distinction between *L. fermenti* and homofermentative lactobacilli with respect to amino acid needs. A summary of other differentiating metabolic and cultural characteristics and their significance with respect to classification of the genus *Lactobacillus* has been given by Rogosa *et al.*(3).

The results have a possible bearing, too, on assay of cystine by microbiological methods. Not only is the rate of growth of many different lactobacilli accelerated by ascorbic acid in the presence of both suboptimum and optimum amounts of cysteine, but an apparent cysteine requirement of some cultures, mostly *L. fermenti,* disappears in the presence of ascorbic acid or certain other reducing agents.

Summary. 1. The requirement of some lactobacilli for cysteine, as determined in growth tests with usual chemically defined media, disappears when ascorbic acid in appropriate amounts, or other reducing substance, is incorporated in the medium. Of 5 strains of lactobacilli in this category encountered in a collection of 36 cultures, one was *L. casei,* the other 4 were heterofermentative *L. fermenti.* For these organisms the apparent cysteine requirement appears to be a need for a reducing substance and not for cysteine *per se.* Many *L. fermenti* strains evidently do not require cysteine though this may not always be apparent with some of the commonly-used synthetic media. 2. In contrast to *L. fermenti,* other lactobacilli—most *L. casei,* lactose-negative *L. casei, L. acidophilus,* and the majority of *L. plantarum*—require cysteine for growth in the presence of ascorbic acid. The rate of growth of these lactobacilli often is accelerated by ascorbic acid in the presence of either suboptimal or optimal amounts of cysteine. 3. Some implications of these findings concerning microbiological assay of cysteine and the distinguishing characteristics of heterofermentative *L. fermenti* and homofermentative lactobacilli are discussed.

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Comparative Study of Orosomucoid Preparations from Sera of Six Species of Mammals.* (22064)

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The presence of an electrophoretically and ultracentrifugally homogeneous acidic protein of very high carbohydrate content has been demonstrated in normal human plasma(1-4). This glycoprotein, designated orosomucoid (5,6), is one of the major components(5) of the "mucoprotein fraction" of human serum (6,7). "Mucoprotein" fractions have been demonstrated in the serum of many animal species(8-16). It appeared of some interest to determine whether proteins with characteristics of orosomucoid are also present in the blood of other species. In the present studies orosomucoid has been prepared from normal, pooled beef, guinea pig, horse, rabbit, and rat serum, and has been compared with that isolated from human plasma.

Materials and methods. Serum orosomucoid was separated by a procedure previously reported(1). To 500 ml of pooled serum from normal animals was added 500 ml of 0.1 M sodium acetate and 360 g of ammonium sulfate.† The precipitated proteins were

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† Erroneously reported previously(1) as 2.73 Molar.

removed by filtration after 16 hours at 4°C. The pH was reduced first to 4.9 and then to 3.7, the precipitated proteins again being removed by filtration after 16 hours at 4°C following each adjustment. Precipitation of the orosomucoid preparations was achieved by saturation of the filtrate with ammonium sulfate, the precipitate then being dialyzed and lyophilized. Since the animal sera were usually hemolyzed, it was necessary to refractionate 1% solutions of the orosomucoid preparations at least once to obtain a white product. Due to the loss of material during refractionation, an accurate estimate could not be made of the amount of orosomucoid in the sera of the different species. The isolated proteins were readily soluble in distilled water, in solutions of neutral salts, and in dilute or concentrated solutions of acids and alkalis. Acidified solutions were not coagulated by boiling.

Methods. The hexose content of the preparations was determined by the orcinol method of Sörenson and Haugaard(17), using a 1:1 galactose-mannose standard. Hexosamine was determined by the Rimington(13) modification of the Elson-Morgan method. "Sialic acid" was determined by the direct Ehrlich reaction as described by Werner and Odin(18), using "sialic acid" isolated from submaxillary mucin as a standard.‡ Nitrogen was determined by digestion and direct nesslerization(19). Protein was determined by the biuret method of Mehl(20), employing bovine serum albumin as a standard. All values are expressed on a moisture-free basis and were obtained by drying each preparation to constant weight at 105°C. Electrophoresis was carried out with a modified Tiselius apparatus, or with an Antweiler micro-electrophoresis apparatus using a veronal-sodium chloride buffer at pH 8.5, and an acetate-sodium chloride buffer at pH 4.5, ionic strength 0.1, as previously described(1).

TABLE I. Electrophoretic Mobilities of Orosomucoid at pH 8.6 and 4.5.

Species	Mobility × 10^{-5} cm²/volt/sec pH 8.5	pH 4.5
Human	−7.2*	−4.7*
Beef	−6.2*	−3.2*
Rabbit	−5.2†	−2.4†
Horse	−5.4†	−1.9†
Guinea pig	−5.8*	− .8*
Rat	−6.2*	− .8*

* Ascending boundaries with Tiselius apparatus.
† " " " Antweiler "

‡ We wish to thank Doctors I. Werner, L. Odin and G. Blix for samples of "sialic acid."

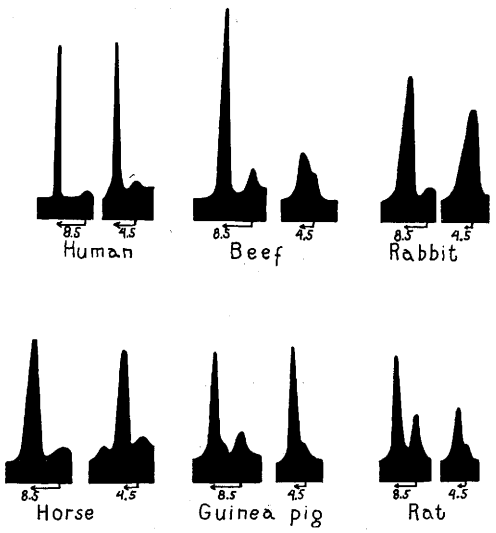

FIG. 1. Electrophoresis of orosomucoid preparations at pH 8.5 and 4.5. Ascending boundaries. Preparations from human, beef, guinea pig and rat with modified Tiselius apparatus. Rabbit and horse preparations with Antweiler micro-electrophoresis apparatus.

Results. The results of the electrophoretic studies are shown in Table I and Fig. 1. Although electrophoretic homogeneity cannot be claimed for any of the preparations from animal sera, it is clear from the results at pH 4.5 that each preparation had an acidic protein as the major component.

Marked differences were apparent in the electrophoretic mobilities of the major component of different species, these differences being especially pronounced at pH 4.5. The fraction from human plasma had the greatest negative mobility at this pH (−4.7 x 10^{-5} cm²/v/sec), while the proteins from rat and from guinea pig serum had the lowest mobility at pH 4.5 (−0.8 x 10^{-5} cm²/v/sec). The major component of all preparations, however, was negatively charged at pH 4.5 and resembled, in this respect, the orosomucoid isolated from human plasma.

The results of the chemical analyses are

TABLE II. Partial Chemical Composition of Orosomucoid Preparations.*

Species	Hexose	Hexosamine	"Sialic acid"	Nitrogen	Protein
			%		
Human	16.5	12.0	11.2	10.1	63.0
Beef	13.9	7.9	10.9	11.9	77.0
Rabbit	12.3	9.1	6.9	12.0	72.0
Horse	12.3	7.0	6.8	12.5	73.0
Guinea pig	11.1	6.6	6.2	13.2	84.0
Rat	9.9	6.4	5.1	13.6	88.0

* Moisture-free basis.

summarized in Table II. The orosomucoid preparations of each species were characterized by high hexose, hexosamine, and "sialic acid," and by low nitrogen and protein contents relative to the major components of serum.

The current study has demonstrated that the blood of several species of animals contains a glycoprotein analogous to the orosomucoid of human plasma. The most pronounced features of this similarity are the high hexose, hemosamine, and "sialic acid" contents; the stability to heat; and the acidic nature of all the preparations. While the similarities in the fractions from various species are marked, it is apparent that there are notable differences among them. The carbohydrate contents, the ratios of hexose to hexosamine or "sialic acid" and of protein to carbohydrate show considerable variation from species to species. Although the electrophoretic data are far too limited to permit definite conclusions, it seems probable that there are significant species differences in the isoelectric points of orosomucoid, the I.E.P. of the rat and the guinea pig preparations apparently being higher than that of human orosomucoid.

Summary. Highly soluble, acidic, carbohydrate-rich proteins have been isolated from the serum of 6 animal species by a fractionation procedure employing ammonium sulfate. All of the preparations contained large amounts of hexose, hexosamine, and "sialic acid," and were not coagulated by heat. The major component of each preparation had an isoelectric point below pH 4.5, and resembled the orosomucoid isolated from human serum. Differences in chemical composition and in electrophoretic mobility were observed in preparations from different species.

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