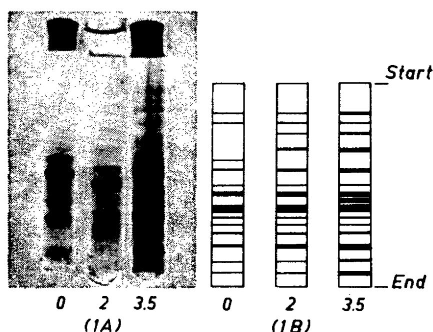

Fig. 2. Fractionation of *Escherichia coli* proteins with disc electrophoresis. On the left are the photos from the separated bands (1A) and on the right a visual reading of original bands (1B). Samples were the same as in Fig.1.

better resolution of bands than starch gel electrophoresis. Distinct differences can be seen in the protein bands using this method. The sample harvested in the retardation phase contains more bands than the previous samples. Upon visual inspection 16 bands are observed, especially high is the appearance of new bands at both ends of the electropherogram. A similar phenomenon is observed in Fig. 1, where the resolution conditions were similar.

The results obtained in the gel filtration experiments are presented in Fig. 3,

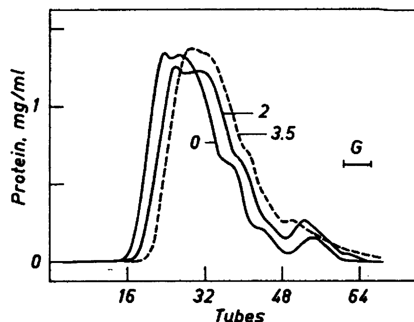

Fig. 3. Molecular sieving of proteins of *Escherichia coli* cells harvested from the active growth phases of growth. 2-ml fractions were collected and protein was estimated. G = tubes containing D-glucose. It was added to the samples before fractionation and tested by the use of Test-tape papers.

where Sephadex G 100 Superfine gel was used. No remarkable differences were found using this method. This result favors the opinion that no remarkable differences are found in molecular weights of the protein components.

The following conclusion can be drawn from the above results: during the active growth phases of *E. coli* remarkable differences can be found in protein composition in certain bands after electrophoretic separation. However, these differences are not reflected in the molecular weight spectrum obtained with the molecular sieve method.

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Molecular Weight of the Albumin Fraction of Barley

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In textbooks the molecular weight of barley albumin is still stated to be 35 000. To any one working on barley proteins, however, it is evident that this cannot be correct, as the water-soluble protein fraction of seeds is very heterogeneous. The heterogeneity of barley albumin has been confirmed by a great number of workers, using modern methods such as ultracentrifugation,[1] various types of electrophoresis, [2-7] chromatography on DEAE cellulose,[8] and exclusion chromatography on Sephadex.[9,10]

Our present investigation on sedimentation coefficients of barley albumins is based on a preliminary fractionation using a Sephadex G-100 column. The albumins were extracted with distilled water, precipitated at the concentration interval of 15–40 % saturation of ammonium sulphate and thereafter subjected to chromatography on Sephadex G-100. The albumin fractions obtained were collected and further analysed in the ultracentrifuge.

The interval of 15–40 % saturation was chosen for the preparation because of the definition of barley albumin used by Quensel,[11] who proved that the albumins are precipitated from the salt extracts of barley at this interval of ammonium sulphate concentration. In the present work the extraction was performed with water, however, in order to avoid the interfering presence of globulins in the solutions.

Experimental. 200 g of acetone-treated barley (Balder) was extracted with 400 ml of distilled water for 1 h at +4°C. Ammonium sulphate was added to the centrifuged extract to give a concentration of 70 % of the saturation concentration. The precipitate obtained was dissolved in 200 ml of water and the undissolved polysaccharides removed by centrifugation. Precipitation was repeated and the precipitate dissolved in 50 ml of distilled water, centrifuged and purified by gel filtration on a water-packed Sephadex G-25 column (Fig. 1). Elution was performed as described earlier.[12] The albumins were thereafter precipitated at the concentration interval of 15–40 % saturation with ammonium sulphate. The precipitate was dissolved in 6 ml of 0.010 M sodium phosphate buffer (pH 6.6) containing 2.5 % of sodium chloride. The proteins were then further fractionated on a Sephadex G-100 column (Fig. 2). The albumin fractions obtained were collected as indicated in the drawings. The final chromatography on Sephadex G-100 was highly reproducible. The corresponding fractions from three runs (600 g of barley) were combined and dialysed against a phosphate buffer (0.2 M sodium chloride, 0.01 M phosphate, pH 7.3, ionic strength 0.23).

Every dialysed albumin fraction was examined in the Spinco model E analytical ultracentrifuge at 59 780 rpm at 19–21°C, a wedge cell being used for the simultaneous running of two samples. Every fraction was studied at two concentrations, using the original concentration and a dilution of 1:2.

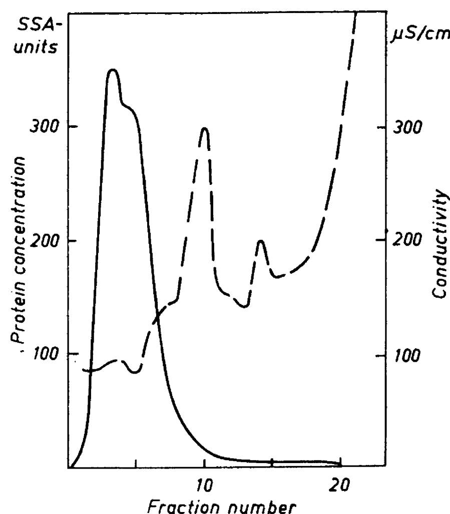

Fig. 1. Purification of barley albumins on Sephadex G-25 packed in water. Column size 4 × 66 cm. Fraction size 14 ml.

——— = Protein concentration;
– – – = conductivity.

The sedimentation coefficients of these fractions were calculated according to the conventional methods. The observed sedimentation coefficient was corrected to the value it

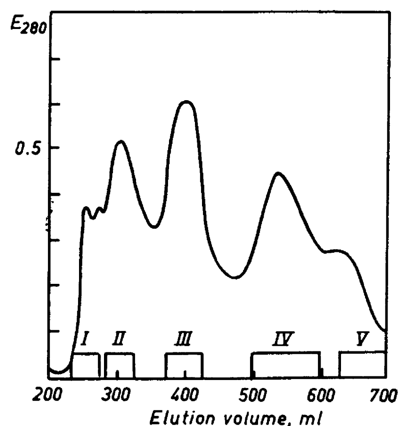

Fig. 2. Fractionation of barley albumins on Sephadex G-100 packed in 2.5 M sodium chloride solution buffered to pH 6.6. Column size 4 × 65 cm.

would have in a solvent having the density and viscosity of water at 20°C. This corrected value ($S_{20,w}$) was extrapolated to infinite dilution in order to obtain $S_{20,w}°$. The sedimentation coefficients are given in Svedberg units (S).

The diffusion coefficients were measured by the immunodiffusion technique.[13]

Results and discussion. The sedimentation coefficients of the barley albumin fractions at infinite dilution ($S_{20,w}°$) are given in Table 1.

Table 1. Sedimentation coefficients of barley albumin fractions.

Albumin fractions	$S_{20,w}°$
I	7.9 S
II	6.1 S
III	4.7 S
IV	2.8 S
V	1.6 S

The boundaries of all the albumin fractions except fraction I were symmetrical throughout the run. The boundary of fraction I was slightly skewed, so that we can suspect the presence of faster-moving material in this fraction (Fig. 3).

The diffusion coefficients ($D_{20,w}$) of fractions I and V were found to be 2.75×10^{-7} and 11.4×10^{-7} cm^2 s^{-1}. When molecular weights are calculated (according to Svedberg's equation, using the value of 0.75 cm^3/g for the partial specific volume) for these two fractions, *i.e.* the fractions having the highest and the lowest molecular weight, they are found to be 280 000 and 13 700. Fraction I, however, contains the largest β-amylase component which according to our earlier experiments [14] has a tendency to dissociate. This phenomenon during the lengthy analytical procedures may cause errors in the evaluation of the molecular weight of this labile fraction. It can be concluded that the albumin of barley consists of a large number of different proteins having molecular weights between 13 000 and 300 000.

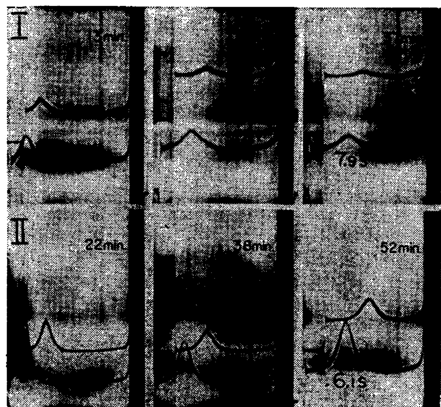

Fig. 3. Sedimentation patterns of barley albumin fractions I and II. The times given are minutes after reaching full speed.

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