under the polarizing microscope. Frozen sections of the tissues fixed in Orth's fluid were stained with Sudan III and by Van Gieson's method; paraffin sections with Van Gieson's method, hematoxylin and eosin, and in some cases with Weigert's elastic tissue stain and Mallory's connective tissue stain.
EXPERIMENTAL.

Rabbit 1.—Control. Received 1,216 cc. of cottonseed oil in 248 days. For short periods no oil was given; the remainder of the time it took from 3 to 23 cc. of oil per day. At autopsy no gross lesions were found. The left adrenal was larger than normal, the right very small, measuring but 2 mm. in diameter. The combined weight was normal, 0.28 gm. On microscopical examination the aorta, liver, spleen, and kidneys were found normal. The liver contained a very small amount of isotropic fat in the peripheries of the lobules. No anisotropic fat was found except in the adrenals.

Rabbit 2.—Received 0.01 gm. of cholesterol per day with the exception of an occasional day. Took 0.29 gm. of cholesterol in 33 days.

Gross Appearances.—Aorta, pulmonary artery, spleen, liver, and kidneys normal. Adrenals normal size; combined weight, 0.37 gm.

No anisotropic fat was found in the aorta, spleen, liver, or kidneys.

Rabbit 3.—Received 0.01 gm. of cholesterol per day with the exception of an occasional day. Took 0.5 gm. in 55 days.

Gross Appearances.—Aorta, pulmonary artery, spleen, liver, and kidneys normal. Adrenals normal size; combined weight, 0.35 gm.

No anisotropic fat was found in the aorta, liver, spleen, or kidneys.

Rabbit 4.—Received 0.05 gm. of cholesterol per day with the exception of an occasional day. Took 3.8 gm. in 84 days.

Gross Appearances.—Aorta, pulmonary artery, spleen, liver, and kidneys normal. Adrenals slightly enlarged; combined weight, 0.41 gm.

No anisotropic fat was found in the aorta, liver, or kidneys; a very small amount in the spleen.

Rabbit 5.—Received 1 egg yolk per day with the exception of an occasional period of a few days. Took 49 yolks in 78 days.

Gross Appearances.—Pronounced intimal lesions in aorta and pulmonary artery. Spleen about three times normal size. Liver slightly enlarged, surface smooth, cuts with somewhat increased resistance, shows pronounced fine deep yellow mottling. Adrenals greatly enlarged; combined weight, 1.35 gm. Kidneys show a few small scars on the surface; in the medulla, mainly in the outer zone, but extending into the inner zone are very numerous yellow opaque streaks and nodules. Large amount of anisotropic fat in the aortic lesions, liver, spleen, and kidneys.

Rabbit 6.—Received from 0.1 gm. to 0.5 gm. of cholesterol per day with the exception of a few days. Took 16.4 gm. of cholesterol in 83 days.

Gross Appearances.—Moderate intimal lesions in the aorta, confined to arch and thoracic portion, and in pulmonary artery. Spleen about normal size. Liver normal size, surface smooth, cuts with somewhat increased resistance, shows pronounced fine yellow mottling. Adrenals slightly enlarged; combined weight, 0.4 gm. Kidneys show a few small scars on the surface, a few opaque yellow lesions in the outer zone of medulla. Large amount of anisotropic fat in the aortic lesions, liver, spleen, and kidneys.
Rabbit 7.—Received 1 egg yolk per day with the exception of an occasional period of a few days. Took 58 yolks in 85 days.

Gross Appearances.—Surface of aorta dull, but no definite lesions apparent here or in the pulmonary artery. Spleen normal. Liver slightly enlarged and yellow. Adrenals enlarged; combined weight, 0.69 gm. Kidneys show no scars on surface, a few very faint yellow lines in outer zone of medulla. Moderate amount of anisotropic fat in intima of aorta, large amount in liver, very small amount in spleen and kidneys.

Rabbit 8.—Received 1 egg yolk per day with the exception of an occasional day. Took 26 yolks in 30 days.

Gross Appearances.—Pronounced intimal lesions in aorta, most numerous in arch and thoracic portion, but present throughout, also in pulmonary artery. Spleen normal in size. Liver shows pronounced fine yellow mottling, otherwise normal. Adrenals slightly enlarged (not weighed). Kidneys show no scars on surface; medulla is a diffuse yellowish color with a few definite yellow streaks in the outer zone.

Anisotropic fat in aortic lesions, liver, spleen, and kidneys.

Rabbit 9.—Received 1 egg yolk per day with the exception of an occasional period of a few days. Took 47 yolks in 98 days.

Gross Appearances.—Very marked intimal lesions throughout aorta, extending into large vessels of neck, renal arteries, and iliacs; also in pulmonary artery. Spleen moderately enlarged. Liver appears normal. Adrenals enlarged; combined weight, 0.56 gm. Kidney shows numerous scars on surface, and in outer zone of medulla very thickly set and pronounced yellow nodular lesions, extending as fine yellow lines into inner zone.

Large amount of anisotropic fat in aortic lesions and kidney, moderate amount in liver and spleen.

Rabbit 10.—Received 0.5 gm. of cholesterol per day with the exception of an occasional day. Took 14.5 gm. in 36 days.

Gross Appearances.—Moderate intimal lesions in aorta, mostly in arch and thoracic portion, a few in abdominal portion; also in pulmonary artery. Spleen moderately enlarged. Liver shows pronounced fine yellow mottling. Adrenals slightly enlarged (not weighed). Kidneys show a few small scars on the surface, numerous yellow lesions in outer zone of medulla extending into inner zone. Large amount of anisotropic fat in aortic lesions, liver, spleen, and kidneys.

Rabbit 11.—Received 1 egg yolk per day with the exception of an occasional interval of a few days. Took 54 yolks in 103 days.

Gross Appearances.—Very marked intimal lesions throughout aorta, most marked in arch and thoracic portion, also in iliacs; marked lesions in pulmonary artery. Spleen moderately enlarged. Liver shows pronounced yellow mottling. Adrenals enlarged; combined weight, 0.58 gm. Kidneys show numerous scars on surface; in outer zone of medulla numerous very pronounced yellow lesions, extending also into inner zone. Large amount of anisotropic fat in aortic lesions, liver, and kidneys; small amount in spleen.
Rabbit 12.—Received ½ to 1 egg yolk per day with the exception of an occasional interval. Took 68 yolks in 138 days.

Gross Appearances.—Marked lesions in the arch of the aorta, smaller lesions in the thoracic and abdominal portions; moderate lesions in the pulmonary artery. Spleen moderately enlarged. Liver about normal size, surface smooth, cuts with somewhat increased resistance, shows pronounced yellow mottling. Adrenals enlarged; combined weight, 0.72 gm. Kidneys appear normal. Large amount of anisotropic fat in aortic lesions and liver, a moderate amount in spleen, very little in kidneys.

Rabbit 13.—Received 1 egg yolk per day with the exception of an occasional day. Took 27 yolks in 34 days.

Gross Appearances.—Moderate intimal lesions in aorta and pulmonary artery. Spleen about normal size. Liver appears normal. Adrenals enlarged; combined weight, 0.55 gm. Kidneys show numerous irregular depressed scars on surface, in outer zone of medulla a moderate number of yellow opaque streaks. Anisotropic fat abundant in aortic lesions and kidney, small amount in liver and spleen.

Rabbit 14.—Received 0.1 to 0.3 gm. of cholesterol per day with the exception of an occasional interval of a few days. Took 8.6 gm. of cholesterol in 97 days.

Gross Appearances.—Very pronounced intimal lesions from valves to exits of renal arteries, a few small lesions below; marked lesions in pulmonary artery. Spleen normal size. Liver shows slight yellow mottling, otherwise normal. Adrenals greatly enlarged; combined weight, 1.4 gm. Kidneys show no scars on surface, in outer zone of medulla a slight diffuse yellow color. Large amount of anisotropic fat in aortic lesions and liver, moderate amount in kidney, very little in spleen.

Rabbit 15.—Received 1 egg yolk per day with the exception of an occasional day. Took 65 yolks in 79 days.

Gross Appearances.—Marked intimal lesions in aorta and pulmonary artery. Spleen about twice normal size. Liver shows pronounced yellow mottling, otherwise normal. Adrenals enlarged, combined weight, 0.84 gm. Kidneys appear normal. Large amount of anisotropic fat in aortic lesions, liver, and spleen; only a very few fine droplets in kidney.

Rabbit 16.—Received ¼ to ½ egg yolk per day with the exception of an occasional interval of a few days. Took 32½ yolks in 134 days.

Gross Appearances.—Slight intimal lesions in arch and upper thoracic portion of aorta, none elsewhere; slight lesions in pulmonary artery. Spleen moderately enlarged. Liver shows slight fine yellow mottling. Adrenals enlarged; combined weight, 0.62 gm. Kidneys appear normal. Large amount of anisotropic fat in aortic lesions and liver, a moderate amount in the kidney, little in the spleen.

The previously mentioned authors agree in the main in their descriptions of the aortic lesions. To summarize very briefly, they describe the production of yellowish white raised plaques and stripes
Atheroma produced by cholesterol feeding

mainly in the arch of the aorta. Microscopically these lesions were mainly intimal and showed a pronounced thickening due to a collection of cells, prominent among which were very large cells filled with doubly refracting fat. Similar fat was also found lying outside the cells. In the later lesions considerable production of elastic fibers was observed with some degeneration of the large cells. A small amount of doubly refracting fat was present in the upper layers of the media. The adventitia was not affected.

The preceding brief summary of our experiments shows that in eleven of the fifteen rabbits lesions of both aorta and pulmonary artery were visible in the gross. One additional rabbit, No. 7, in which lesions were not evident macroscopically, showed very numerous characteristic but relatively slight lesions microscopically. The gross lesions varied greatly in number, size, and severity, but were always quite evident (Fig. 1). They were invariably most marked in the arch and thoracic portion and in some of the less severe cases were found only here. In several of the latter the lesions were especially prominent about the exits of the large vessels arising from the arch or of the intercostals; in others a tendency to this localization was not apparent. The lesions consisted of small (in the early cases a fraction of a mm.) raised, yellowish white, rather sharply defined spots, or less frequently of fine streaks lengthwise of the vessel. In more marked cases larger irregular areas, several mm. in diameter, were seen evidently formed by the confluence of many smaller spots. These in cases involved a large portion of the arch and thoracic portion, leaving only comparatively small irregular areas of normal intima. The lesions of the pulmonary artery were similar to those of the aorta, but in most cases not so pronounced.

The intima of the rabbit's aorta is normally very thin, consisting of a single layer of endothelium and possibly an occasional fibroblast lying on the internal elastic layer of the media. The early lesions produced by cholesterol feeding consist of a cellular thickening of the intima, most of the cells present showing small droplets of doubly refracting fat. This fat is seen in spindle cells, a few drops appearing at either end of the nucleus, and in large, swollen, round or somewhat irregular cells which are loaded with the fat. The nuclei of these
cells vary, some being small, round, and deeply staining, others larger, round or oval, and vesicular.

There has been considerable discussion as to the nature of these fat-containing cells, as there has been concerning the similar cells seen in human atheroma. Large cells similar in appearance and loaded with doubly refracting fat are seen in various localities in these rabbits, in the bone marrow, in the spleen both in the pulp and free in the venous sinuses, and in the kidney in the capillaries of the medulla and in interstitial lesions to be described. These large fat-containing cells in the situations mentioned are apparently of endothelial type (histiocytes of Kiyono), and it would seem probable that the similar cells in the aorta are of the same nature. In marked cases, however, the fat is also seen in cells which are evidently fibroblasts, as in the epicardial connective tissue, about the bronchi in the lungs, and in the capsule and trabeculae of the spleen. In these cells the fat is present as a few small droplets at either end of the nucleus. The fat content of the cells in these situations is always small and the large swollen cells are not seen. It is believed that in the intimal lesions of the aorta also fibroblasts take some part in the absorption of the fat.

Anitschkow (19) calls attention to the similarity in the distribution of the doubly refracting fat to that of the dye in vital staining. In the latter process also fibroblasts show a few granules of the dye at either end of the nucleus, while endothelial cells are very deeply stained. Tschaschin (20) has made an extensive study of these vitally staining cells, and also Aschoff and Kiyono (21). Tschaschin shows that these cells correspond to the resting wandering cells of Maximow, and a further source, especially in inflammation, he believes to be the hematogenous lymphocytes. These vitally staining cells he terms polyblasts. Anitschkow (19) believes that in part at least the fat-containing cells in the aortic lesions of cholesterol-fed rabbits are of this origin. He applies to them the term cholesterol ester-phagocytes. Aschoff and Kiyono (21) introduced the term histiocytes for these vitally staining cells, and believe that they are ultimately derived from the endothelium and certain cells of the reticulum of the blood-forming organs.

The fat-containing cells, especially in the early lesions, vary considerably in size, form, and fat content, and it seems possible to follow gradations from the spindle cells with a few fat droplets to the large cells filled with fat. As previously stated, it is believed that
the fibroblasts here as elsewhere play a part in taking up the fat, but from the appearance of gradations mentioned it does not necessarily follow that fibroblasts swell up to form the large cells described.

By this collection of cells an enormous thickening of the intima is produced, frequently to nearly twice the thickness of the media. In the earliest lesions observed the greater part of the fat was intracellular. A collection of fine fat droplets, apparently extracellular, is, however, sometimes seen lying along the inner surface of the internal layer of elastic tissue as described by Anitschkow. As the lesions progress there is a pronounced proliferation of fibroblasts and an abundant formation of collagen and fine elastic fibers, especially in the upper portion of the lesion. The large collection of fat-containing cells in the lower portion breaks down, and areas of degeneration are formed in the lower intima with an abundant deposit of cholesterol crystals, and in some cases calcification, capped by fibrous tissue (Fig. 2). Calcification was observed in the aortic lesions in four rabbits, Nos. 9, 11, 12, and 16. Two rabbits, 11 and 14, received calcium lactate with some of their feedings; Rabbit 11 about 0.5 gm. in each of 7 feedings, and Rabbit 14 a similar amount in 31 feedings. Since No. 11 showed a deposit of calcium, while No. 14 did not, and since three rabbits which received no additional calcium in their food showed such a deposit, it is evident that the addition of calcium to the rabbits' regular diet is not necessary to obtain calcification, and it would seem that the process is not facilitated by such addition.

Although the lesion is mainly intimal, it is not so limited. Even in the earlier cases some fat may be seen in the media, mostly in the upper third, but occasionally as deep as the middle, both in cells and extracellular between the layers of elastic tissue. In the more marked lesions the upper layers of elastic tissue are raised by the collection of fat and fat-containing cells in this region, and the same degeneration as described in the intima is seen here.

In Rabbits 12 and 16, which were fed longer than any of the other rabbits but with smaller doses, the aortic lesions differed somewhat from those in the other rabbits. While the intimal lesions were of the same character with many large fat-filled cells, the intimal thickening was not nearly as pronounced, fibrosis was slight, and areas of
degeneration with the deposit of cholesterol were not found. The media, however, was more markedly affected than in the rabbits receiving larger doses for a shorter period. Underlying the intimal lesions the media showed many nodular areas and longitudinal streaks, between the elastic layers, of granular fatty material with a considerable deposit of calcium salts. These were present in places, in Rabbit 12, as far as the middle of the media.

Wesselkin (9) noted lesions at the beginning of the pulmonary artery similar to those in the aorta. These lesions were present in eleven of fifteen rabbits in this series. In eight of these rabbits lesions were present not only at the beginning of the vessel but in its branches in the lungs. These lesions consisted of pronounced nodular intimal thickenings containing many large cells filled with fat (Fig. 3). They were very similar to those in the aorta. Degeneration with the deposit of cholesterol crystals, fibrosis, and calcification were present in some of the more pronounced lesions in the larger branches (Figs. 4 and 5), but were not as marked as in the aorta. The media was affected to a varying extent. In the severe cases some of the small branches showed similar intimal thickenings, occasionally so pronounced as apparently completely to obliterate the lumen.

The splenic lesions have been described by Ignatovski (22, 23) Anitschkow (24, 19), and Wesselkin (9). The findings in our series confirm in the main the observations of these authors. The spleen in Rabbit 5 was about three times normal size, in No. 15 somewhat over twice normal size. In five others there was moderate enlargement. Doubly refracting fat was present in all spleens except those of Nos. 2 and 3, but varied considerably in amount. The fat was contained in large cells in the pulp, similar large cells free in the venous sinuses, and in the endothelial lining cells of the sinuses. The cells free in the venous sinuses also contained considerable yellow granular pigment. The Malpighian bodies showed very little fat but an occasional large fat-containing cell was seen. Fat was sometimes present in small amount in the endothelium of the arteries, and their walls sometimes gave a diffuse orange stain with Sudan III. No intimal thickenings were observed. In the severe cases the fibroblasts in the capsule and trabeculae showed a few fat droplets at either end of the nucleus.

Enlargement of the adrenals has been noted by several of the previously mentioned investigators and also by Rothschild (25), who reports experiments on the relationship of the adrenals to cholesterol metabolism and hypercholesterinemia. Enlargement of the adrenals appears to be a consistent finding, having been present in all rabbits except Nos. 2 and 3. In Rabbits 5 and 14 these organs were about four times the normal size. They were of a uniform, almost white, color except for a very small pinkish medullary portion. Beyond the excessive anisotropic fat content no lesions were observed.

The liver lesions have been especially studied by Chalatow (26). He obtained somewhat different results in rabbits fed on pure cholesterol from those in rabbits
feated on egg yolk. In the former the doubly refracting fat was mainly in the peripheries of the lobules and there was marked increase of connective tissue here, which, however, did not invade the lobule. In one case the fat was present in both the peripheries and centers of the lobules and there was an increase of connective tissue in both places. In the rabbits fed on egg the doubly refracting fat was in the centers of the lobules. In these rabbits there was an increase of periportal connective tissue, which, however, was more diffuse and invaded the lobules. He describes a marked proliferation of the bile ducts with subsequent regeneration of the liver cells from them. In the older cases the liver surface was irregular, but the organ was not reduced in size.

In our series the livers, with a few exceptions noted in the summary, were of a deep yellow color; they were of normal size or slightly enlarged, and the surface was always smooth. Doubly refracting fat was present in all cases except Nos. 2, 3, and 4; in most in very large amount. The author cannot confirm Chalatow’s observation as to the different localization of the fat in the rabbits fed on cholesterol and those on egg yolk. No differences were noted between the two series either as to location of fat or production of connective tissue. A very large portion of the fat was situated in the central portions of the lobules. It was contained both in the Kupffer cells and in the parenchymal cells. Some fat was also present in the periportal spaces, contained in fibroblasts and large endothelial cells. There was a considerable degeneration of the liver cells about the central veins, even in the earlier cases. Small focal areas of degeneration were frequent in the neighborhood of the central veins or midway in the lobules. Cirrhosis was present in six rabbits, Nos. 5, 6, 7, 10, 11, and 12. In Nos. 5 and 12 there was a pronounced formation of fibrous tissue; in the other four the process was slight. In all these cases there was a formation of fibrous tissue about the portal spaces and also in the centers of the lobules. In the former region the connective tissue encroached on the periphery of the lobule, and small islands of liver cells were isolated by the fibrous overgrowth. Fibrous tissue was formed about the central veins and extended radially outward in fine strands into the lobule (Fig. 6). The small areas of focal degeneration mentioned become fibrous in the more pronounced cases.

Chalatow (26) believes that the proliferation of interlobular connective tissue occurs only in part as a reactive phenomenon, as is the case in atrophic liver
cirrhosis where proliferating connective tissue takes the place of liver cells in the peripheries of the lobules. In great part it proliferates, stimulated by the new-formed epithelium of bile ducts, to form a stroma for them.

It is true that the degenerated cells in the centers of the lobules and in the areas of focal degeneration are ultimately replaced by fibrous tissue. In the peripheries of the lobules, however, little if any degeneration of the liver cells is apparent, as a primary process at least, and in the author's rabbits the strands of cells in the connective tissue have the appearance of included liver cells rather than proliferating bile ducts. It is also worthy of note in this connection that the fat is present in the peribiliary areas in endothelial cells and fibroblasts. It would seem, therefore, that the fibrosis occurs as a result of the irritative action of the anisotropic fat on the connective tissue itself and its consequent proliferation rather than as a secondary reaction to the destruction of liver cells or for the purpose of forming a stroma for proliferating bile ducts, as believed by Chalatow.

Spontaneous cirrhosis is a common lesion in rabbits as pointed out by Ophuls (27), and it is possible that the periportal increase of connective tissue is in part to be thus accounted for. It is believed, however, that the intralobular formation of connective tissue, as seen in these rabbits, is not observed as a spontaneous lesion and there seems no room for doubt that this at least is due to the cholesterol feeding.

Little mention is made of the kidneys by any of the writers on the subject. Chalatow (28) says: "As the connective tissue phagocytes are scattered through all organs one can find in a rich circulation of cholesterol in the blood of rabbits, an isolated cell in nearly all organs which contains anisotropic fat. . . . So one can, for example in the kidney in the interstitial cells, oftenest in the region of the collecting tubules, sometimes observe single cell elements of the type of connective tissue cells which are infiltrated with anisotropic fat." This author considers the process of anisotropic fatty infiltration of organs under two divisions: an infiltration of the stroma, in which the fat is present in phagocytic cells, which he terms xanthomatosis, and an infiltration of the parenchymatous cells, which he terms myelinosis. For the latter process he considers a preexisting deposit of isotropic fat to be necessary to bind the cholesterol, and he says (28): "Under the usual conditions a myelinosis of the kidney does not occur experimentally. And it is apparent why this is so, since in order to produce a myelinosis of the kidney it is necessary first to produce a deposit of isotropic fat in the kidney epithelium of longer or shorter duration. . . . This, however, is very difficult to produce experimentally." Weltmann and Biach (12) had previously, with this same idea in mind, fed rabbits on cholesterol and in the course of the feeding injected uranium nitrate intraperitoneally. They thus obtained a deposit of anisotropic fat in the tubular epithelium.

The gross appearance of the kidneys in our series has been briefly given in the outline of the experiments. The kidneys of eight rabbits showed characteristic macroscopic lesions which, however, varied
considerably in severity. These consisted of opaque yellowish white stripes and nodules up to 1 mm. in breadth situated in the outer zone of the medulla, standing out sharply from the pink of the surrounding kidney tissue. In the more pronounced cases, Rabbits 5, 9, and 11, these were large and so numerous as to cover a large portion of the outer zone (Fig. 7). In these more severe cases fine yellow lines extended from the large lesions in the outer zone into the deeper portions of the medulla. In Rabbits 6, 7, 8, 10, and 13, lesions of the same character were present, but smaller and fewer in number.

These yellow lesions are due to a localized deposit of anisotropic fat. The kidneys show a small amount of doubly refracting fat in the tubular epithelium, which, however, is not confined to any particular region, but is found in the tubules of both cortex and medulla. Whether or not a neutral fatty degeneration preexisted would be difficult to decide. The striking lesion microscopically in these kidneys, however, and that to which the nodular lesions seen in the gross are due is not that of the tubules but of the interstitial tissue. Accepting the classification of Chalatow, it is a xanthomatosis rather than a myelinosis. The fat is contained for the most part in endothelial cells, both those of the capillaries and those of the lymph spaces, but especially the latter. The fat is seen in the endothelial cells of the capillaries to some extent throughout the kidneys, less abundant, however, in the capillaries of the glomeruli and elsewhere in the cortex than in the medulla. The fat so situated is small in amount, a few fine drops being seen in the cells at either end of the nucleus. Three rabbits, Nos. 12, 14, and 16, in addition to the eight mentioned above, showed this diffuse distribution of fat but without the nodular lesions seen in the latter. These nodular deposits are confined to the outer zone of the medulla in the less pronounced cases; in the more severe they extend into the deeper medulla but never outward into the cortex. In these areas the endothelial cells of the lymph spaces appear as large round or irregular cells filled with doubly refracting fat, very similar in appearance to the cells described in the intima of the aorta (Fig. 8). In more pronounced cases there are large collections of these swollen anisotropic fat-filled cells. In two rabbits, 9 and 11, many of these lesions show in their centers large areas of degeneration of the fat-containing cells, with an abun-
dant deposit of cholesterol crystals (Fig. 9). In No. 11 there is also some deposit of calcium in these degenerated areas. The tubules are for the most part destroyed, but an occasional collapsed tubule is seen passing through the lesions. Some of the capillaries in the deeper medulla, underlying these lesions, contain large numbers of round swollen cells filled with fat (Fig. 10). In only one rabbit, No. 11, a single nodular lesion of the intima of one of the larger vessels in the cortex was found, similar to those described in the vessels of the lung. The kidney lesions, therefore, do not appear to be due to lesions of the large vessels.

That some relationship exists between scars in these kidneys and the cholesterol lesions is evident. It will be noted from the outline that of the eight rabbits whose kidneys showed cholesterol lesions in the gross, six showed also scars on the surface of the kidneys, and that none of the kidneys of the other rabbits showed scars. These scars are similar in appearance to those seen in the so called spontaneous nephritis of rabbits, appearing in the gross as small, slightly depressed, purplish areas, and microscopically as cellular or fibrous interstitial lesions with collapse or disappearance of the tubules. In these kidneys nearly every scar shows at its base, in the outer zone of the medulla, a cholesterol lesion as described above. These collections of large fat-containing cells are never seen in the scars in the cortex but always at their base in the medulla. Rarely a scar is seen in the cortex at whose base no cholesterol lesion is apparent in the sections as cut; and occasionally a cholesterol lesion is seen with no overlying scar in the cortex. In Rabbit 8 scars extending through the cortex are not seen either in the gross or microscopically. There are, however, a considerable number of cholesterol lesions in the outer zone of the medulla. These are early; that is, they are made up of large fat-containing cells without areas of degeneration. Between the large cells are seen a few connective tissue fibrils. In Rabbit 7 there are no scars in the cortex. In the outer zone of the medulla, situated between the tubules, are a few very small longitudinal cellular scars. These show none of the large fat-containing cells described, but many spindle cells, apparently fibroblasts, contain a few fat droplets.

From the appearance of the tissue immediately surrounding the
older lesions it seems apparent that the cholesterol deposited in these areas in the kidney produces some proliferation of fibroblasts as it does in the aorta and in the liver. Whether, however, the cholesterol lesions are responsible for the scars in the cortex or whether the scars preexisted, is an important but difficult matter to decide from these few rabbits. If the scars preexisted the percentage of spontaneous nephritis in these rabbits was very high. On the other hand, it is to be noticed that a few scars were seen at whose base no cholesterol lesions were found, that in one case, No. 13, the scarring was very pronounced in a rabbit which had been fed for but 34 days, and that two rabbits, Nos. 12 and 15, whose kidneys showed no scars, and who, from a comparison with other rabbits, would seem to have been fed a sufficient amount of cholesterol and for a sufficient length of time to produce lesions, nevertheless showed none. These facts are in favor of the view that the scars preexisted and that a preexistent scar or perhaps merely a nodular infiltration of endothelial cells is necessary for the formation of these lesions. If this is so it remains to be explained why the large anisotropic fat-containing cells do not appear and cholesterol lesions are not formed in scars or portions of scars lying in the cortex. Small scars in the cortex about the glomeruli and elsewhere show no lesions, and scars extending through the cortex show cholesterol lesions only at their base in the medulla.

Considering the results as a whole it is found that the feeding of cholesterol-containing food to rabbits produces a widely distributed deposit of doubly refracting fat. This process is primarily one of infiltration, not of degeneration. For the production of such a deposit exceedingly large doses of cholesterol are necessary relative to the rabbit's body weight. This is shown by the results in Rabbits 2, 3, and 4, which were purposely given small doses to test this point. In none of these rabbits were any lesions found or any deposit of doubly refracting fat, except in the adrenals, which in No. 4 were slightly enlarged, and in the spleen of No. 4 which showed a very few large cells in the pulp containing the fat. Where this fat is deposited in large amounts, characteristic lesions are secondarily produced, notably an atheroma of the aorta, lesions of other vessels, mainly intimal, especially of the branches of the pulmonary artery, a cirrhosis of the liver, and lesions in the kidney as described.
Aschoff (16), Anitschkow (24, 17), Zinserling (29), and others discuss the possible relationship between the aortic lesion and human atheroma. Such a discussion would necessarily be at the present time largely theoretical and beyond the province of this paper. It seems, however, worthy of note and of possible importance for human pathology that in the rabbit an experimentally produced deposit of doubly refracting fat in the aorta finally results in a lesion practically identical with that seen in human atheroma. On the other hand, these lesions in the aorta are constantly associated with conditions in the spleen, liver, and pulmonary vessels which are not commonly found in human atheroma, but which seem to be an important part of the general picture of an overloading of the animal body with cholesterol. These additional manifestations could not be eliminated by feeding smaller doses. The rabbit is evidently an animal unable to cope with large doses of cholesterol, although small doses are comparatively innocuous.

SUMMARY.

The feeding of egg yolk or pure cholesterol to rabbits produces an abundant deposit of anisotropic fat in various organs. From this deposit characteristic lesions secondarily result in certain organs. Prominent among these lesions is an atheroma of the aorta very similar in the gross and histologically to the human lesion. Lesions of other vessels are also produced, conspicuous among which are those of the branches of the pulmonary artery. There is a large deposit of anisotropic fat in the liver which produces a cirrhosis. Enlargement of the adrenals occurs, probably due to the storage of an excessive amount of anisotropic fat. In a certain proportion of rabbits conspicuous lesions are produced in the kidneys consisting of nodular deposits of anisotropic fat in the medullary portion, the fat being contained for the most part in endothelial cells and fibroblasts in the interstitial tissue. Later softening occurs in these areas as in the aorta; the cells break down; there is an abundant deposit of cholesterol crystals, some calcification, and a proliferation of connective tissue. Scars frequently extend outward from these lesions through the cortex, but the nodular deposits of anisotropic fat are
never seen in the cortex. It is impossible to determine definitely from these experiments whether or not these deposits are dependent on preexisting interstitial lesions.

In conclusion I wish to acknowledge my indebtedness to Dr. Ophüls for his advice and assistance in this work, and to Dr. Jean Oliver for suggestions and for his assistance in taking the photomicrographs.

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Fig. 3.

Fig. 4.

(Bailey: Atheroma Produced by Cholesterol Feeding.)
Fig. 5.

Fig. 6.

(Bailey: Atheroma Produced by Cholesterol Feeding.)
FIG. 7.

FIG. 8.

(Bailey: Atheroma Produced by Cholesterol Feeding.)
Fig. 9.

Fig. 10.

(Bailey: Atheroma Produced by Cholesterol Feeding.)
EXPLANATION OF PLATES.

PLATE 15.

FIG. 1. Rabbit 9. Thoracic portion of aorta. Shows large elevated plaques in the upper part; smaller lesions below.

FIG. 2. Rabbit 9. Aorta. Shows the edge of an area of atheroma with fibrosis on the surface and degeneration with cholesterol crystal spaces below. Slight invasion of media.

PLATE 16.

FIG. 3. Rabbit 11. Branch of pulmonary artery. Shows a pronounced nodular intimal thickening made up of the large cells which contain the anisotropic fat.


PLATE 17.

FIG. 5. Rabbit 9. Branch of pulmonary artery. Shows lesions composed of large cells, with fibrosis on surface, cholesterol crystal spaces, and some calcification below. Mainly intimal, but invading media.

FIG. 6. Rabbit 5. Liver. Shows a central vein with fine connective tissue fibrils extending outward into the lobule.

PLATE 18.

FIG. 7. Rabbit 9. Kidney. Shows the scars on the surface and the nodular lesions involving a large portion of the outer zone of the medulla and extending as fine lines into the inner zone.

FIG. 8. Rabbit 5. Kidney. Shows one of the nodular lesions in the outer zone of the medulla, which consist of large cells loaded with anisotropic fat.

PLATE 19.

FIG. 9. Rabbit 11. Kidney. One of the more pronounced lesions in the outer zone of the medulla. Many of the large cells are seen which contain the anisotropic fat; also areas of degeneration with numerous cholesterol crystal spaces. Surrounding the lesion there is moderate fibrosis. In the lesion a few collapsed tubules still persist.

FIG. 10. Rabbit 5. Kidney. Shows a capillary in the inner zone of the medulla containing many large cells. These cells are filled with anisotropic fat.