synthesize fatty acids at a considerably slower rate than do liver slices; an approximate estimate would place the rate of kidney slice lipogenesis at about 10-20% that of the liver slice. Kidney tissue appears to have a lower level of fatty acid synthesizing enzymes than liver but the difference in lipogenic rate of the intact cell is probably related to other factors as well. Kidney cytoplasmic particles suppress lipogenesis in kidney particle-free supernatant while liver cytoplasmic particles stimulate kidney particle-free supernatant lipogenesis. Thus, the presence of cytoplasmic particles in the kidney that inhibit rather than stimulate fatty acid synthesis probably is also related to difference in lipogenic activity between liver and kidney slices.

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Sequestration of Serum Low-Density Lipoproteins in the Arterial Intima by Complex Formation.* (30053)

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Low density serum lipoprotein has recently received considerable attention because of its probable role in the genesis of atherosclerosis. In general it is thought that it may act as a carrier of cholesterol and other lipids that accumulate in the diseased vessel. Previous studies have indicated that this class of protein, alone of all the serum proteins, is selectively bound in the aortic intima(1). But the material extracted from the intima, although antigenically identical with low density serum lipoprotein, was not entirely characteristic of it. Its immunoelectrophoretic pattern was unique. This study is concerned with the nature of this alteration of lipoproteins in extracts of arterial intimas.

The serum lipoproteins with molecular density 1.006 to 1.063 in the ultracentrifuge are subdivided into beta and alpha-2, named for their electrophoretic mobilities in numerous media including agar gel. Each of these has its own immunoelectrophoretic pattern, yet the protein portions of these subclasses have eluded all attempts to distinguish them antigenically with the more recently developed immunological techniques(1,2). They

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appear to differ only in the kind and quantity of various lipids associated with them. Nevertheless, it is important to determine which of these subclasses is the one that collects in the arterial wall. If they are antigenically identical and the material extracted from the vessel has its own unique characteristics it is important to develop new techniques to characterize further the lipoproteins from arteries.

Two observations have given impetus to the present approach to these related questions. First Hanig *et al*(3), using the ultracentrifuge, identified specifically the alpha-2 lipoprotein in the aortic intima. Second, Amenta and Waters(4) and more recently Bihari-Varga *et al*(5) have shown that glycoprotein prepared from human aorta forms a firm physical complex with low density serum lipoprotein. Anderson(6) has extended these studies using chondromucoprotein and fibrinogen. We have developed a simple approach that might link these observations with our own. In the ultracentrifuge, low density lipoproteins are prepared in strong salt solution. Since strong salt solution is known to disrupt the complex between lipoprotein and such polyanions as mucopolysaccharide(7) we have studied the effects of salt (NaCl) on the lipoprotein separated from diseased human aortic intima.

Materials and methods. Preparation of lipoproteins in the ultracentrifuge, antisera against these in the rabbit, and immunoelectrophoresis in agar gel at buffer pH 8.7 have been described(1). Human aortas obtained up to 3 days after autopsy were graded with respect to extent of atherosclerosis as mild, moderate, or severe. After washing the endothelial surface by gentle rubbing with the fingers under running tap water, the left and right halves of the intima were stripped separately, avoiding all areas of advanced plaques. One half of the stripped intima was homogenized with twice its volume of 12% NaCl, the other with 0.9% NaCl. These preparations are referred to as "with" and "without" salt respectively. They were then subjected to centrifugation. Only the opalescent middle layer was used for agar gel electrophoresis. Anti-low density lipoprotein serum was allowed to diffuse toward the samples for 3 days before washing, drying, and staining with Sudan black. The amount of lipoprotein in any particular extract was roughly estimated as absent, small amount, or large amount, on the basis of the darkness of staining by Sudan black. Ultracentrifugally prepared human serum alpha-2- and beta-lipoproteins were dialyzed for 3 days against daily changes of normal saline at 4°C. After dialysis each was treated in 2 ways: dilution 1:1 with 16% NaCl and with 0.9% NaCl. These 4 preparations are referred to as "with" and "without" salt respectively.

Results. Immunoelectrophoresis of 0.9% NaCl extract of most human aortic intimas produces one precipitin arc when tested against antiserum directed against anti-whole human serum or against anti-low density lipoprotein(1). This arc is in the beta region, is positive for Sudan staining, and resembles a horn off of the well of origin (Fig. 1 [3]). This "horn" does not have the same configuration as the reaction given by ultracentrifugally isolated low density lipoproteins, either alpha-2 or beta (Fig. 1 [5&6]), nor of lipoproteins in whole serum(1,2). In addition, from extracts of some severely sclerotic aortas, a second lipoprotein can be precipitated with these same antisera; this deposits in the pre-albumin region (Fig. 1 [3]). In the present study, intimas from mildly diseased areas of 3 aortas in which the atherosclerosis was considered mild yielded no lipoprotein; 1 severely and 2 moderately sclerotic aortic intimas yielded irregular amounts of lipoproteins in the beta region only; 2 severely sclerotic intimas yielded large amounts of precipitate in the beta region, but also substantial amounts in the pre-albumin region.

Extraction of the aortic intima with 12% NaCl, yielded a lipoprotein which on immunoelectrophoresis showed absence of the beta region "horn" and the pre-albumin reaction. In their stead there was a broad band in the alpha region (Fig. 1 [4]). This new band is again not entirely characteristic of the pattern for ultracentrifugally isolated beta or alpha-2 lipoprotein. But when salt is added to the isolated lipoproteins, the slight alteration seen in the alpha-2 material brings it into nearly perfect resemblance to the pat-

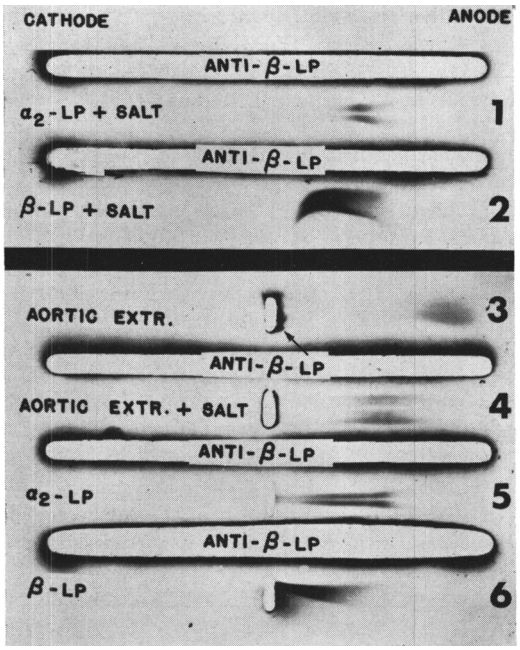

FIG. 1. Two agar gel immunoelectrophoresis plates are stained with Sudan black. Antiserum prepared against human serum beta-lipoprotein (anti-β-lp) was placed in each trough after electrophoresis of the designated substances. *β-lp* is beta-lipoprotein, *α₂-lp* is alpha-2-lipoprotein (both are low density serum lipoproteins); *Aortic extr.* is extract of aortic intima prepared with 0.9% NaCl, *Aortic extr.* + *Salt* is extract of aortic intima prepared with a final NaCl concentration of 8%, *α₂-lp* + *Salt* and *β-lp* + *Salt* are the respective lipoproteins brought to a final concentration of 8% NaCl. Low-density lipoprotein is present in aortic extract in two complex forms (pre-albumin and arrow in well 3); both are disrupted by strong salt to release lipoprotein resembling alpha-2 in its characteristics (wells 1 and 4).

tern developed from the salt-treated intimal extract.

Discussion. That the quantity of lipoprotein in extracts of aortic intima appears to increase with severity of atherosclerosis agrees with our earlier impression(1). In the previous study this trend was observed when comparing areas both with and without grossly visible plaques.

The lipoprotein in intimal extracts, designated here as a "horn" in the beta region, has been described(8,9). However, those reports also include descriptions of other unidentified serum proteins in the extracts. We have found it possible to eliminate all of these extraneous reactions simply by freeing the endothelial surface of adherent serum by washing the intima before preparing the extracts.

Bihari-Varga *et al*(5) isolated mucopolysaccharides from human aorta and added this to whole serum. Immunoelectrophoresis then showed the presence of a Sudan positive (lipid) pre-albumin arc with the antigenicity of low density serum lipoprotein. Thus, the *in vitro* reaction of low density lipoprotein with aortic mucopolysaccharide yielded a complex with characteristics resembling those of a natural material we have extracted from some severely sclerotic aortas. The failure to confirm this result(10) may relate to the use of paper electrophoresis rather than gel immunoelectrophoresis.

Low density serum lipoprotein has been identified in human arterial walls in tissue sections stained by fluorescein and ferritin labeled antibodies(11). The latter technique in combination with electron microscopy revealed lipoprotein within cells as well as in interstitial tissue, although no estimate of the proportionate distribution between the two compartments was possible.

The exact nature of the distinction between the alpha-2 and beta subclasses of the low density lipoproteins is still not defined. That they differ in kinds and quantity of fat in proportion to protein is abundantly clear(1). But no antigenic differences can be distinguished between these 2 subclasses(1). Perhaps if these molecules have a transport function, the beta might be likened to the empty boxcar while the alpha-2 represents the filled one. The proteins would then in fact be identical. Yet, of these 2 subclasses, it appears to be largely the alpha-2 that is bound by the aorta in the mild lesions.

Conclusions. The selective sequestration of serum low density lipoproteins by the aortic intima, which occurs in greater quantities with increasing severity of atherosclerosis, appears to involve the formation of a pair of complexes. One of these has the pre-albumin electrophoretic mobility which has been described for complexes of aortic mucopolysaccharide with low density lipoproteins(5), and both are disrupted by a high salt concentration, which is a property of complexes of beta lipoprotein with sulfated polysaccharides(7). Whether such complexes actu-

ally exist in the living vessel wall remains to be determined.

Summary. Extracts of human aortic intima were made in 0.9% NaCl and in 12% NaCl. Ultracentrifugally isolated human serum beta and alpha-2 lipoproteins were placed in similar salt concentrations. These were then examined in the immunoelectrophoresis system using antiserum against serum beta lipoprotein (which is equally effective against alpha-2 lipoprotein). Extract of aorta gave 2 lipoprotein arcs with this antiserum, one in the beta and one in the pre-albumin region, when prepared with 0.9% NaCl; there was only one arc (in the alpha-2 region) in extracts with 12% NaCl. This is interpreted as suggesting that serum alpha-2 lipoprotein is bound in the aortic intima in a pair of complexes both of which can be disrupted with strong salt solution.

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Enhancement by Parathyroidectomy of Glycoprotein Mobilizing Property of Parathyroid Extract.* (30054)

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In the absence of the parathyroid glands the effects of bovine parathyroid extract (PTE) on Ca^{++} metabolism of rats are enhanced. Ca^{++} elevation is more pronounced, metastatic calcification is more severe and the animals die rapidly from the general toxic effects of PTE(1). Parenthetically this enhancement of the Ca^{++} mobilizing action of PTE by parathyroidectomy (PTX) is a useful phenomenon for bioassay of parathyroid hormone preparations(2). The effect of PTX on other properties of PTE has not been studied. In addition to mobilizing Ca^{++}, PTE increases serum glycoproteins and alters serum protein and glycoprotein fractions of rats (fall in serum albumin and rise in a_1, a_2 and β globulins)(3). Concurrently mucopolysaccharide material is deposited in the kidneys and the amount of mucoprotein in the gastric mucosa(4) increases. The significance of these changes is not known. They have been attributed to depolymerization of bone matrix following bone demineralization (5). The purpose of the studies described here was to find out if the effects of PTE on mucopolysaccharide metabolism are also enhanced in absence of the parathyroids.

Materials and methods. One hundred thirty male Wistar rats, weighing from 150 g to 180 g, were divided into 4 groups and were treated as follows: *Group I*: Cautery PTX under ether anesthesia. This was followed immediately by the first of 1, 2, or 3 injections of 100 U of PTE (Lilly) given at 8-hour intervals. Rats were sacrificed at 8, 16 or 30 hours after the beginning of the experiment, having thus received 100, 200, or 300 U of PTE respectively. *Group II*: Sham operation under ether anesthesia. The

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