Brief Notes

Sedimentation Studies of Epidermal Keratins.* Keratin A and Keratin B.

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A previous paper (1) described a fractionation method by which pulverized cornified epithelium of the human skin was divided into two main fractions: a "soluble fraction" and an "insoluble residue." From the "soluble fraction" a protein component was isolated which was found to be electrophoretically homogeneous, with an isoelectric point of pH 4.1. This component was named *keratin A*. After solubilization of the "insoluble residue" in weak alkali, a soluble epidermal keratin derivative was obtained. This was also electrophoretically homogeneous, with an isoelectric point of pH 4.1, as found for keratin A. This alkali-dissociated portion of the cornified epithelium was named *keratin B*.

For the purpose of a more extensive characterization of epidermal keratins, electrophoretically homogeneous keratin A and keratin B preparations were subjected to ultracentrifugal analyses. In this brief note, some observations are reported on the sedimentation behavior of these preparations.

EXPERIMENTAL

Keratin A and keratin B were prepared from thickened cornified epithelium of human plantar skin (calluses) in essentially the same way as described in an earlier paper (1). Keratin A was extracted from the pulverized cornified epithelium with Sørensen's standard phosphate buffer of pH 7.1 and precipitated once with 0.1 N hydrochloric acid at about pH 4.1. The precipitate was resuspended in 0.1 M glycine buffer[1] of pH 8.9 and dialyzed against 2 liters of the same buffer for 24 hours at 5°C. Subsequently the sample was cleared by high-speed centrifugation for 1 hour at 20,000 R.P.M. at 0°C.

Keratin B was prepared from the residue of the cornified epithelium remaining after extraction with Sørensen's standard phosphate buffer of pH 7.1. This residue was solubilized by shaking for 48 hours in 0.02 N sodium hydroxide. The dissolved material was precipitated once, redissolved in glycine buffer,[1] and cleared by high-speed centrifugation as described for keratin A.

The electrophoretic homogeneity of both keratin A and keratin B preparations was checked in a Perkin-Elmer Tiselius electrophoresis apparatus model 38. Electrophoresis was carried out using an E.M.F. of 200 volts and a current of 3.5 ma. with 0.1 M glycine buffer of pH 8.9 (ionic strength, 0.01) at 0°C. Photographs were taken by the scanning method at 1, 10, 20, and 30 minute intervals after the start of the experiment. Each sample revealed a single moving boundary.

A Spinco analytical ultracentrifuge, model E, was used for the sedimentation studies. It was operated at a speed of 59,100 R.P.M. at a temperature between 19 and 21°C. Sedimentation constants were calculated

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[1] Mixture of 9.0 ml. of 0.1 M glycine solution and 1.0 ml. of 0.1 N sodium hydroxide.

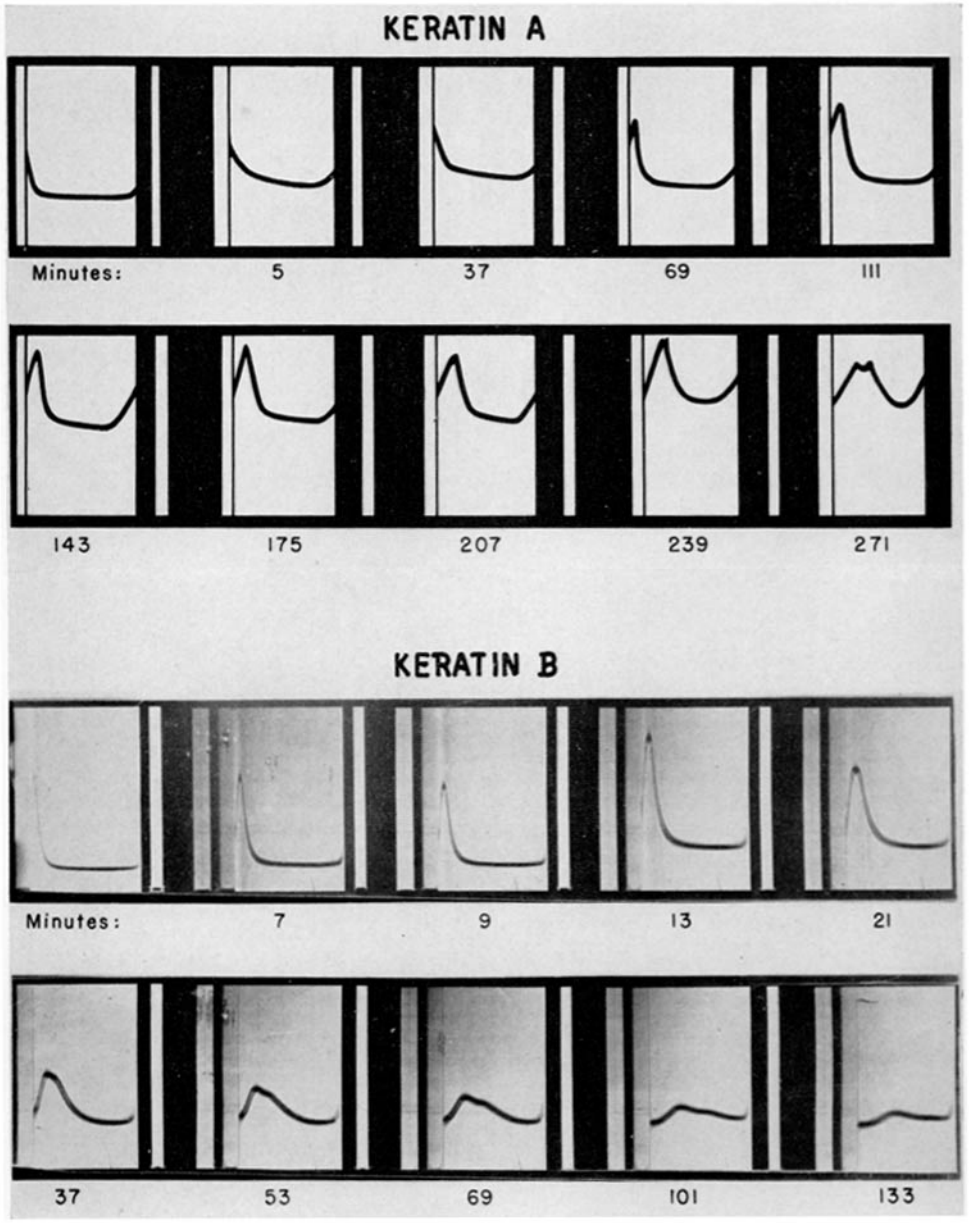

FIG. 1. Upper picture shows tracings[2] of schlieren diagrams of keratin A. Solvent: 0.1 M glycine buffer of pH 8.9. Keratin A concentration: 1.5 per cent. Lower picture shows a photograph of schlieren diagrams of keratin B. Solvent: 0.1 M glycine buffer of pH 8.9. Keratin B concentration: 1.0 per cent.

[2] Tracings are shown because the original photographs were not suitable for publication.

according to the conventional method, corrected to salt-free medium at 20°C., and expressed in Svedberg units. The relative percentage of each fraction represents the area under the corresponding curve obtained by resolving the enlarged tracings of the schlieren diagrams.

TABLE I

Data Obtained in Ultracentrifugation Studies of keratin A and keratin B

Preparation	Distribution	Sedimentation constant
	per cent	*Svedberg units*
Keratin A		
Rapidly sedimenting fraction	40.0	1.7
Slowly sedimenting fraction	42.0	1.2
Residual aliquot*	18.0	—
Keratin B		
Rapidly sedimenting fraction	35.0	3.8
Slowly sedimenting fraction	56.0	2.2
Residual aliquot*	9.0	—

* Represents the area not covered by the gaussian curves of resolved tracings.

RESULTS

The schlieren diagrams derived from ultracentrifugation of keratin A and keratin B are shown in Fig. 1. It can be seen that both preparations exhibited two maxima in the curve of refractive index gradient *versus* radius. The distribution of the rapidly and slowly sedimenting fractions, and their average sedimentation constants, are shown in Table I. From the available data, it is difficult to judge the homogeneity of each fraction. The asymmetric patterns, particularly the pattern of keratin B, suggest that each fraction is polydisperse. In the case of keratin A, it is not possible to decide how much of the spread is due to diffusion and how much is due to polydispersity.

SUMMARY

Electrophoretically homogeneous keratin A and keratin B were studied in the ultracentrifuge. Both preparations revealed two fractions: one which sedimented rapidly and another which sedimented slowly. This indicated that both preparations are heterogeneous with respect to particle size.

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