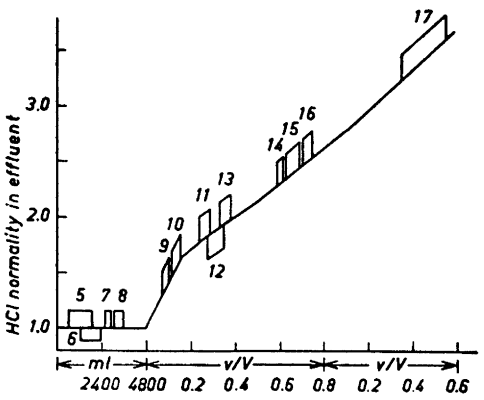

Fig. 2. Elution of the Dowex 50 column. (5) Ser; (6) Thr; (7) Gly; (8) Ala; (9) Val; (10) Pro; (11) Ileu; (12) Met; (13) Leu; (14) Cys; (15) Lys; (16) His; (17) Arg + Phe.

volume above the resin, this volume was kept at a minimum. This was achieved by application of a pressure equivalent to the difference in level between the mixing chamber and the top of the resin, *via* a side tube to the air space above the resin before the eluent was allowed to flow into the column. The flow rate was continuously kept at 108 ml/h by means of a capillary. The results are plotted in Fig. 2. Phenylalanine and arginine were separated on a cellulose column.

Purity of compounds rather than a high yield was the main aim in the separation. From Table 1 the total yield from 7 g of protein is seen to be about 40 %.

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Crystalline Leghemoglobin

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The red pigment from the soya bean root nodules was first demonstrated by Kubo [1] to have a hemoglobin nature. Ellfolk and Virtanen [2] showed that this pigment was not electrophoretically homogeneous after repeated ammonium sulphate fractionations, but contained two hemin components which seemed to occur in approximately equal concentrations. This was recently confirmed by Thorogood [3] who also found a third component in addition to the above two components and possibly a fourth.

Studying different techniques the present author has found that a cellulose ion exchange column was the most convenient one to separate these different hemin components. Diethylaminoethylcellulose according to Peterson and Sober [4] was used as adsorbent. The preparation of the individual components in this way was as follows:

After crushing the nodules, leghemoglobin was extracted twice into distilled water and fractionated once with ammonium sulphate between 55–80 % saturation (23°C). After dialysis against phosphate buffer (pH 8) and then against distilled water the protein solution was put on a cellulose ion exchange (DEAE-SF) column buffered to pH 5.2 with an acetate buffer of ionic strength 0.01 μ. All the hemin proteins remain on the top of the column as a sharp band. By elution with an acetate buffer (pH 5.2, μ = 0.01) one component moved down the column. This component was found to be the electrophoretically slowest one. Using buffer of this ionic strength the other components hardly moved at all and therefore, in order to increase the elution speed, the ionic strength of the acetate buffer was increased to 0.02 μ or 0.03 μ by addition of sodium chloride.

The second component was found to be the electrophoretically faster component. Between these two bands moved a faint, broad band which electrophoresis showed to be a mixture of several proteins with no

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distinct peaks. It evidently contained parts of the first and second components and also some oxidized component (choleglobin). This component was too small to allow any decision whether it contained a distinct new fraction or not. After the second band a fourth small band was eluted, which separated distinctly and the mobility of which in electrophoresis was found to be slightly higher than that of the main fast component. All the spectra of these three components showed the acid hemiglobin nature of the components.

On the top of the column there still remained a brown band which did not move though the ionic strength was increased. It was partially eluted by 3 M NaCl and its spectrum did not show any maxima or minima but the absorption increased steadily toward the ultraviolet. This component was therefore assumed to be a denaturation product of the hemin proteins.

The two main components were homogeneous in the ultracentrifuge and their sedimentation constants were the same, about 1.9 *S*. They were also homogeneous in long run electrophoresis at two different pH values, *i. e.* pH 6.1 and 9.0. The iron content of the two fractions was slightly different, the electrophoretically faster one had an iron content of 0.320 % and the electrophoretically slower one a value of 0.287 % of iron per dry weight. The diffusion coefficient $D_{20°}$ of the fast component was found to be about 11.1 *F* and that of the slower one about 8.5 *F*. On the basis of iron content the fast component has a molecular weight of 17 500 and the slower one 19 500. It is evident that the faster component is not far from spherical in shape, whereas the slower one is somewhat assymetric or highly hydrated.

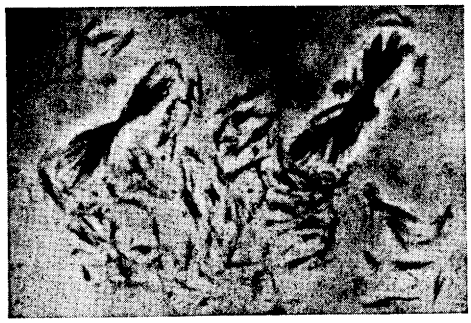

Fig. 1. Crystalline leghemoglobin, faster component, 1 130 ×.

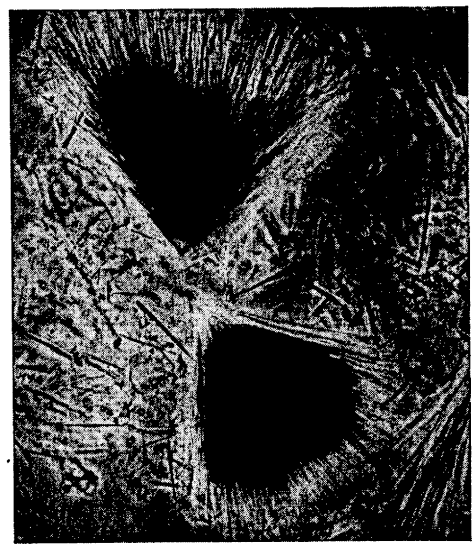

Fig. 2. Crystalline leghemoglobin, slower component, 230 ×.

Ellfolk and Virtanen [2] assumed that the slower component was not a homogeneous protein but probably an artefact of the faster component adsorbed to a neutral or basic protein. From these findings it is evident that this assumption was not correct.

Both the components were found to crystallize from ammonium sulphate solutions, 0.65 saturation, at slightly acid pH (4.8 and 4.7). Fig. 1 shows the crystals of the faster component and Fig. 2 those of the slower one.

A detailed report on these findings will be given in this journal.

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