Comparative Study of Soluble Proteins of Corneas from Three Mammalian Species.* (22514)

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There have been few studies on the nature of the water soluble proteins of corneal tissue. The first report on the composition of the soluble proteins of cornea(1) showed that there is a small amount of water extractable protein in bovine corneas equal to approximately 0.15% of the wet weight of this tissue. Temperature denaturation studies suggested that these extracts contained 2 distinct globulins in addition to an albumin.

The present study was undertaken to determine whether any similarity exists in the composition of extracts of corneas from various species. Sedimentation studies were conducted to determine the number of protein fractions and the particle size distribution of these fractions in the various extracts.

Materials and methods. A. *Source of Corneas.* The swine, bovine, and New Zealand White rabbit corneas were obtained soon after death of the animals. The corneas were removed, washed in 1% saline solution to remove any adhering blood and then placed on cracked ice until the collection was complete. Within 4-5 hours the corneas were either immediately extracted or frozen until extraction could be accomplished. The corneas from the mongrel rabbits were obtained from rabbit heads which were frozen immediately after death of the animals and shipped in the frozen state. Removal of these corneas occurred before the tissue thawed completely. B. *Extraction.* Extracts were made by adding the tissue to the phosphate buffer and mincing for 15 minutes at 10°C in an Eskimo Blender, producing a gel from which the extract could be separated by centrifugation at 110,000 x g for 5 minutes. The precipitate was then extracted once more by the same procedure and the 2 extracts combined. When mincing swine, bovine, and mongrel rabbit corneas, 100 ml of buffer and 25 g of tissue were used for each extraction. When mincing New Zealand White rabbit corneas, 24 ml of buffer were used for each 2 g of corneal tissue. C. *Concentration of proteins in extracts.* Two methods of concentrating the extracts were employed. One method consisted of complete precipitation of all proteins in the extract by saturating with ammonium sulfate. The precipitated protein was redissolved in a small amount of buffer and dialyzed in the cold against pure buffer until the solution was free of sulfate. The second method of concentrating the extracts consisted of centrifuging in a preparative rotor for 5 hours at a mean gravitational field of 173,400 x g. This sedimented the proteins so that they were contained in the lower half of the solution in each centrifuge tube. The upper half of the solution in each centrifuge tube (which was protein free) was removed and discarded. The remaining extract was combined and the same procedure applied 2 more times, thus increasing the concentration of the proteins of the original extract by a factor of 8. D. *Sedimentation constants.* The sedimentation constants of the proteins in the above concentrates were determined with the Specialized Instruments Co. Model E analytical ultracentrifuge at a speed of 52,640 rpm (201,320 x g). The standard Spinco Analytical Rotor "A" was employed. The temperature of the rotor was 24° to 25° C. Photographs of the sedimentation diagrams were taken at 16 minute intervals after reaching full speed. A microcomparator was utilized to measure the boundaries on the photographic plates. The sedimentation values reported were determined in the phosphate buffer described at 24-25°C and are not

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TABLE I. Sedimentation Constants of 3 Soluble Protein Fractions of New Zealand White Rabbit Corneas Obtained under Various Conditions of Preparation.

Source of protein	Method of concentration of extract		$S \times 10^{13}$ I	II	III
Fresh corneas	Ammonium sulfate precipitation	a.	3.2	3.9	5.9
		b.	3.1	4.1	6.2
Fresh "	Ultracentrifugal		3.3	4.1	6.3
Frozen "	Ammonium sulfate precipitation		3.0	4.0	6.2

corrected to standard conditions. E. *Concentration measurements.* The areas under the curve in the sedimentation diagrams, which are proportional to the concentration of the individual protein fractions, were measured by planimetry.

Results. A. *Effect of concentration methods on proteins obtained.* The effect of the 2 methods of concentrating the extracts from New Zealand White rabbit corneas on the amount and nature of the proteins in the final solutions was investigated. It was observed in all preparations that 3 protein fractions were obtained from this tissue. The sedimentation values of the 3 components in the mixtures were measured for extracts concentrated by the 2 methods (Table I). It was observed that freezing the corneas for as long as 3 months before extraction, or concentrating the extracts by either the ammonium sulfate precipitation method or the centrifugal method had no significant effect on the sedimentation values of the 3 protein components in the mixture. These components are labeled I (lightest), II (intermediate) and III (heaviest component). The relative concentrations of the protein fractions in the various preparations, based on the measurement of the areas under the respective curves on the schlieren patterns were determined (Table II). The differences in concentration for the fractions in the various preparations were within experimental error and therefore are not significant. Similar studies with extracts from ox cornea also suggest that tissue may be frozen for a considerable length of time before extraction without affecting the amount or the nature of protein extracted. The method of concentrating these extracts also had no effect on the proteins in the final preparation.

TABLE II. Relative Concentrations of Protein Fractions of Various Preparations of Soluble Proteins from Fresh Corneas of New Zealand White Rabbits.

Method of concentration of extract	Fraction	% of total area of Schlieren diagram
Ammonium sulfate precipitation	I	46–51
	II	24–21
	III	24–26
Ultracentrifugal	I	53
	II	18
	III	27
Ammonium sulfate precipitation	I	54
	II	21
	III	26

B. *Comparison of particle size distribution of soluble corneal proteins from 3 species.* The sedimentation constants (each an average of 4 separate determinations) for the protein components in bovine, swine, New Zealand White and mongrel rabbit corneal extracts are shown in Table III. The preparations from mongrel rabbit corneas showed a difference in the number of protein fractions as compared to preparations from New Zealand White rabbit corneas. Only 2 definite fractions could be distinguished. The lighter fractions from both rabbit preparations showed similar sedimentation characteristics. The second fraction from the mongrel rabbit preparations, however, sedimentated at a rate significantly greater than either the second or third fractions of the New Zealand White rabbit preparations.

The bovine preparations also contained 3 components. Two of these showed sedimentation constants comparable to the second and third components of the New Zealand White rabbit preparations; the third component, however, was considerably heavier and showed the highest sedimentation constant of all components in the preparations

TABLE III. Distribution of Protein Components in Extracts of Corneas from Various Sources.

Source of tissue	$S \times 10^{13}$
NZW rabbits	3.2, 4.0, 5.9
Bovine	3.7, 6.3, 16.6
Swine	2.2, 3.3, 5.8, 11.7
Mongrel rabbits	3.0, 7.7

studied. The swine cornea preparations contained a total of 4 components, 2 of which sedimented at rates different from components in the other preparations. The above sedimentation values for the components in the mixtures cannot be taken as sedimentation coefficients of the respective components because these values were not corrected to conditions which would obtain in a theoretical medium having the density and viscosity of water at 20°C at zero protein concentration (standard conditions).

Conclusions. These studies show that there are fundamental differences in number of protein fractions and particle size distribution in extracts of water soluble corneal proteins from 3 different mammalian species. A marked difference was also noted in number of fractions and particle size distribution in preparations from New Zealand White rabbits and mongrel rabbits.

Summary. 1. Methods of concentrating soluble proteins are reported for aqueous extracts of cornea. These methods apparently do not denature the proteins. 2. These methods were applied to increase concentration of protein from aqueous extracts of bovine, swine, New Zealand White rabbit and mongrel rabbit corneas to a level which would permit ultracentrifugal analysis of these extracts. 3. Fundamental differences in number of protein fractions and particle size distribution were noted in these extracts. This suggests a species difference in soluble corneal proteins. 4. Differences were noted in sedimentation diagrams of extracts from New Zealand White rabbits and mongrel rabbits, suggesting differences in soluble corneal protein content of different strains of the same species.

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1. Krause, P. C., *Am. J. Ophthal.*, 1932, v15, 422.

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Comparative Fat and Fatty Acid Intestinal Absorption Test Utilizing Radioiodine Labeling—Results in Normal Subjects.* (22515)

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Disorders of intestinal absorption may be divided arbitrarily into two groups: (a) those with deficiency of digestive enzymes; and (b) a group with normal enzyme pattern but impaired absorption due to intestinal dysfunction. The most direct way to distinguish between (a) and (b) is by analysis of duodenal contents for enzymes(1,2). Another method, admittedly indirect, involves measurement of products of digestion and absorption in the blood. In theory, a person with a normal enzyme pattern and intestinal absorption would show a comparable response to ingestion of both unhydrolyzed and hydrolyzed substances; a person lacking enzymes but possessing normal absorptive ability should show a normal response only to the hydrolyzed form. This hypothesis has been thoroughly tested in starch-sugar(3) and in protein-amino acid(4,5) tolerance tests. We have devised a similar test for fats utilizing I^{131}-labeled fats and fatty acids. This report describes the technique and results of its use in normal human subjects.

Methods. The whole fat selected was commercial olive oil since it is not unpalatable, has a high iodine number, 79-88, and an absorption coefficient of 98. It consists of approxi-

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