cord). When tested against a suspension of heat-killed pneumococci at the end of the incubation period, the ferment was found to have lost at least 90% of its lytic activity. The control ferment solution also incubated at pH 6.0 for the same length of time in the absence of the polysaccharide retained all its activity.

The enzymatic hydrolysis of the polysaccharide yields about 70% of the theoretical amount of reducing sugar. The reaction appears specific, since commercial trypsin, saliva, taka-diastase and emulsin do not hydrolyze the carbohydrates. The pneumococcus ferment, on the other hand, is without action on chondroitin sulfuric acid or on a polysaccharide isolated from gastric mucin[3] containing acetylglucosamine and galactose.

So far the only other source of an enzyme capable of hydrolyzing the two polysaccharides is a tissue hash from rabbit iris and ciliary body. Since aqueous humor also contains the polysaccharide acid,[4] it is probable that the ferment plays a rôle in the fluid exchange of the eye.

Typical results of the hydrolysis of the polysaccharides by the pneumococcus enzyme are shown in the text figure. Ten mg. amounts of the acids in 1.0 cc. of citrate buffer, pH 6.0, were incubated with 1.0 cc. of respectively 0.05, 0.1, 0.5, and 1.0 percent solutions of pneumococcus ferment. Two drops of toluene were added. As controls the same concentration of the acids were incubated with 1.0 cc. of saline and 1.0 cc. of citrate buffer with 1.0 cc. of ferment. Reducing sugar was estimated on aliquot portions of the mixture by the Hagedorn-Jensen method. The control values were subtracted.

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Attempt to Produce Atherosclerosis in Chickens by Feeding Cholesterol.*

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In view of the inability of various workers throughout the world

[3] Meyer, Karl, Palmer, John W., and Smyth, Elizabeth M. (unpublished experiments).

[4] Meyer, Karl, and Palmer, John W., *Am. J. Ophthal.* (in press).

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to produce experimental arteriosclerosis comparable in pathogenesis and pathology to that occurring in man, other animals besides the commonly used rabbit have been under investigation in this laboratory. One of these is the chicken, accustomed to ingesting and excreting cholesterol, in contrast to the rabbit, which is herbivorous. This report covers an attempt to produce atherosclerosis in chickens by the administration of cholesterol in large amounts.

Duff,[1] in a recent review of experimental cholesterol arteriosclerosis, found insufficient evidence that cholesterol feeding affects the arteries of chickens. Yamaguchi[2] has reported the occurrence of atherosclerosis in the aorta of chickens fed egg yolk or lanolin over long periods, and Uchiyama[3] believed that the aortic intimal changes observed following cholesterol feeding were more advanced than in control chickens, but emphasis remains on the frequent spontaneous occurrence of lipid-containing fibrous lesions in the intima and inner media of the aorta of relatively old chickens. Younger birds, in which spontaneous vascular lesions presumably should be relatively infrequent, have not been used for this type of experiment.

Further support of Uchiyama's conclusions, however, was obtained when cholesterol was fed to 17 five-months-old White Leghorn roosters. Nine of the 14 were fed daily for 1½ to 4 months 0.3 gm. of cholesterol attached to scratch corn by dissolving in ether and a little olive oil, and stirring with the grain until the ether had completely evaporated. (The basic diet of this group consisted of mixed scratch grain and oats and was cholesterol free). The remainder received 0.8 gm. or 1.6 gm. doses of cholesterol mixed with olive oil and administered by capsule 4 times a week for 2½ to 3 months. (The diet of these chickens was composed of scratch grain and egg mash, the latter adding approximately 0.03 gm. of cholesterol to the daily intake. This diet was used for the second group of chickens because of the difficulty experienced in keeping the first group in good condition in the laboratory on a grain diet alone). An elevation of serum cholesterol (up to 300 mg. per 100 cc.; normal: 80-160) was produced in only ⅓ of the fowls, indicating inadequate absorption, or, more probably, the ability of the omnivore to metabolize cholesterol. Seven of the 14 roosters, when killed at the end of the feeding period, had small yellow longitudinal plaques

[1] Duff, G. L., *Arch. Path.*, 1935, **20**, 81 and 259.

[2] Yamaguchi, M., *Hokuetsu Igakukwai Zasshi*, 1922, **37**, quoted by Kawamura, R., *Neue Beiträge zur Morphologie und Physiologie der Cholesterinsteatose*, Jena, Gustav Fischer, 1927.

[3] Uchiyama, T., *Virch. Arch. f. Path. Anat.*, 1930, **277**, 642.

or streaks in the abdominal or muscular portion of the aorta, consisting of intimal fibrosis and thickening, with infiltration of the thickened intima by fine and coarse droplets of lipid, which was in part doubly refractive. Some of this fat was within large mononuclear phagocytes. There were also more or less fan-shaped radiating deposits of similar lipid extending outward in the media beneath the intimal lesions with occasional interruption of the internal elastic lamella (Fig. 1). When these deposits were extensive a

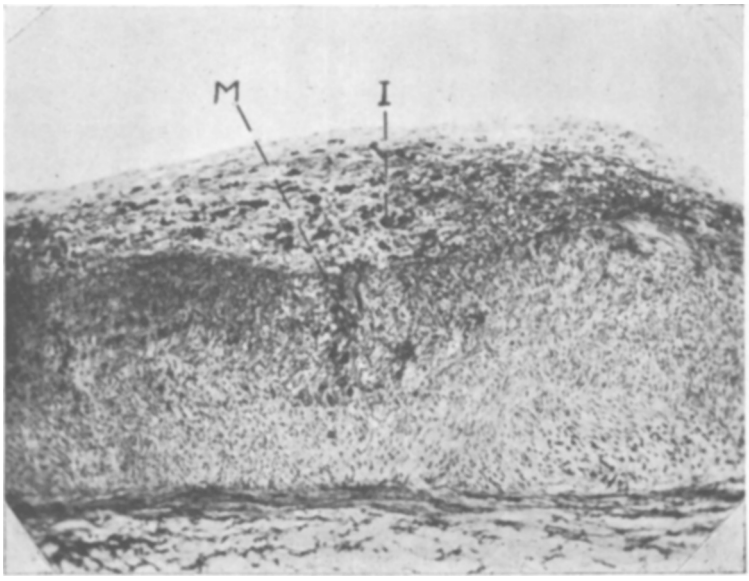

FIG. 1.
Aorta of rooster fed cholesterol, showing intimal sclerosis; also lipid in intima (I) and underlying media (M).

few muscle cells had been replaced by the accompanying fibrous tissue. In 3 other chickens grossly invisible patches of fibrosis of the intima without stainable fat were noted in sections. There was no correlation between the arteriosclerotic change and the blood cholesterol concentration, but somewhat less lipid was noted in the aortas of the birds treated only 1½ months. Gross lesions were absent from the hearts of all birds.

Twenty-five control roosters of the same age were kept on the same standard laboratory diets (9 received the cholesterol free diet, 16 that containing egg mash) but were fed no additional cholesterol. Areas of intimal fibrosis were present in the abdominal aorta of 14 and these were accompanied by lipid deposits in 9 (Fig. 2). Fat

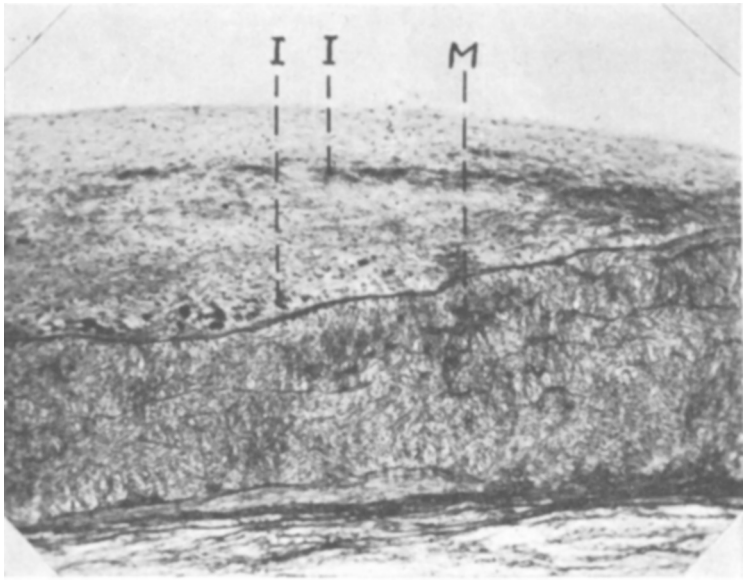

FIG. 2.
Same in control chicken. (Combined Weigert-Sudan stain.)

was present in the underlying media in only 4, however, and these medial deposits were rather less extensive and without the fan-shaped radiating distribution suggestive of rapid infiltration from the intima noted in some of the cholesterol-fed birds. Small deposits of lipid were present in the mid-portion of the media, unassociated with an intimal plaque, in the thoracic aorta of one control chicken, but no other independent medial lesions were observed.

Conclusion. The aorta of the young adult rooster commonly exhibits areas of intimal fibrosis, with an infiltration of lipid into the thickened intima and the underlying media. Feeding of cholesterol for periods up to 4 months fails to increase the incidence of such lesions, but may slightly augment the deposition of lipid, particularly in the media. For the production of intimal lipid deposits in the aorta by feeding cholesterol, the chicken, therefore, appears to be an unsatisfactory animal.