Brief Notes
Sedimentation Studies of Epidermal Keratins.* Keratin A and Keratin B. By
A. Gedion Matoltsy, M.D. From the Department of Dermatology, Harvard
Medical School, and the Dermatological Research Laboratories, Massachusetts
General Hospital, Boston.†

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 ho-
mogeneous, with an isoelectric point of pH 4.1. This component was named
keratin A. After solubilization of the "insoluble residue" in weak alkali, a sol-
uble 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 sub-
jected 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 hu-
man 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 Søren-
sen's standard phosphate buffer of pH 7.1 and precipitated once with 0.1 N hydro-
chloric acid at about pH 4.1. The precipitate was resuspended in 0.1 M glycine buffer1
of pH 8.9 and dialyzed against 2 liters of the same buffer for 24 hours at 5°C. Subse-
quently 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 Sörensen'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 centri-
fugation 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

1 Mixture of 9.0 ml. of 0.1 M glycine solu-
tion and 1.0 ml. of 0.1 N sodium hydroxide.
Fig. 1. Upper picture shows tracings 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.

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

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Distribution</th>
<th>Sedimentation constant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>Svedberg units</td>
</tr>
<tr>
<td>Keratin A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapidly sedimenting fraction</td>
<td>40.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Slowly sedimenting fraction</td>
<td>42.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Residual aliquot*</td>
<td>18.0</td>
<td>—</td>
</tr>
<tr>
<td>Keratin B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapidly sedimenting fraction</td>
<td>35.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Slowly sedimenting fraction</td>
<td>56.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Residual aliquot*</td>
<td>9.0</td>
<td>—</td>
</tr>
</tbody>
</table>

* 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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BIBLIOGRAPHY