tion of glycoprotein tubular casts produced by PTE administration. The data suggest that the liver is the major source of the elevated serum glycoproteins found in rats receiving PTE. In addition, it is possible that PTE may have a direct stimulating action on biosynthesis of glycoprotein by the kidney.

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Biochemical and Histochemical Changes in Aorta of Chicks Fed Vegetable Oils and Cholesterol. (30382)

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We reported earlier(1) that feeding vegetable oils and cholesterol to chicks for 8 weeks increased total plasma cholesterol, β-lipoprotein cholesterol, β-lipoprotein percentage, triglycerides, and free fatty acids in plasma. The rise in plasma cholesterol was maximum in chicks fed sesame oil with cholesterol or hydrogenated groundnut oil with cholesterol, minimum in chicks fed mustard oil with cholesterol with intermediate value when fed coconut oil with cholesterol. Hypercholesteremia did not depend on saturation or unsaturation of the oils. Clinical and experimental evidences indicate a direct relationship of atherosclerosis to the altered lipid metabolism characterized by hypercholesteremia and hyper β-lipoproteinemia. The origin of lipid in atheromatous plaques might be due to filtration from circulating plasma (2) or arterial synthesis of cholesterol(3-5) and phospholipids(6). Significant quantities of mucopolysaccharides containing hexosamine present in the arteries(7) are increased in rabbits fed cholesterol(8). Earliest change observed in the development of atherosclerosis was the marked accumulation of acid mucopolysaccharides(9).

We wished to investigate the interrelation, if any, between dietary fat, plasma lipids and changes in the various parts of the aorta. The distribution of elastic tissue, acid mucopolysaccharides and lipids was, therefore, determined histochemically and cholesterol, phospholipids, hexosamine and hydroxyproline were determined chemically in the different segments of the aorta in chicks fed vegetable oils and cholesterol.

Materials and methods. Two-week-old male white Leghorn chicks were fed a basal diet(10) for 3 weeks, then divided into groups of 6 birds (Table I). Oils when fed were mixed with the diet at 10% level. Cholesterol when administered was mixed with the diet at 1% level. Average daily food consumption by each chick was 30 g. After the diets were fed for 8 weeks, birds were killed

TABLE I. Plasma Cholesterol and Arterial Cholesterol, Phospholipids, Hexosamine and Hydroxyproline in Chicks Fed Different Diets for 8 weeks.

Treatments	Total plasma cholesterol, mg %	Arterial cholesterol* (mg/100 g wet wt) Arch	Tho + abd	Arterial phospholipid* (mg/100 g wet wt) Arch	Tho + abd	Arterial hexosamine† (mg/100 g dry wt) Arch	Tho + abd	Arterial hydroxyproline† (mg/100 g dry wt) Arch	Tho + abd
Basal diet	141 ±12	70	97	149	130	1.62 ±.05	1.14 ±.04	1.33 ±.09	1.52 ±.08
Idem + cholesterol	259 ±29	97	132	178	148	1.60 ±.06	1.28 ±.03	1.55 ±.07	1.42 ±.03
Basal + mustard oil	158 ± 7	68	48	191	192	1.26 ±.11	1.07 ±.04	1.41 ±.06	1.54 ±.14
Idem + cholesterol	400 ±59	190	240	176	205	1.30 ±.05	1.09 ±.08	1.50 ±.06	1.39 ±.11
Basal + coconut oil	174 ±10	71	60	223	213	1.25 ±.04	1.12 ±.03	1.29 ±.02	1.34 ±.13
Idem + cholesterol	634 ±83	158	207	137	255	1.39 ±.03	1.04 ±.03	1.51 ±.14	1.85 ±.10
Basal + sesame oil	164 ±20	75	92	196	161	1.54 ±.04	1.19 ±.03	1.37 ±.02	1.49 ±.10
Idem + cholesterol	735 ±82	198	299	293	364	1.58 ±.12	1.14 ±.13	1.55 ±.06	1.93 ±.11
Basal + hydrogenated groundnut oil	158 ±11	64	72	173	175	1.34 ±.10	1.11 ±.07	1.59 ±.14	1.57 ±.13
Idem + cholesterol	1036 ±127	173	251	140	271	1.15 ±.05	1.01 ±.02	1.65 ±.17	2.06 ±.10

* Mean of 2 determinations from pooled aorta. † Values are Mean ± S.E. (5 chicks in each group).

by decapitation after withdrawal of blood by cardiac puncture. The aorta was removed, separated into arch and thoracico-abdominal portions, freed from adherent blood and connective tissue and cut into various pieces. A few pieces were used for estimation of hexosamine(11) and hydroxyproline(12). The aortic segments of 2 or more birds were pooled, dried, extracted in a hot extractor with pretroleum ether for 12 hours and the extract used for estimation of cholesterol and phospholipids(13). Plasma cholesterol was also estimated(13). The aorta of one chick in each group was used for histochemical studies. Arterial segments were fixed in 10% neutral formol. Frozen sections were stained with Sudan Black B for lipids(14) and paraffin sections were stained for elastic tissue(15) and acid mucopolysaccharides(16).

Results. Cholesterol: In both the control and experimental chicks the arch of aorta contained more cholesterol than the thoracico-abdominal portion. Minimum changes were produced when chicks were fed cholesterol or oil. Aortic cholesterol increased 2 to 3 times when cholesterol was fed along with oils. *Phospholipids:* The distribution was uniform in the different segments of aorta. Feeding oils with or without cholesterol, increased phospholipids in most of the chicks. Maximum increase was observed in chicks fed sesame oil and cholesterol, the thoracico-abdominal portion containing a higher amount than the arch of the aorta. *Hexosamine:* In both control and experimental chicks hexosamine was higher in the arch of the aorta than in the thoracico-abdominal aorta. Hexosamine decreased in the aortic arch when chicks were fed coconut oil, mustard oil and hydrogenated groundnut oil with or without cholesterol and in the thoracico-abdominal aorta when cholesterol was fed with coconut oil or hydrogenated groundnut oil. Aortic hexosamine did not change when sesame oil was fed with or without cholesterol. *Hydroxyproline:* In control and most of the experimental groups hydroxyproline of aortic arch was less in the arch of aorta than in the

TABLE II. Distribution of Acid Mucopolysaccharides and Lipids in Aorta of Chicks Fed Different Diets for 8 Weeks.

Treatments	Acid mucopolysaccharides Arch	Thor	Abd	Lipids Arch	Thor	Abd
Basal diet	+	+	+	—	—	—
Idem + cholesterol	+	+	+	—	—	—
Basal + mustard oil	+	++++	+	—	—	—
Idem + cholesterol	++	++++	+	—	+	—
Basal + coconut oil	++	++	+	—	—	—
Idem + cholesterol	+	+	+	++	++	—
Basal + sesame oil	+	+	+	—	—	—
Idem + cholesterol	+++	+	+	+	+	++
Basal + hydrogenated groundnut oil	+	+	++++	—	—	—
Idem + cholesterol	+	++++	+	+++	++++	++

thoracico-abdominal aorta. Hydroxyproline increased in the aortic arch when cholesterol was fed with sesame oil, and in the thoracico-abdominal portion when cholesterol was fed with coconut oil, sesame oil or hydrogenated groundnut oil. Mustard oil feeding did not alter arterial hydroxyproline, neither did it change when oils or cholesterol were fed alone. *Elastic tissue:* Feeding of cholesterol or oils did not affect the elastic tissue of the aorta. Slight proliferation of intima with little disruption of elastic tissue was seen in the arch and thoracic aorta when cholesterol was fed along with coconut oil, mustard oil and hydrogenated groundnut oil. Feeding of cholesterol with sesame oil produced atheromatous plaque, fragmentation of elastic tissue throughout intima and complete disappearance from the media of the arch of aorta with slight intimal proliferation and fraying of the intimal elastic tissue in the thoracic aorta. No change in the abdominal aorta was seen after the chicks were fed different diets. *Lipid:* Lipid was either absent or present in a very low concentration in the aorta which could be detected by histochemical methods. Feeding vegetable oils or cholesterol did not produce any change. Lipid was deposited when cholesterol was fed with oils in the intima. The deposition was marked in the arch and thoracic aorta when coconut oil was fed, in abdominal aorta when sesame oil was fed and in thoracic aorta when mustard oil or hydrogenated groundnut oil was fed. *Acid mucopolysaccharides:* Feeding of cholesterol alone did not produce any change in its distribution. Coconut oil feeding increased it in intima and media of arch and thoracic aorta and decreased it in abdominal aorta. Sesame oil fed with cholesterol increased acid mucopolysaccharide in the aortic arch. Mustard oil fed alone or with cholesterol increased acid mucopolysaccharide only in the aortic arch. Feeding hydrogenated groundnut oil increased it in abdominal aorta and in the thoracic aorta when cholesterol was fed simultaneously. Detailed results are given in Tables I and II and in Fig. 1-21.

Discussion. Arterial cholesterol and phospholipids: Increased aortic cholesterol and phospholipids in chicks fed cholesterol with or without vegetable oils confirmed earlier reports(17). Elevation of arterial lipids might be due to increased lipogenesis(18), increased transfer of plasma lipids(19) or decreased destruction as a result of decreased lipolytic activity consequent to deficient production of heparin(20).

Arterial hexosamine and hydroxyproline: Hexosamine value represented the combined hexosamine content of mucoproteins, mucoids and glycoproteins of the aorta. Diminution of arterial hexosamine after feeding the oils with or without cholesterol indicated that changes were not related with the hypercholesteremia. Mucopolysaccharides act as precursors in formation of collagen fibrils(21). Intimal and subintimal fibrosis characteristic of atheromatous lesions has been suggested to be due to depolymerization of mucopolysaccharides(22) and as a result mucopolysaccharides are decreased. We have also obtained decreased hexosamine in the aorta. Decreased mucopolysaccharide in aorta will

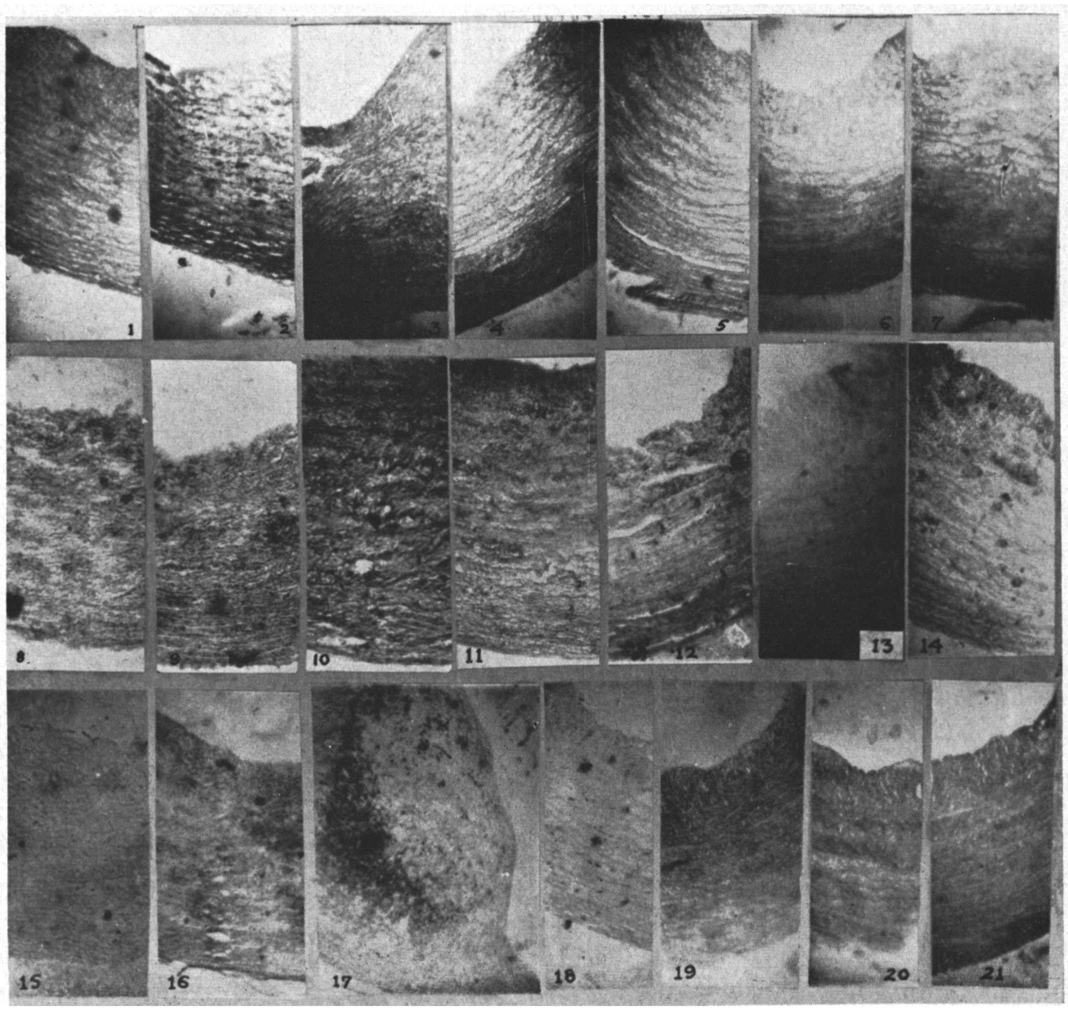

FIG. 1-7 showing distribution of elastic tissue in aorta. × 56.

Fig. 1. Arch of aorta in a chick fed basal diet.

Fig. 2. Arch of aorta in a chick fed coconut oil + cholesterol showing slight intimal proliferation and disruption of elastic tissue.

Fig. 3. Arch of aorta in a chick fed sesame oil + cholesterol showing atheromatous plaque, fragmentation of the elastic tissue in the intima.

Fig. 4. Arch of aorta in a chick fed mustard oil + cholesterol showing similar changes as in Fig. 2.

FIG. 5. Arch of aorta in a chick fed hydrogenated groundnut oil and cholesterol showing similar changes as in Fig. 2.

FIG. 6. Thoracic aorta in a chick fed coconut oil and cholesterol showing little fragmentation of the elastic tissue in intima and proliferation of the intima.

FIG. 7. Thoracic aorta in a chick fed sesame oil and cholesterol showing intimal proliferation and fraying of intimal elastic tissue.

FIG. 8-14 showing distribution of lipid in aorta. × 56.

FIG. 8. Arch of aorta from a chick fed basal diet showing absence of lipid staining material.

FIG. 9. Arch of aorta from a chick fed coconut oil and cholesterol showing lipid deposition in the intima.

FIG. 10. Arch of aorta from a chick fed sesame oil and cholesterol showing deposition of lipid in the intima.

FIG. 11. Arch of aorta from a chick fed hydrogenated groundnut oil and cholesterol showing deposition of lipid in the intima.

FIG. 12. Thoracic aorta of a chick fed coconut oil and cholesterol showing similar changes as in Fig. 9.

result in decreased diffusion of nutrients to the intima and the inner half of the media, leading to aggravation of the condition. Increased deposition of collagen in the aorta as evidenced by increased hydroxyproline content in chicks fed cholesterol with oils possibly indicates the existence of a mechanism to strengthen the arterial wall in areas of fragmentation of the elastic tissue.

Histochemical changes: Disruption and degeneration of the elastic tissue and increase in the acid mucopolysaccharides were noticed in the aorta of hypercholesteremic chicks. Intimal proliferation, thickening of intima and complete disappearance of elastic tissue with subsequent production of atheromatous plaques were the changes observed. Increase of acid mucopolysaccharide and decrease of hexosamine in the aorta indicated a reduction of neutral hexosamine containing polysaccharide after the feeding of oils and cholesterol. Deposition of lipid and degeneration of elastic tissue of the aorta were closely related. The elastic tissue changes coupled with disturbances in the metabolism of mucopolysaccharides and collagen might alter the permeability of the arterial wall resulting in formation of atheroma.

The appearance of atherosclerotic lesions in the arch of the aorta earlier than thoracic and abdominal aorta indicated a close similarity in the pattern of development of the condition in man(23). The sites of atheromatosis are also the sites at which stress occurs(24) and the breakdown of elastic tissue is more severe in the region than elsewhere. Therefore the arch of aorta is likely to be more susceptible than other regions of the aorta. Diminished succinic dehydrogenase and succinic oxidase activities were observed in the arch of aorta of hypercholesteremic chickens without any change in the thoracic or abdominal aorta(25). This suggested that metabolic activity of the arch was decreased in atheromatosis.

Results indicated that the severity of the arterial lesion was not parallel to the degree of hypercholesteremia. Although maximum hypercholesteremia was observed in chicks fed hydrogenated groundnut oil and cholesterol, atheromatous lesions were more severe in the arch of aorta of chicks fed sesame oil and chlesterol. The nature of the fat might be responsible for the atheromatous changes observed.

Summary. Portions of aorta of chicks fed coconut oil, sesame oil, mustard oil and hydrogenated groundnut oil, with or without simultaneous administration of cholesterol, for 8 weeks were analyzed for cholesterol, phospholipids, hexosamine and hydroxyproline. The distribution of elastic tissue, acid mucopolysaccharides and lipids in the aorta was also studied by histochemical methods. Feeding of cholesterol with or without oils increased cholesterol and phospholipids in different portions of aorta. Oils when fed alone increased the phospholipids without changing the cholesterol of the aorta. Hexosamine content of the aorta diminished when vegetable oils other than sesame oil were fed either alone or in combination with cholesterol. This indicated a decrease in mucopolysaccharide content of the aorta. Hydroxyproline of aorta increased when chicks were fed vegetable oils with cholesterol. The increase in arterial collagen, as evidenced by its hydroxyproline content, appeared to be proportional to the degree of hypercholesteremia. Feeding of oils with cholesterol increased lipid and

FIG. 13. Thoracic aorta of a chick fed sesame oil and cholesterol showing deposition of lipid in the intima.

FIG. 14. Thoracic aorta of a chick fed hydrogenated groundnut oil and cholesterol showing marked deposition of lipid in intima.

FIG. 15-21 showing distribution of acid mucopolysaccharides in aorta. $\times$ 56.

FIG. 15. Arch of aorta of a chick fed basal diet showing slight reaction.

FIG. 16. Arch of aorta of a chick fed coconut oil, increased reaction in intima.

FIG. 17. Same from a chick fed sesame oil and cholesterol, very marked reaction in the intima.

FIG. 18. Same from a chick fed mustard oil and cholesterol, slight reaction.

FIG. 19. Thoracic aorta from a chick fed mustard oil, increased reaction.

FIG. 20. Thoracic aorta from a chick fed mustard oil and cholesterol, increased reaction for mucopolysaccharide in the intima.

FIG. 21. Thoracic aorta from a chick fed hydrogenated groundnut oil and cholesterol, increased reaction.

acid mucopolysaccharide contents of the aorta, proliferation of the intima, and fragmentation or complete disappearance of the elastic tissue in the intima and media. The changes were most marked in the arch of aorta. Elastic tissue changes coupled with disturbances in the metabolism of mucopolysaccharides and collagen might alter the permeability of the arterial wall resulting in atheroma formation. The severity or degree of the different changes was neither related to the plasma level of cholesterol nor to the saturation or unsaturation of the vegetable oils used.

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Quantitative Estimation of 7,12-Dimethylbenz(a)Anthracene in Rat Mammary Tissue by Gas Liquid Chromatography.* (30383)

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The distribution of carcinogenic polycyclic hydrocarbons in animal tissues and their concentration at different intervals following administration have been studied mainly by spectrofluorometric measurements on tissue extracts(1-4). Autoradiographic techniques have also been used for study of the metabolism of radioactively-labelled hydrocarbons and their distribution in different organs of the rat(5,6).

In our laboratory, 7,12-dimethylbenz(a) anthracene (DMBA) is used for induction of mammary tumors in female rats by a single intragastric administration of the carcinogen dissolved in sesame oil, as advocated by Huggins and Yang(7). In connection with these

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