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European Research Office (9851 DU)
U.S. Department of the Army
Frankfurt am Main, Germany
APO 757, US Forces

FTR No. 3, May 31st 1961

Subject of Research: Investigations of the methods for separating biologically and medically important substances of large molecular weight

Contractor: Institute of Biochemistry, Uppsala, Sweden

Statement: The research reported in this document has been sponsored in part by the Office, Chief of Research and Development, U.S. Department of Army, through its European Office under contract number DA-91-591-EUC-1462

Institute of Biochemistry, Uppsala, Sweden/A. Tiselius.
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Institute of Biochemistry, Uppsala, Sweden / A. Tiselius
Summary of the reported work.

The present report is to be considered as a direct continuation of FTR No. 2, May 30th 1960, (Contract number DA-91-591-ECU-1025).

The material has been grouped in the following main sections:

1. **Separation methods based upon partition in two-phase systems containing water soluble polymers** (Dr. P. A. Albertsson and collaborators)

2. **Separation methods based upon electrophoresis**
   Drs. J. Porath, S. Hjertén and collaborators

3. **Separation methods based upon chromatography and similar procedures (molecular sieving)** (Drs. J. Porath, S. Hjertén and collaborators)

4. **Separation methods based upon stratification by density difference in packed ultracentrifugal sediments.** (Dr. Ö Levin and collaborators)

Although the main part of the research work has been directed towards the development and improvement of separation methods this has been done with much attention to possible applications and many such examples are reported, for example new possibilities of virus purification, separation of bacteria and other microbiological materials, etc. Some procedures which originally were introduced chiefly for particle separation have been extended also to smaller molecules, whereas other procedures which have their main application in the low molecularweight field have now been modified to be useful also with large molecules and particles. In both cases a surprisingly high degree of specificity has often been observed. It is believed that the new methods for the separation of biological particles, being both highly specific and comparatively gentle (non-distractive) will prove of value not only for preparative purposes but also as an approach to the study of the micro-structure of biological materials of all kinds.
1. Two-phase partition

The investigations of Albertsson and his collaborators form the most extensive part of the project under the contract. A detailed report covering the results obtained up to November 1960 was published as Dr. Albertsson's thesis and was submitted in QTSR number 2. Further copies are available on request. The monograph is also distributed by John Wiley and Sons, Publishers, New York. References are given in this publication also to a number of applications of the method by Albertsson and by other authors.

Recently Albertsson and Baird (from the University of Cambridge, England, guest of the Institute) have carried out fractionation experiments with various microorganisms using aqueous two-phase systems. It has been shown that many different microorganisms may be separated from each other by one single distribution step. When different, but closely related strains, of the bacterium Escherichia coli has been tested a partial separation is obtained by one single distribution. However, by applying a multistep procedure such as the liquid-interface counter current distribution (see the thesis by Albertsson, page 172) it is possible to fully separate even very closely related organisms. Thus Fig. 1 shows a separation of a mixture of the two strains K 12 Hfr and ML 3081 of Escherichia coli. The lower curve of Fig. 1 shows the extinction in each tube and the upper curve shows the number of colonies of the strains in each tube. The two strains could be distinguished by the fact that the one, K 12 Hfr, is Lac+ and that the other ML 3081 is Lac−.

As a whole the introduction of multi-step procedures in this type of separation (counter-current distribution) has been one of the main points in our most recent work. It has been possible in this way to increase the resolution quite considerably and it is interesting that counter-current procedures thus can be applied also to whole cells.
cell fragments, bacteria and other biological particles.

The following is a survey by Albertsson of the present results, as quoted from the summary in the above mentioned monograph, p. 228-229:

The distribution of various cell particles and biological macromolecules in aqueous polymer two-phase systems is described. The particles studied include whole cells, microsomes, bacteriophages, and viruses and the macromolecules studied include proteins in the molecular weight range 50 000 - 10 000 000 and nucleic acids.

The purpose of this study has been to develop partition methods for characterisation and fractionation of biological particles and macromolecules.

The phase systems are constructed by mixing aqueous solutions of two different polymers. A large number of such phase systems have been analyzed in detail; the phase compositions and phase diagrams are given in Chapter 2. The polymers include both non-ionic polymers and polyelectrolytes.

The distribution of a particle depends on the nature and size of its surface and the nature of the phase system. Theoretically, it can be shown that the larger the particle size the more one-sided the distribution. It can also be shown that adsorption of particles at the interface is favoured the larger the particle size and the larger the interfacial tension between the phases.

The experimental results are in qualitative agreements with those expected from theory. Thus, in polymer-polymer two-phase systems, smaller molecules, like the smaller protein molecules, partition evenly between the phases, while larger molecules or particles such as viruses and highly polymerized nucleic acids collect predominantly in one of the phases or at the interface. A relation between the surface area and the partition coefficient of proteins and viruses in a dextran-methyl
Fig. 1. Separation of two strains of the bacterium *Escherichia coli* by counter current distribution. The lower curve shows the extinction times the volume in each tube. The upper curve shows the number of colonies in each tube. The one strain, K12 Hfr, is Lac\(^+\) (\(\ominus\) - \(\Theta\) -) and the other strain, ML3081, is Lac\(^-\) (\(\ominus\) - \(\Theta\) -).
The phase systems have been used for fractionation of rat brain microsomes and for countercurrent distribution of proteins. The partition of a protein follows the Nernst partition law, that is its partition coefficient is independent of protein concentration and the presence of other proteins. The value of the partition coefficient may thus be used for the characterization of a protein. Considerable fractionation of protein mixtures may be achieved by countercurrent distribution using a dextran-polyethylene glycol system with a suitable salt content.

The phase systems have also been applied for concentration and purification of viruses. By adding polymers in certain proportions to a virus culture, the virus activity may be almost completely transferred to a small volume phase. This procedure can be carried out in several steps. Thus, a virus activity may be concentrated as much as $10000$ times. Other substances than viruses, such as proteins and cell fragments, distribute in a different manner in the phase system; a purification of virus is therefore also obtained.

The viruses tested include bacteriophages and a large number of animal viruses. Methods for the preparation of almost pure phage T2 and Echo virus are described.

The distribution of antigen-antibody mixtures in two-phase systems is described. The results show that an increased sensitivity in measuring an immunological reaction in the antigen excess can be achieved by two-phase systems.
2. Electrophoresis

The optimal conditions for the electrophoretic separation of mono- and oligosaccharides have been studied by Drs. J. Porath and B. Waligora, using zone-column type of apparatus described in earlier reports and borate or germane buffers. The resolution is satisfactory and it is interesting that by use of micro-columns, the method can be used as an analytical procedure.

The results will be published in Journal of Chromatography.

Also an extensive investigation on electrophoresis in gels has been made, chiefly by Dr. S. Hjertén and his collaborators. It has been shown that agarose, a component of common agar, is much superior to agar as a medium. This is particularly important in so-called immunoelectrophoresis. A description of the procedure will appear in Biochimica et Biophysica Acta.

Suspensions of certain weak gels (e.g. agar or agarose) are convenient media also for separation of very large molecules and particles, as shown by Hjertén. Hjertén has further improved his revolving tube apparatus for free zone electrophoresis, especially with regard to the optical observation methods (see also FTR No. 2 pp. 15-17).

3. Chromatography

Much attention has been given to various applications of the new "Sephadex" materials, which may act as molecular sieves in the separation of substances of molecular weights up to about 100,000. So far the results have been particularly useful with polypeptides and similar substances.

Thus Dr. Porath (in collaboration with Miss E. B. Lindner) has developed methods for fractionation of low molecular weight peptides. They are based on molecular sieving in Cross-linked
dextran gels (Sephadexes) and ought to find application in other fields.

Separation of tripeptides or even higher peptides from amino acids is not easily achieved in Sephadex when ordinary aqueous media are used. One of the new methods is based on restricted swelling of the gel substance in mixed organic solvents.

Another method utilizes both molecular sieving and ion exclusion and permits rapid fractionation of substances which differ in charge but not in molecular weight or vice versa. Presumably, it should be well suited both for analytical applications and for large scale fractionations.

Similarly Hjertén and Mosbach have examined the possibilities of using a bed of cross-linked polyacrylamide for chromatography of proteins by one-step elution. A large number of proteins have been chromatographed on this polymer, and these experiments show that there is a clear correlation between the molecular weight of the proteins and their emergence from the column. In this respect a column of cross-linked polyacrylamide resembles one of starch /G.H. Lethe and C.R.J. Ruthven, Biochem. J., 62, 665 (1956)/ or "Sephadex /J. Porath and P. Flodin, Nature, 183, 1657 (1959/). An important difference is, however, that polyacrylamide has the ability to separate substances of much higher molecular weights, for instance, phycoerythrin (m.w. 290 000) is characterized by an $R_f$-value of 0.9 when chromatographed on a column packed with this polymer. Table 1 demonstrates the relation between $R_f$-values of a substance and its molecular weight.
Table 1.

The $R_f$-values of some substances chromatographed on cross-linked polyacrylamide.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Molecular weight</th>
<th>$R_f$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phycoerythrin</td>
<td>290,000</td>
<td>0.9</td>
</tr>
<tr>
<td>Phycocyanin</td>
<td>135,000</td>
<td>0.8</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>68,000</td>
<td>0.6</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>31,000</td>
<td>0.5</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>13,000</td>
<td>0.4</td>
</tr>
<tr>
<td>DNP-aspartic acid</td>
<td>285</td>
<td>0.3</td>
</tr>
<tr>
<td>Naphtol green</td>
<td>253</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Fig. 2 shows a separation of laccase (m.w. 57,000) and carbonic anhydrase (m.w. 31,000). The latter enzyme was labelled with $^{65}$Zn. This experiment was performed on a column with the dimensions 1.5 x 22 cm. The volume of the sample was 1 ml., and the total amount applied was about 7 mg. The chromatogram was developed with 0.05 M sodium phosphate buffer pH 7.3. The flow rate was 1.8 ml/h. and fractions of about 0.3 ml. were collected. The laccase was localized by enzymatic and the carbonic anhydrase by radioactive measurements.

In an earlier report Hjerten showed the usefulness of a suspension of agarose as anticonvection agent in zone electrophoresis. He has also used beds of such suspensions for chromatography of cell particles and high molecular weight substances. A relation between molecular weight and $R_f$-value, similar to that obtained for cross-linked polyacrylamide is valid also for agarose suspensions, though these suspensions show their maximal resolution for substances of much higher molecular weights. Thus phycoerythrin migrates on a column filled with agarose suspension
with an $R_f$-value of 0.6, while the corresponding $R_f$-value on a column of polyacrylamide is 0.9. That even cell particles can be fractionated on these columns is evident from Fig. 3, which shows the chromatographic behaviour of microsomes from E. coli bacteria.

About 1 ml. of the sample was filtered into a column (1.5 x 22 cm) of 1% agarose suspension. The elution was performed with 0.01 M TRIS-HCl buffer, pH 7.2 containing 0.001 M Magnesium sulfate. The first peak corresponds to material which is strongly opalescent and therefore probably contains comparatively very large particles; it is devoid of absorption maximum in the region 200-300 μm, while the fractions of the main peak show a strong absorption at 260 μm.

It appears particularly important that in these new methods of chromatography of proteins and other large molecular weight substances it is possible to elute in a medium of constant composition thus avoiding the step-wise or gradient elution which is normally necessary in earlier methods.

4. Stratification (Dr. Ö. Levin)

This method of particle separation is based upon mutual displacement of particles of different densities in packed ultracentrifugal sediments. The main principles were described in FTR No 2 pp. 35-37. The following are some results obtained during the last year, as reported by Dr. Levin.

**Displacement effects in centrifugation of particles of the same density.**

Two samples of polystyrene latex particles have been used for studies of the displacement effect between chemically identical substances. One of the samples had a particle diameter of 880 Å, while the diameter of the other was 1380 Å. Both samples sedimented in the analytical ultracentrifuge with a single sharp boundary. The sedimentation coefficients were 98 and 219 s respectively.
Fig. 3

Optical density at 260nm
Fig. 4

The shape of a zone of phycoerythrin after 21.5 hours at 25,000 r.p.m. in rotor SW 25.
0.90 ml of a 1% solution was put on the tube.
Separation of erythrocrurin from breakdown products, 120 minutes at 30,000 r.p.m. in rotor SW 39. Six mg in a volume of 0.20 ml was put on.
Centrifugation of 70 s ribosomes from E. coli. 120 minutes at 25,000 r.p.m. in rotor SW 25. 0.60 ml of a 2 % suspension was put on.
The following amounts of these two suspensions were mixed with each other:

- 0.40 ml 880 Å with 0.40 ml 1380 Å
- 0.16 ml 880 Å with 0.64 ml 1380 Å
- 0.08 ml 880 Å with 0.72 ml 1380 Å

These mixtures were placed in the three tubes of rotor SW 39 together with 2.0 ml of water. The tubes were fitted with liners having a radius of curvature of 51 mm. The rotor was spun at 15,000 RPM for 300 minutes. After this time all the material had sedimented.

As both suspensions contained 10% solids, the total amount in each tube was 80 mg.

The sediments were cut into about 10 sections, which were suspended in water. A drop of these suspensions was placed on an electron microscope grid, a picture was taken in the microscope, and from the prints the number of 1380 Å and 880 Å particles were estimated. A small portion had been withdrawn from the starting suspensions before centrifugation, and were investigated in the same way. The results are given in the following tables.

<table>
<thead>
<tr>
<th>Tube I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section nr</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>Starting suspension</td>
</tr>
</tbody>
</table>
### Tube II

<table>
<thead>
<tr>
<th>Section nr</th>
<th>% 1380 Å particles</th>
<th>% 880 Å particles</th>
<th>Nr of particles counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>82.7</td>
<td>17.3</td>
<td>209</td>
</tr>
<tr>
<td>4</td>
<td>87.7</td>
<td>12.3</td>
<td>603</td>
</tr>
<tr>
<td>5</td>
<td>80.7</td>
<td>19.3</td>
<td>155</td>
</tr>
<tr>
<td>6</td>
<td>80.6</td>
<td>19.4</td>
<td>170</td>
</tr>
<tr>
<td>7</td>
<td>86.1</td>
<td>13.9</td>
<td>43</td>
</tr>
<tr>
<td>8</td>
<td>86.1</td>
<td>13.9</td>
<td>326</td>
</tr>
<tr>
<td>9</td>
<td>Too much aggregates to allow any conclusions.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Starting suspension 50.8  49.2  621

### Tube III

<table>
<thead>
<tr>
<th>Section nr</th>
<th>% 1380 Å particles</th>
<th>% 880 Å particles</th>
<th>Nr of particles counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>27.6</td>
<td>72.4</td>
<td>347</td>
</tr>
<tr>
<td>3</td>
<td>90.8</td>
<td>9.2</td>
<td>329</td>
</tr>
<tr>
<td>4</td>
<td>92.6</td>
<td>7.4</td>
<td>421</td>
</tr>
<tr>
<td>5</td>
<td>87.3</td>
<td>12.7</td>
<td>448</td>
</tr>
<tr>
<td>6</td>
<td>91.2</td>
<td>8.8</td>
<td>439</td>
</tr>
<tr>
<td>7-10</td>
<td>Contained aggregates.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Starting suspension 66.1  33.9  278

**Discussion of the experiment.** When the sedimentation coefficients of the two components in the suspension, and their relative amounts are known, the composition of the sediment may be calculated if ideal sedimentation is assumed.

Assuming the cross section of the tube to be $a$, the concentration of the 880 Å latex particles to be $c_1$ and their sedimentation coefficient to be $s_1$, the number of particles $dn_1$ sedimented during the time interval $dt$, is

$$dn_1 = c_1 \cdot s_1 \cdot dt \cdot a.$$
Denoting the $1380\,\AA$ particles with subscript $2$, one obtains for the same reasons
\[ \frac{dn_1}{dn_2} = \frac{c_1 \cdot s_1}{c_2 \cdot s_2} \cdot dt \cdot a. \]

The composition of the sediment formed during the time interval $dt$ is given by the ratio
\[ \frac{dn_1}{dn_2} = \frac{c_1 \cdot s_1}{c_2 \cdot s_2}, \]
and is obviously constant until one of the particle species is completely sedimented.

If these equations are applied for calculation of the compositions of the sediments in the centrifuge tubes, one obtains the following results.

**Tube I:** \[ \frac{dn_1}{dn_2} = \frac{81.2}{18.8} \cdot \frac{98}{219} = 1.931, \]
which means that the sediment should consist of 66.0% 880\,\AA particles and 34.0% 1380\,\AA particles.

**Tube II:** \[ \frac{dn_1}{dn_2} = \frac{49.2}{56.8} \cdot \frac{98}{219} = 0.433, \]
which means 30.3% 880\,\AA particles and 69.7% 1380\,\AA particles.

**Tube III:** \[ \frac{dn_1}{dn_2} = \frac{33.9}{66.1} \cdot \frac{98}{219} = 0.229, \]
which means 18.6% 880\,\AA particles and 81.4% 1380\,\AA particles.

There is a great difference between the calculated amounts of 880\,\AA particles in the lower part of the sediments, and the amounts found. Instead of the calculated 66.0%, the last sections of tube I contains only a little more than 30%. For tube II and III these figures are 30.3 vs. 14%, and 18.6 vs. 10%.

In earlier reports it has been reported about stratification of cellular and subcellular components. Then it was found that each stratum consisted of one particle species. In the experiments described here this is not the case, but the differences between the calculated and the actually found amounts of 880\,\AA particles in the 1380\,\AA particles sediment are so great that one must assume some sort of displacement effect also between particles of the same density.
The difficulties usually met with in zone centrifugation in ordinary density gradient columns are well known. They consist of the appearance of swirling motions caused by the shearing forces perpendicular to the radial ones during acceleration and deceleration, and of the so-called droplet sedimentation.

The swirling motion, of course, causes convective mass transport. During acceleration it disturbs the initial zone on top of the column, during deceleration it tends to sweep out the separation obtained.

The droplet sedimentation depends on the difference in diffusion velocity of the low molecular weight substance used for formation of the gradient, and the high molecular weight substances in the zone. The gradient forming substance diffuses more rapidly into the protein zone than the protein diffuses out of it. Eventually one gets a higher density in this zone than in the liquid layer just below it, and at this point the lower edge of the zone starts sedimenting in the form of small drops.

The disturbing forces acting are much smaller than the centrifugal force, and may be eliminated simply by filling the tube with a stabilizing medium of the same kind as that used in zone electrophoresis experiments, together with the gradient forming solutions. These packed columns are very easy to handle, and also seem to stabilize rather large amounts of material. In the following a description of the application of such "doubly stabilized" tubes to zone centrifugation will be given.

Packing materials. Several packing materials have been tried, e.g. plastic beads, glass beads, starch from different sources, and cellulose powder. Of these materials, plastic and glass beads gave a too small void volume, and did not give sufficient stabilization. Cellulose
powder showed elastic compression and was discarded. Of the
different starches investigated unhydrolyzed potato starch gave the
best result. The void volume is between 35 and 40 % and the
elasticity is negligible even at 40,000 R. P. M., when the tube has
been properly packed.

**Packing of the centrifuge tubes.** The following sucrose solutions have
been used for creating the density gradient: 15 %, 20 %, 25 %, 30 %,
35 %, and 40 % w/v. The solutions also contain the buffer salts.
Twentyfour ml of these solutions is mixed with 20 g of starch, so that
6 starch pastes with different sugar concentrations are obtained. The
pastes are filled into the centrifuge tubes with the paste having the
highest sugar concentration at the bottom and the one with the lowest
at the top. Each portion shall occupy about one sixth of the tube if
linear density gradient is desired.

The tubes are placed in rotor SW 25 and centrifuged in Spinco
Model L ultracentrifuge at 25,000 R. P. M. for a few minutes. The
liquid squeezed out of the starch column is sucked off and replaced by
starch paste with the lowest sugar concentration. The tubes are then
centrifuged for 30 minutes and the liquid above the starch column is
removed. On top of the column are then placed two or three thick
sheets of cellulose, and the tubes are centrifuged once more for
30 minutes. After removing the cellulose sheets the sample may be
placed on top of the tubes and centrifugation started.

When rotor SW 39 is used and operated at 40,000 R. P. M., the
compression is more pronounced. For this reason it has usually been
necessary to introduce fresh starch paste twice, and also to repeat
the centrifugation with cellulose sheets once more. In addition it has
been preferable to use a lower speed than 40,000 R. P. M. during the
very first run, in order to obtain a continuous starch column.

After this packing procedure the compression with concomitant
back streaming of liquid is very small. Usually the back streaming
amounts to about 0.1 ml, when a 5 ml tube is centrifuged in rotor SW 39 at speeds between 35,000 and 40,000 R.P.M. When rotor SW 25 is used it is proportionally smaller.

**Elution.** Elution is carried out in the same way as by starch block electrophoresis. The tubes are first cut into two halves and the starch columns are sectioned with a razor blade into slices of a few mm thickness. In the first experiments these sections were weighed before elution. The ordinate in fig. 2 is therefore given as amount of protein per g of starch slab. This procedure is, however, unnecessarily tedious and weighing can usually be omitted. Some scattering of the points occurs, however, depending on the difficulty to obtain sections of exactly the same thickness.

**Capacity and recovery.** The capacity of each tube of rotor SW 39 was investigated with hemoglobin. When 3 mg was put on the tube, 90% of it was recovered after 23 hours at 36,000 R.P.M. in 5 sections out of 26. This equals a zone broadness of 1 cm. If, instead, 9 mg was put on, 90% was recovered in 8 sections, which means a zone broadness of 1.6 cm.

The concentration of hemoglobin in the sample put on the column has been varied from 1% to 18%, without affecting the stability of the migrating zone.

The recovery of material from the starch column is usually quantitative, even when such large particles as ribosomes from E. coli are centrifuged.

**Shape of the zone.** Zone centrifugation is not carried out in sectorial but in cylindrical tubes, which gives rise to an increased number of wall impacts by the sedimenting molecules. These impacts favour the formation of clusters of molecules. The density of a volume element containing these clusters may be higher than the density of
the surrounding liquid, causing transport by convection instead of sedimentation.

This effect may be easily studied when centrifugation is carried out in packed tubes, and is illustrated in fig. 4. As is seen, the front of the zone is curved, and the parts of the zone situated closest to the walls are the leading ones. If the sedimentation had taken place in an ideal way, these parts should have been the trailing ones, situated a small distance behind the front of the zone at the centre of the tube. This indicates a rather great disturbance of the migration due to the cell wall impacts.

Some systems investigated. Erythrocrue irin. This strongly coloured protein has a sedimentation coefficient of 58 s, but it easily breaks down to smaller units. A separation of intact erythrocrue irin from breakdown products with a sedimentation coefficient of 16 s is shown in fig. 5. Six mg of protein in a volume of 0.20 ml was put on a tube to rotor SW 39 and run at 30.000 R. P. M. for 120 min. A good separation was obtained.

Phycoerythrin - phycocyanin. These proteins were investigated in phosphate buffer of pH 7, where the sedimentation coefficient is 12.0 s for phycoerythrin and 6.2 s for phycocyanin. They were separated from each other after 300 min. at 36.000 R. P. M. In addition to the ordinary proteins a zone with the colour of phycoerythrin was found to sediment more slowly than phycocyanin. This zone certainly consists of split products with sedimentation coefficients around 3 s.

Ceruloplasmin - albumin. The sedimentation coefficients of these proteins are 7.1 and 4.6 s respectively. In order to make the albumin easily visible it was conjugated with fluorescein. Experiments were carried out with total amounts of protein between six and nine mg per tube. The rotor was run at 35.000 R. P. M. for 17 hours and 20 min. Complete separation was not obtained but the front was always free
from albumin and the back was free from ceruloplasmin, as far as could be judged by colour.

Ribosomes from E. coli. These particles have a sedimentation coefficient of 70 s, but may be dissociated into subunits which sediment at 50 and 30 s. 0.60 ml of a 2% suspension of 70 s particles was centrifuged at 25,000 R. P. M. for 120 min. in SW 25. The result is shown in fig. 6. When the dissociation products were investigated, only a small amount could be eluted from the starch column.

The experiments by Dr. Levin have quite recently given some very interesting results, and as the present report is a final one it was considered desirable to describe this work in some detail.

Implication of the results

I would like to quote the following from FTR Number 2, p. 40, which is valid also for the results obtained during the last year:

"The present report gives a number of specific applications of the methods developed or improved, which may serve to illustrate their usefulness. The demand for highly specific but very gentle separation methods in biochemistry is increasing rapidly and there are a number of problems which for their study are highly dependent upon such methods. Recent examples are (to mention only a few) genetic differences as reflected by differences in specific proteins, and the increasing attention paid to submicroscopical particles in connection with the fundamental problem of protein biosynthesis. Naturally to almost any of the more classical problems in biochemistry and to many problems in basic medical research methods of the type studied are of key importance. Thus it seems probable that the particle separation methods described here will become of practical importance e.g. in the production of viruses for vaccination purposes. Also the two-phase method may become of importance in plasma fractionation because of the ease with which it can be per-
formed even on a very large scale."

The material presented in the present report further strengthens these points. It is obvious that we have not yet fully utilized the possibilities of developing highly specific separation methods, based upon simple physicochemical phenomena, and applicable to biological systems. The possible applications are manifold and only a small fraction of them has been explored.

Summary of personnel utilized during the period.

Research Associates
Dr. P. Å. Albertsson
Dr. J. Porath

Research Assistants
Dr. S. Hjertén
Dr. Ö. Levin

Technical Personnel
Chief Technician        Mr. T. Dahm
Instrument maker       Mr. L. Eriksson
Laboratory assistans   Miss E. Nolander
                       Miss B. Brantefors
Laboratory attendants  Mr. S. Lövgren
                       Mr. L. Ling

Office etc.
Mrs. M. Rudberg
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<th>Man hours</th>
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Uppsala, June 11, 1961

Institute of Biochemistry

Arne Tiselius
Director
Professor of Biochemistry
According to the contract DA-91-591-EUC-1462, Modification No. 1, April 19, 1960, the principal investigator Prof. A. Tiselius was to undertake a visit to the U.S.A. in order "to discuss the results and applications of the research done under the subject contract".

The following institutes were visited:

- **Sept. 29, 1960 New York.** Visit Rockefeller Institute, Drs. Moore, Stein, Craig, Kunkel.
- **Oct. 1st.**
- **Oct. 3.** Washington Lecture at the National Academy of Sciences on invitation by President Bronk. Dinner at the Cosmos Club.
- **Oct. 4.** " Visit Walter Reed Institute (Col. Mason, Dr. Hahn, Dr. Knoblock and others). Dinner at the invitation of General Trudeau. Discussions also with Dr. Weiss of the Army Research Office.
- **Oct. 5.** " Visit Fort Detrick. (Dr. Maxfield and several others)
- **Oct. 6.** " Visit National Institutes of Health (Drs. Anfinsen, Sober, Peterson, Saroff and others).
  Visit Naval Research Laboratories (Drs. Friess, Benzinger) Discussions also with Drs. Frederick Stone and Carl R. Brewer.

Oct. 8. New York Visit Rockefeller Institute (Drs. Longsworth, Shedlovsky, McLnnes)

Oct. 10 " Rockefeller Institute (Dr. Lipmann)
Lecture at the Rockefeller Inst.

Oct. 11. Indianapolis Visit and lecture at the Eli Lilly's laboratories (Dr. Davis and others)


Oct. 13 Natick (Mass.) Visit Natick. (Dr. Sieling, Col. Whitney, Dr. Cole, Dr. Hasselstrom, Dr. Long and others).
Lecture at the Organic-Chemical Symposium.


Oct. 15. Leave Boston for Stockholm

Total expenses in connection with the above travel: $1,220
(as stated in contract).

Uppsala June 10, 1961

Institute of Biochemistry

Arne Tiselius
Director
Prof. of Biochemistry

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