compared with data reported by Kirk(7,8). LDH, G-6-PDH and 6-PGDH activities are remarkably similar in human and rat aortas.

Summary. (1) Extracts of arteriosclerotic aortas of female Sprague-Dawley breeder rats were assayed for lactic dehydrogenase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconic dehydrogenase. (2) Extracts obtained from moderately and severely arteriosclerotic aortas contained significantly less lactic dehydrogenase than extracts of aortas grossly normal or only slightly affected. TPNH production by combined action of glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase was significantly depressed in extracts from moderately and severely arteriosclerotic aortas. No significant differences were observed in levels of 6-phosphogluconic dehydrogenase.

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Comparison of Ultracentrifuge and Polyanion Precipitation Methods for Serum β-Lipoproteins.*† (25927)

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Isolation of human serum β-lipoproteins by precipitation of a complex with polyanions has been described by Burstein(1) and confirmed by Oncley(2), Bernfeld(3) and other authors. The polyanion precipitation method yields an insoluble complex which, in the human β-lipoprotein, can be dissociated readily (4). It has the advantage of rapid separation of β-lipoproteins from other serum constituents. This precludes exchange of lipid components between α and β lipoproteins(5) and makes possible kinetic studies of β-lipoprotein, which previously could not be done precisely due to slowness of electrophoretic and ultracentrifugal isolation technics. Completeness of precipitation technic has been checked against electrophoretic methods of β-lipoprotein isolation(6), but this comparison seems insufficient in view of poor quantitation of lipids possible by means of paper electrophoresis(7) and the ease with which mobility of β-lipoproteins can be altered by adsorbed fatty acid anions(8). We therefore studied completeness of precipitation of serum β-lipoproteins by comparison with ultracentrifugal lipoprotein analyses(9). We extended this study to include a number of animal sera, since no adequate evaluation of applicability of the precipitation method to sera of common laboratory animals has been published. Much of published work used dextran sulfate as the polyanion, but this compound is not available in this country. Mepesulfate, the sodium salt of sulfated polygalacturonic acid methyl ester methyl glycoside (Hoffmann - LaRoche,) whose use was described parenthetically by Burstein(4), is the only suitable polysulfonated macromolecule available at reasonable cost. We therefore used it and compared its

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action with that of a sample of rice starch sulfate prepared by Drs. T. Ushiyama and J. Mehl by a slight modification of Grönwall's method(10).

Methods. Sera were obtained from volunteers or laboratory animals or purchased from Colorado Serum Co. They were cooled to 1°C before use. Rice starch sulfate was used at concentration of 1 mg/cc of serum by adding a saturated solution (10 mg/cc) in saline to the serum with constant agitation. Mepesulfate was used at level of 5 mg/cc of serum, and calcium acetate was added to make final concentration 0.17 M. Suspensions thus formed stood half hour before centrifuging. Supernatant was decanted and precipitate washed with 0.10 M calcium acetate. The precipitate was suspended in saline of density 1.063, β-lipoproteins isolated by standard ultracentrifugation technic(9) using Spinco Model E preparative ultracentrifuge, and assayed by Spinco Model L analytical ultracentrifuge. The supernatant solutions were similarly processed after adjusting them to density 1.063 with sodium chloride.

Results. For analysis of β-lipoproteins of human serum, very satisfactory results were obtained using either mepesulfate or rice starch sulfate as the polyanionic precipitating agent.

Using mepesulfate, 3 human sera were investigated, and in 2 of these no β-lipoprotein material could be detected in the supernatant remaining after precipitation, while in a third only 8% of previous β-lipoprotein level remained. The precipitate from either method was readily soluble in 1.6 M sodium chloride (density: 1.063) and lipoproteins could be recovered ultracentrifugally unchanged.

A simple screening test for β-lipoprotein levels in patients was devised, based upon precipitation of β-lipoproteins with mepesulfate and determination of protein content of precipitate with biuret reagent, after washing and defatting with dimethoxymethane. Comparison of total cholesterol level and this test yielded a correlation coefficient of 0.76 for 43 sera, indicating that the test might be of some value clinically. Correlation with other assays for β-lipoprotein is currently in progress.

TABLE I. Recovery of Lipoproteins from Human Serum.

S_f class	0-12	12-20	20-100	100-400
Whole serum	280 mg %	48	77	34
RSS precipitate	260	29	55	30
Supernate	6	2	12	10

With animal sera rather variable results were obtained. Thus, Table II lists completeness of precipitation as judged from lipoprotein analysis of sera and the supernate from precipitation and also the fraction of total β-lipoproteins recovered from the precipitate by flotation at density 1.063.

There appeared no correlation between flotation fractions and β-lipoprotein recovery after precipitation. The material lost during precipitation and dissociation procedure derived equally from all fractions.

Discussion. Precipitation of β-lipoproteins by either rice starch sulfate or mepesulfate is satisfactory with human sera and correlates very closely with ultracentrifugal isolation of the same fractions. β-lipoproteins are readily recoverable from the precipitate by dissociation of the complex in concentrated NaCl solution.

We had better results using mepesulfate as precipitating agent. It is easily used at higher concentration than rice starch sulfate because of greater solubility. With several animal sera it gave more complete precipitation of β-lipoproteins. It does, however, require addition of calcium and therefore cannot be used

TABLE II. Precipitation of Animal β-Lipoproteins.

	Rice starch sulfate		Mepesulfate	
	% precipitated	Recoverable	% precipitated	Recoverable
Calf	74	50	62	62
Cat	90	40	100	10
Chicken	42	40	90	
Ferret	99	30		85
Guinea pig	90	66	100	100
Horse	80	80	"	"
Man	91	85	98	
Monkey	86	86	100	100
Mouse	100	83	"	"
Opossum	66	4	96	
Rabbit	100	39	100	92
Rat	26	24	"	100

with plasma. In the absence of added calcium no precipitate formed and no changes in ultracentrifugal properties of β-lipoproteins occurred. Rice starch sulfate precipitates human β-lipoproteins in absence of added calcium, but for maximal precipitation of several animal β-lipoproteins calcium was required.

Quantitative evaluation of analytical ultracentrifuge patterns is fraught with difficulties which combine to cause a sizable error in determinations, particularly when lipoprotein concentrations are either very low or very high. Thus, the difference from quantitative precipitation values for human sera seen in Table I are well within the experimental error of the method.

Summary. Ultracentrifuge analysis confirms that polyanion-complex precipitation method for human serum β-lipoproteins is adequate and does not denature the lipoproteins when either rice starch sulfate or mepesulfate are used as precipitating agent. The method is applicable to isolation of β-lipoproteins from several but not all species of animals.

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Determination of Ceruloplasmin Oxidase in Serum. (25928)

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Ceruloplasmin is a copper oxidase which can be determined photometrically by rate of oxidation of p-phenylenediamine(1,2,3,4). Oxidation of p-phenylenediamine (PPD) by serum is catalyzed by serum ceruloplasmin and by copper and iron present in serum as well as in water and reagents used in the test. To determine oxidation due to ceruloplasmin alone, it is necessary either to correct for or inhibit catalytic oxidation due to metals. Both approaches have been used. In the method of Ravin(3) a serum blank is determined in which ceruloplasmin is inhibited by azide. In methods of Humoller *et al.*(2) and Broman (4), catalytic oxidation by metals is inhibited by complexing them with ethylenediaminetetraacetate (EDTA). This paper presents (a) a modification of Ravin's method which points out an error in original method, (b) a study of various factors which must be controlled in the procedure, (c) normal values obtained with this procedure and (d) results of study of validity of methods employing EDTA to inhibit metal-catalyzed oxidation of PPD.

Methods. Reagents. 1. Acetate buffer, 0.1 M, pH 6.0. Add 10 ml 0.1 M acetic acid (0.57 ml glacial acid plus water to 100 ml) to 200 ml 0.1 M sodium acetate (1.36 g $CH_3COONa \cdot 3H_2O$/100 ml). pH should be 5.95-6.00. 2. Sodium azide, 0.1% in acetate buffer. pH must be 5.95-6.00. Store in refrigerator. 3. p - Phenylenediamine · 2HCl, 0.25% in acetate buffer. Recrystallize commercial salt as follows: Dissolve in water, add Darco charcoal, warm in water bath at 60°C with occasional mixing and filter. Add acetone to filtrate until turbidity appears, refrigerate several hours, filter off PPD · 2HCl and dry the crystals in the dark in vacuum