The Ultracentrifuge and Its Applications.

By The Svedberg.
(*Fysikalisk-Kemiska Institutionen, Upsala.*)

ZSIGMONDY'S monograph "Zur Erkenntnis der Kolloide" which appeared in 1905 is to be looked upon as one of the mile-stones along the road of advance in colloid science. In this book was collected the experimental material showing conclusively that a great number of apparently homogeneous pseudosolutions or colloids are really heterogeneous in nature, being built up of small particles suspended in a liquid. The new means used for carrying out these observations was the ultramicroscope constructed by Siedentopf and Zsigmondy in 1903. By way of analogy it appeared probable that all colloids are similarly built.

The ultramicroscope, however, has two serious limitations. In the first place, it can only make visible particles the index of refraction of which differs greatly from that of the solvent (or dispersion medium, as the "solvent" of colloids is usually called). Only in especially favourable cases, such as gold, platinum and silver particles, is it possible to penetrate down to sizes of the order of $5m\mu$. The wide and important domain of lyophilic colloids, such as sulphur, ferric oxide, silicic acid, proteins, starch, cellulose, rubber, can only to a very limited extent be explored by means of the ultramicroscope. In the second place, it is to be noted that an ultramicroscopic study even in the favourable case of, say, a gold colloid, can only give rather incomplete information with regard to the statistical distribution of the various particle sizes present in the colloid solution.

Zsigmondy's monograph impressed the writer greatly when he, as a young research student, became acquainted with it. Mainly through its influence his studies were directed towards research in colloids. During these activities the limitations of the ultramicroscope became evident to him and he soon found himself engaged in a search for some other means of attack in cases where the ultramicroscope failed. The brilliant work of Perrin (1908) on the Brownian movement and the sedimentation of small particles suggested some method built on diffusion, osmotic pressure and sedimentation. It should be possible, so the writer argued, to combine these properties so as to dispense with the necessity of making the particles visible.

After some preliminary work on diffusion and sedimentation of colloids (1911) and after Odén had carried out in the writer's laboratory his beautiful researches on the size-distribution of particles by means of a self-recording balance (1916) which measured the accumulation during sedimentation in the field of gravity, the writer decided to try the possibility of studying particle-size and size-distribution curves by means of the centrifuge (1922). Earlier attempts in this direction did not seem very encouraging. The first trials made by Nichols and the writer in the chemistry department at Wisconsin (1923) showed, indeed, that this road would not be an easy one to travel along. In the first place, it was evident that all measurements had to be done while the sample was rotating at constant speed. Accordingly only optical methods could be used for recording the sedimentation. In the second place, the centrifuging had to be conducted at constant or only slightly and uniformly varying temperature. These two conditions have to be fulfilled in order to obtain convection-free sedimentation. In the third place, the centrifugal field created within the solution to be studied should be of high intensity and homogeneity.

The conditions for convection-free sedimentation were studied by Rinde and the writer (1924) using fine-grained gold sols as test objects. It was found that the sample must be sector-shaped, completely enclosed and of not too large dimensions. The friction against the surrounding gas has to be reduced and the heat from the bearings kept away. We found it possible to perform faultless sedimentation in centrifugal fields 5,000 times the force of gravity (mean radius 45 mm., height of column of solution 15 mm., speed 10,000 r.p.m.) and to measure the size-distribution in gold sols down to the most fine-grained ones. The name ultracentrifuge was proposed for this new research tool.

Using the same apparatus Fåhræus and the writer (1925) succeeded in determining the molecular weight of proteins by means of sedimentation measurements. Evidently the ultracentrifuge would be of great value

as a research instrument in the study of high-molecular compounds. Feeling convinced of this the writer decided to do his best to develop further the ultracentrifuge idea.

In 1926 F. Ljungström, A. Lysholm and the writer reached 100,000 times gravity (mean radius 52 mm., height of column of solution 12 mm., speed 45,000 r.p.m.). In the spring of 1931, further improvements of the machinery accomplished by G. Boestad and the writer made possible sedimentation measurements at 200,000 times gravity (or 200,000 g.), (mean radius 65 mm., height of column of solution 12 mm., speed 54,000 r.p.m.). Using the same radius and the same height of column of solution we reached 260,000 g. early in 1932, 300,000 g. in the spring of 1932 and 400,000 g. in the spring of 1933.

measurements made in very intense centrifugal fields using a low column of solution and a small mean radius with such made in somewhat less intense fields using a higher sample situated farther from the centre of rotation has shown that the accuracy is much better in the latter case at least as far as sedimentation velocity measurements are concerned. For a standard equipment, therefore, a large rotor is to be preferred.

From the many different experimental machines built in Upsala two standard types have been developed. The first one is adapted for the region 500 to 15,000 g., the other one for the range 15,000 to 750,000 g. The low-speed machine is driven directly by a high-frequency motor and is provided with ball-bearings. The rotation takes place in hydrogen of atmospheric pressure and the casing is immersed in a

Fig. 1.

Rotor and cell for centrifugal fields up to 750,000 times the force of gravity.

Essentially higher fields cannot be utilised with rotors of this size because of failure of the material. It seemed of interest to try a smaller rotor type capable of giving considerably higher intensities although at the sacrifice of height of column of solution and homogeneity of the centrifugal field. Reducing the mean radius to 36 mm. and the height of sample to 8 mm. sedimentation measurements in fields up to 600,000 g. were made in the autumn of 1933 and up to 900,000 g. in the summer of 1934. The rotors used in these experiments exploded however after a few runs. A further reduction of the mean radius to 32·5 mm. and improvements in the construction have made it possible to do regular measurements in fields up to 750,000 g. The comparison of

water thermostat. It is used for sedimentation equilibrium measurements in solutions of high molecular substances and for sedimentation velocity measurements on heavy particles.

The high-speed machine is driven by oil-turbines and has white-metal bearings with movable, damped pistons. The rotor spins in hydrogen of reduced pressure. It is used for velocity measurements in solutions of high-molecular compounds and for equilibrium measurements on low molecular substances.

A few details concerning the oil-turbine ultracentrifuge might be of interest. The rotor (Fig. 1 and 2R) of chromium-nickel steel is supported by horizontal bearings, B_1 and B_2 (Fig. 2), and kept in rotation by

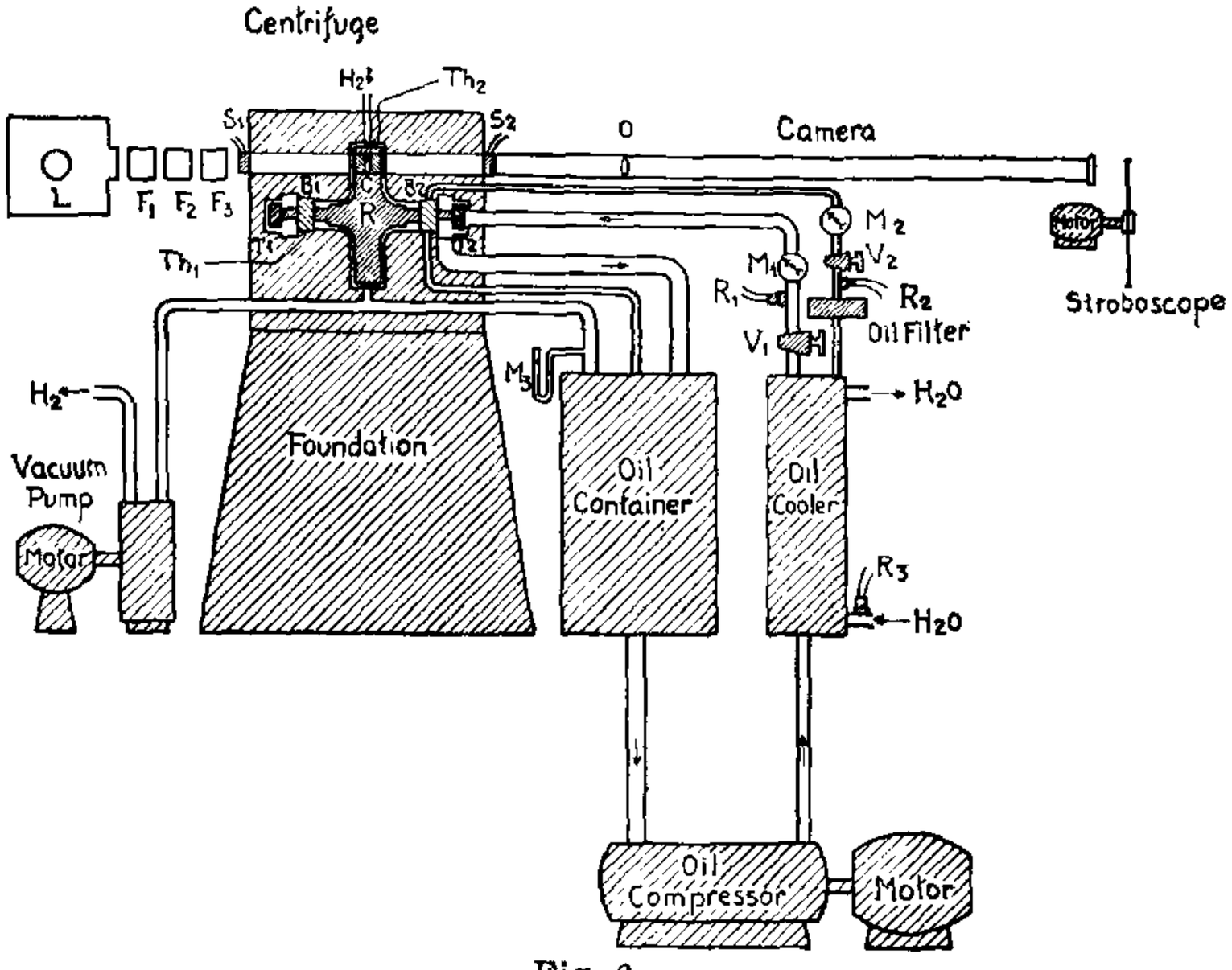

Fig. 2.

Diagrammatic representation of the oil-turbine ultracentrifuge.

means of two small oil-turbines, T_1 and T_2, one on each end of the shaft. Hydrogen is let in at the periphery and constantly pumped off so as to maintain a pressure of about 25 mm. Thermocouples, Th_1 and Th_2, in the bearings and at the inner surface of the heavy steel casing, which surrounds the rotor, serve for temperature control of the centrifuge. A beam of light from a mercury lamp L, filtered through F_1, F_2, F_3, passes the cell C in the rotor on its way to the camera. The exposures are timed by means of the electromagnetic shutters S_1 and S_2. A stroboscope enables the observer to measure the speed of the rotor. The pressure oil which feeds the turbines is produced by a special oil compressor and cooled to a suitable temperature before entering the turbine chambers. The lubricating oil for the bearings passes through an oil filter and is controlled by the valve V_2. By changing the speed of the motor which drives the compressor and by operating the valve V_1 the pressure of the oil entering the turbines may be regulated so as to make possible sedimentation measurements at any desired speed between 5,000 and 140,000 r.p.m. The resistance thermometers R_1, R_2, R_3 and the manometers M_1, M_2, M_3 enable the operator to control temperature and pressure in various parts of the machinery.

A detail section of the centrifuge proper through the axis of rotation (with a previous

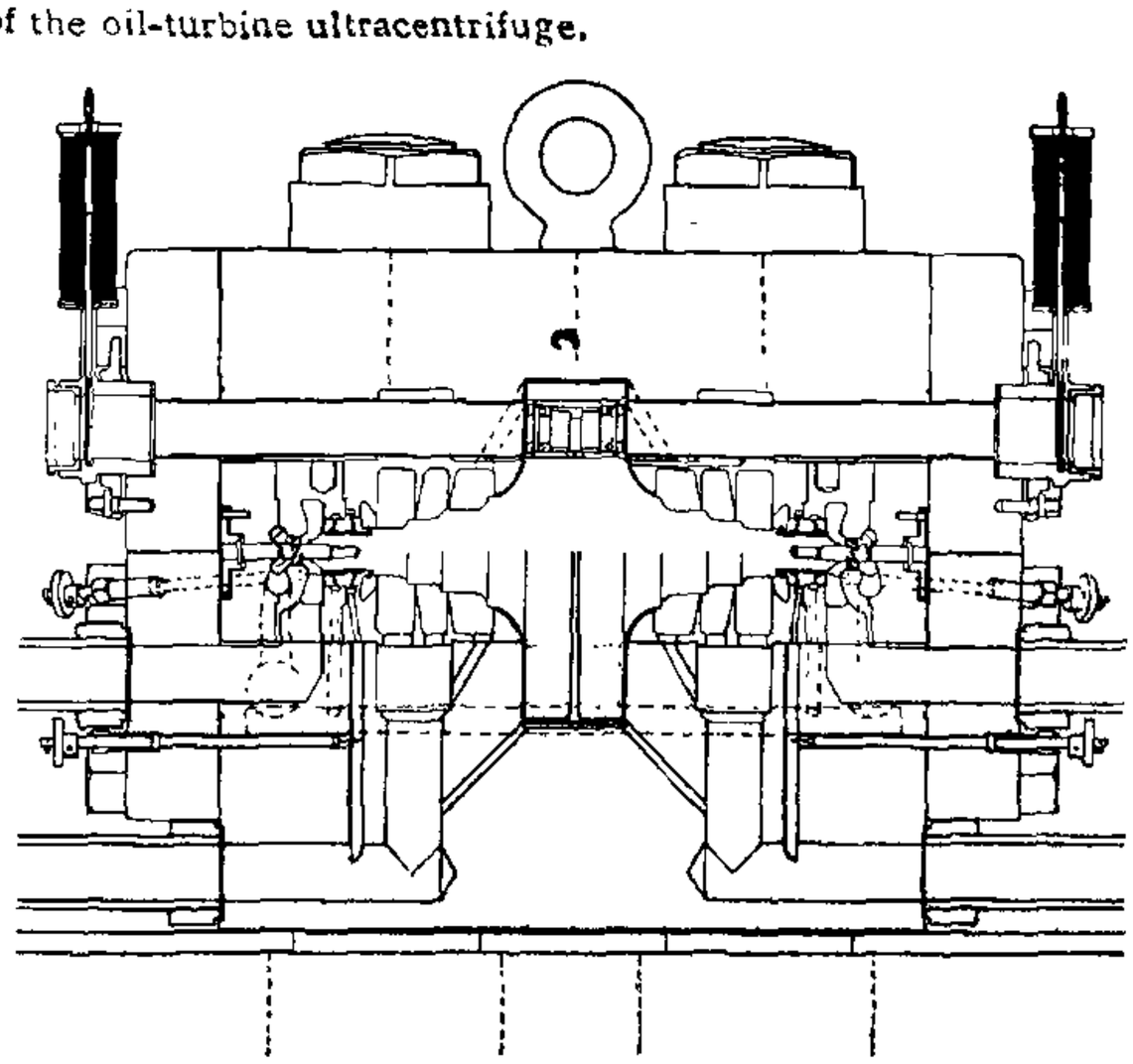

Fig. 3.

Axial section of the oil-turbine ultracentrifuge.

type of rotor) is given in Fig. 3. Fig. 4 shows a picture of it with the upper part of the heavy steel casing lifted, laying bare the rotor and the turbine chambers. The cell with its sector diaphragm is in vertical position upside down. Behind the centrifuge is the lamp house and the light filters. The two halves of the thick steel casing are held together by bolts of chromium-nickel steel firmly anchored in a concrete

Fig. 4.
The oil-turbine ultracentrifuge with the upper part of the casing lifted.

Fig. 5.
Oil-turbine ultracentrifuge installation.

foundation. This arrangement has proved an efficient protection in case of accident (explosion of the rotor caused by overstrain). Fig. 5 gives a total view of the installation showing the stroboscope for measuring the speed, the camera, the centrifuge on its foundation, the oil coolers and, to the left, the switchboard with all the control instruments.

Two kinds of measurements can be done by means of the ultracentrifuge. In the first place, one may centrifuge long enough for a state of equilibrium to be reached between sedimentation and diffusion. Then for each molecular (or particle) species the following formula is valid:

$$M = \frac{2\,RT\,ln\ (c_2/c_1)}{(1-V\rho)\omega^2(x_2^2-x_1^2)}$$

where M = molecular (or particle) weight, R = gas constant, T = absolute temperature, c = concentration of solute, V = partial specific volume of solute, ρ = density of solvent, x = distance from centre of rotation, ω = angular velocity.

In this way one obtains directly the molecular weight. If several molecular species are present in the solution the molecular weight values calculated for different distances from the centre of rotation show a marked drift. Freedom from drift is a criterion of homogeneity with regard to molecular weight.

In the second place one may use a centrifugal field strong enough to cause the molecules or particles to sediment with measurable velocity. This procedure enables

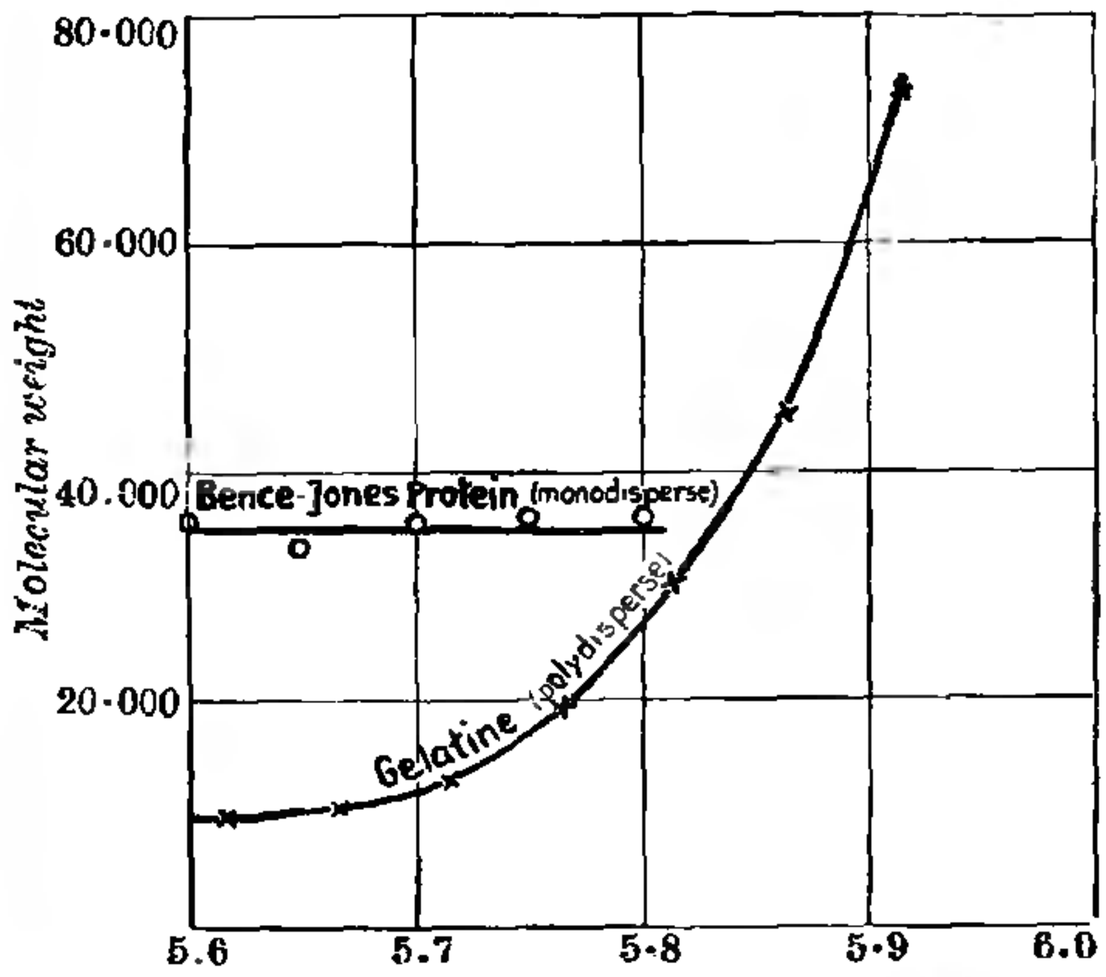

Fig. 6.
Sedimentation equilibrium of Bence-Jones protein (monodisperse) and gelatine (polydisperse).
(B. Sgögren and K. Krishnamurti)

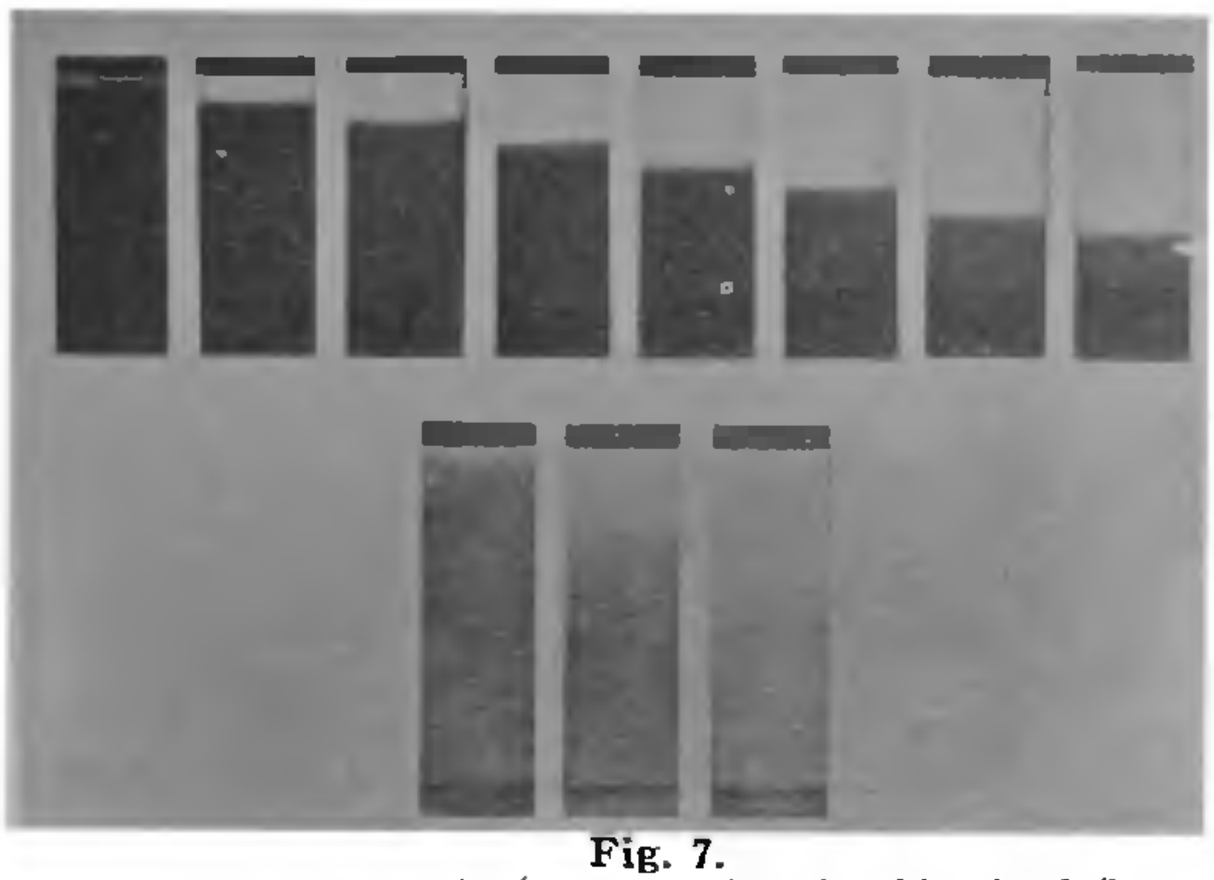

Fig. 7.

Sedimentation of hemocyanin (upper row) and gold colloid (lower row) in a centrifugal field 37,000 times the force of gravity; time between exposures 3 minutes. The former is monodisperse, the latter polydisperse.
(E. Chirncaga and H. Rinde)

Fig. 8.

Sedimentation of hemoglobin in a centrifugal field 900,000 times the force of gravity; time between exposures 3 minutes.
(Inga-Britta Eriksson-Quensel)

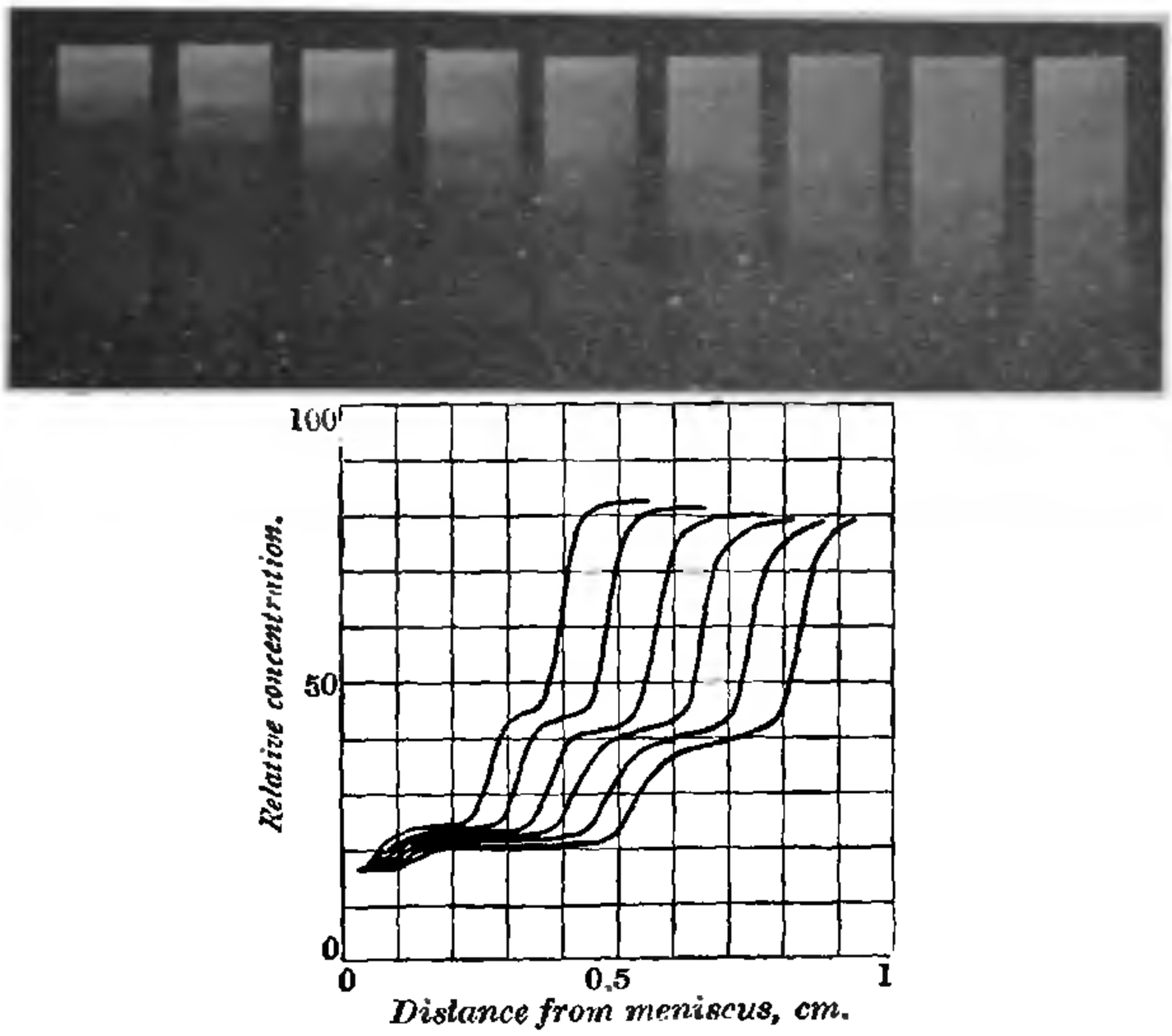

Fig. 9.

Sedimentation analysis of Helix-hemocyanin at the alkaline border of the stability region. Centrifugal field 33,000 g.; time between exposures 5 minutes. (K. O. Pederson)

us to find how many different kinds of molecules are present in the solution. If the sedimentation velocity is referred to unit field and water of 20° C. as solvent, it is called the sedimentation constant. By combining diffusion and sedimentation data the weight of the different molecular species may be calculated according to the formula

$$M = \frac{RT \cdot s}{D(1 - V\rho)}$$

where s = sedimentation constant, D = diffusion constant.

Sedimentation measurements in the ultracentrifuge may also be used for the determination of the weight-distribution or size-distribution of molecules or particles in a polydisperse mixture. The theory being rather complicated we will not go into it on this occasion.

ii.

The ultracentrifuge has a wide range of

application. With the aid of this tool molecular weight determinations have been done from about 10,000,000 (hemocyanin-variety in the blood of the snail Busycon) down to about 40 (lithium chloride). Quite unique is the possibility which this technique offers of carrying out an analysis of the various molecular species or particle sizes present in a solution. The sedimentation constant is a very characteristic molecular

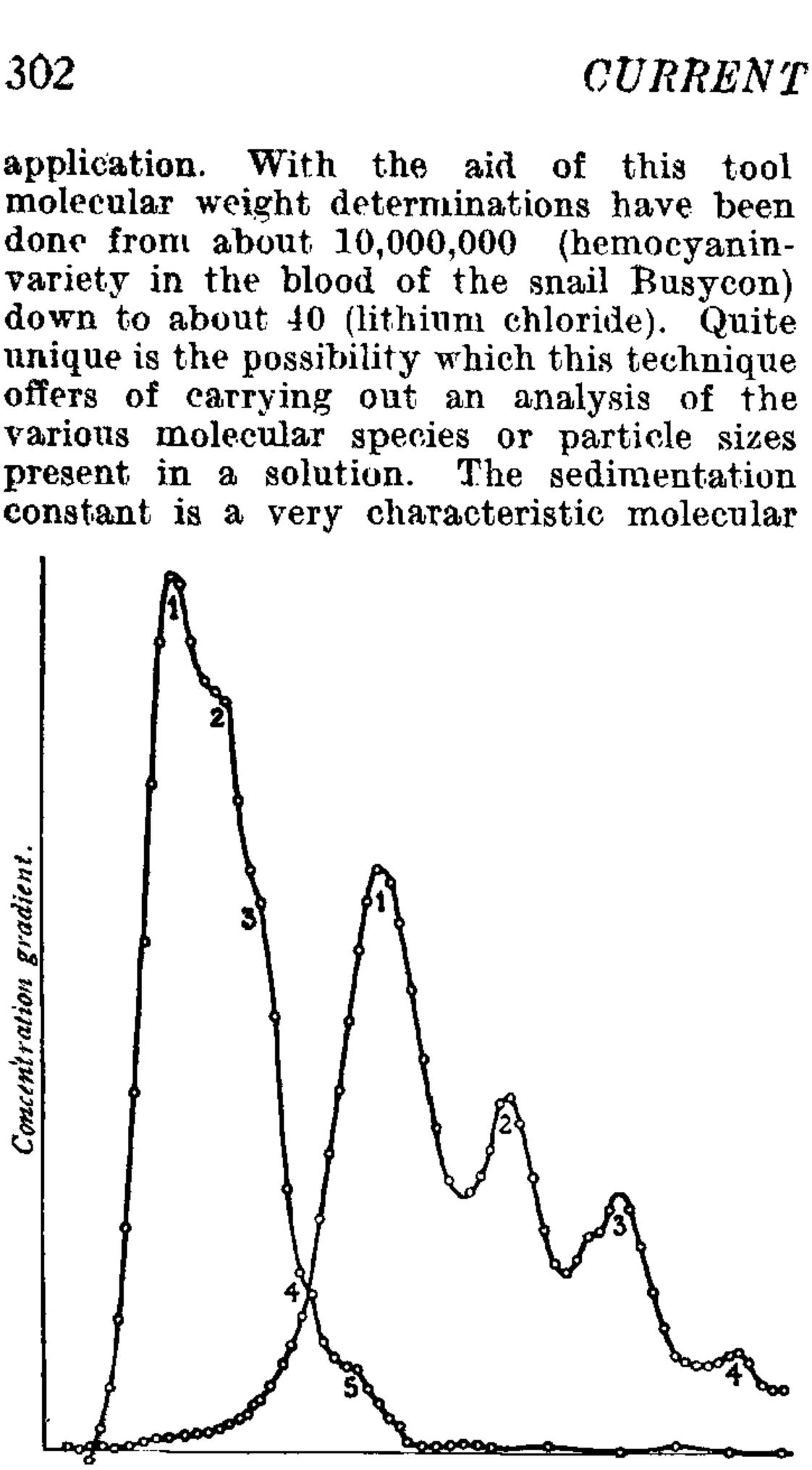

Fig. 10.

Refractometric sedimentation diagram of diluted blood-plasma from a case of myeloma; centrifugal force 260,000 g., time of centrifuging 15 and 60 minutes. (A. S. McFarlane)

property and by means of it, one often finds it possible to follow sensitive aggregation- and dissociation-reactions in biological systems. The combination of sedimentation equilibrium and sedimentation velocity measurements allows of certain conclusions with regard to the shape of the molecules or particles. This is often of importance when investigating high-molecular compounds.

Among the substances studied so far are proteins, polysaccharides, poly-styrols, dye-stuffs and other synthetic organic compounds as well as inorganic colloids and inorganic salts.

Some of the main results of the protein investigations may be mentioned. The native proteins are very homogeneous with regard to molecular weight while artificial colloids as well as proteins extracted from the organisms by rough treatment are polydisperse. As an example of a homogeneity test by means of sedimentation equilibrium measurements the diagram Fig. 6 gives the values of the molecular weight as measured at different distances from the centre

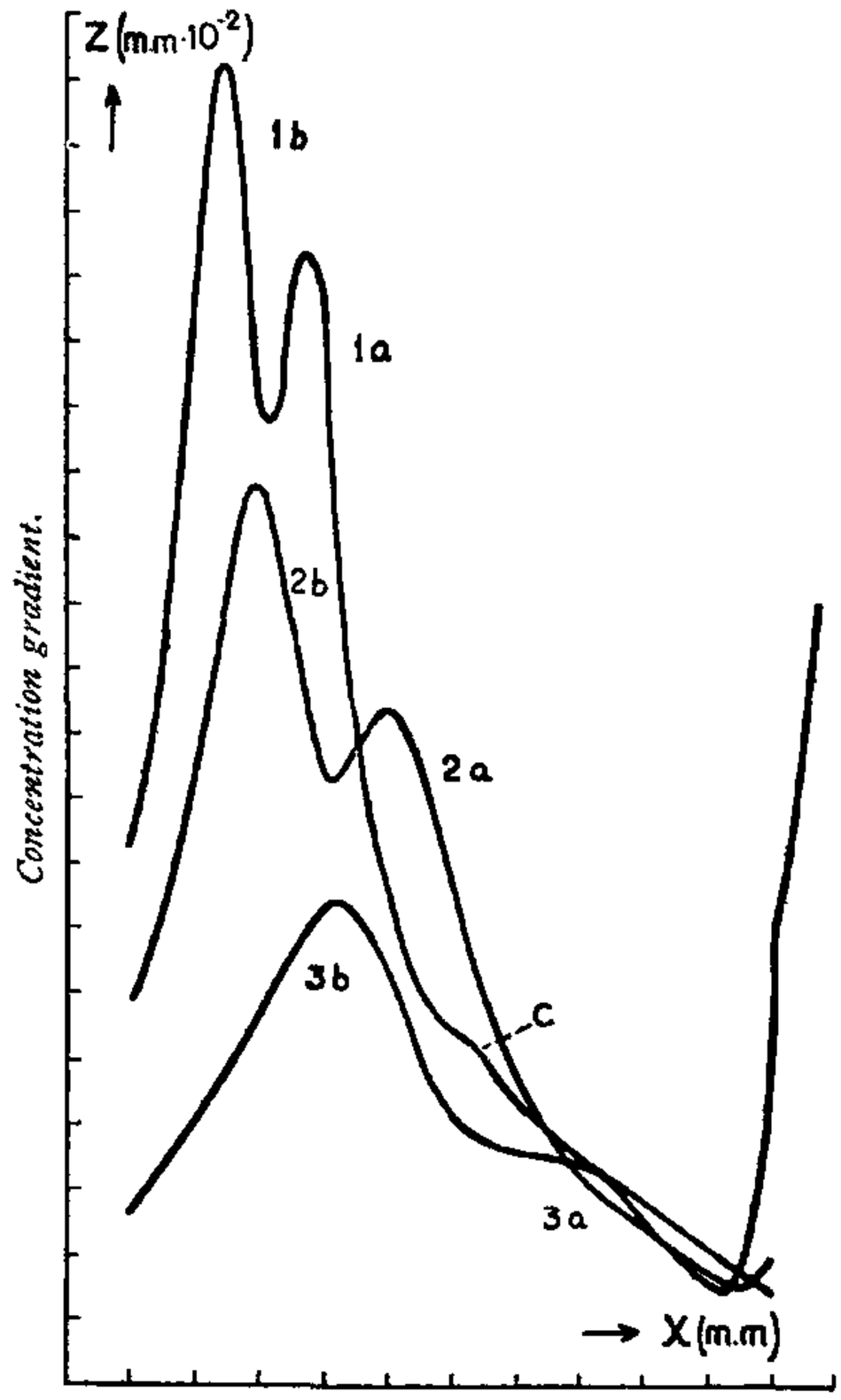

Fig. 11.

Refractometric sedimentation diagram of a solution of starch treated with acid, centrifugal force 145,000 g. (O. Lamm)

of rotation in the case of a homogeneous substance, Bence-Jones protein (B. Sgögren) and an inhomogeneous substance, gelatine (K. Krishnamurti). This is further demonstrated by the sedimentation velocity runs in Fig. 7 which shows in the upper row (E. Chirnoaga) the sedimentation of hemocyanin from the blood of Helix (M = 6,600,000) and in the lower (H. Rinde) the sedimentation of a gold colloid, both of them in a centrifugal field 37,000 g. In the first case the border-line between solution and solvent remains sharp, in the second case it becomes blurred with time because of the different speed with which the gold particles of different sizes are sedimenting. To test the homo-

geneity of a substance of lower molecular weight by a velocity run the centrifugal force has to be increased so as to get sufficient sedimentation before a blurring of the boundary by diffusion takes place. Fig. 8 shows the sedimentation of hemoglobin (M = 69,000) in a centrifugal field 900,000 times the force of gravity (Inga-Britta Eriksson-Quensel). The border-line remains sharp and the protein in question is, accordingly, quite homogeneous.

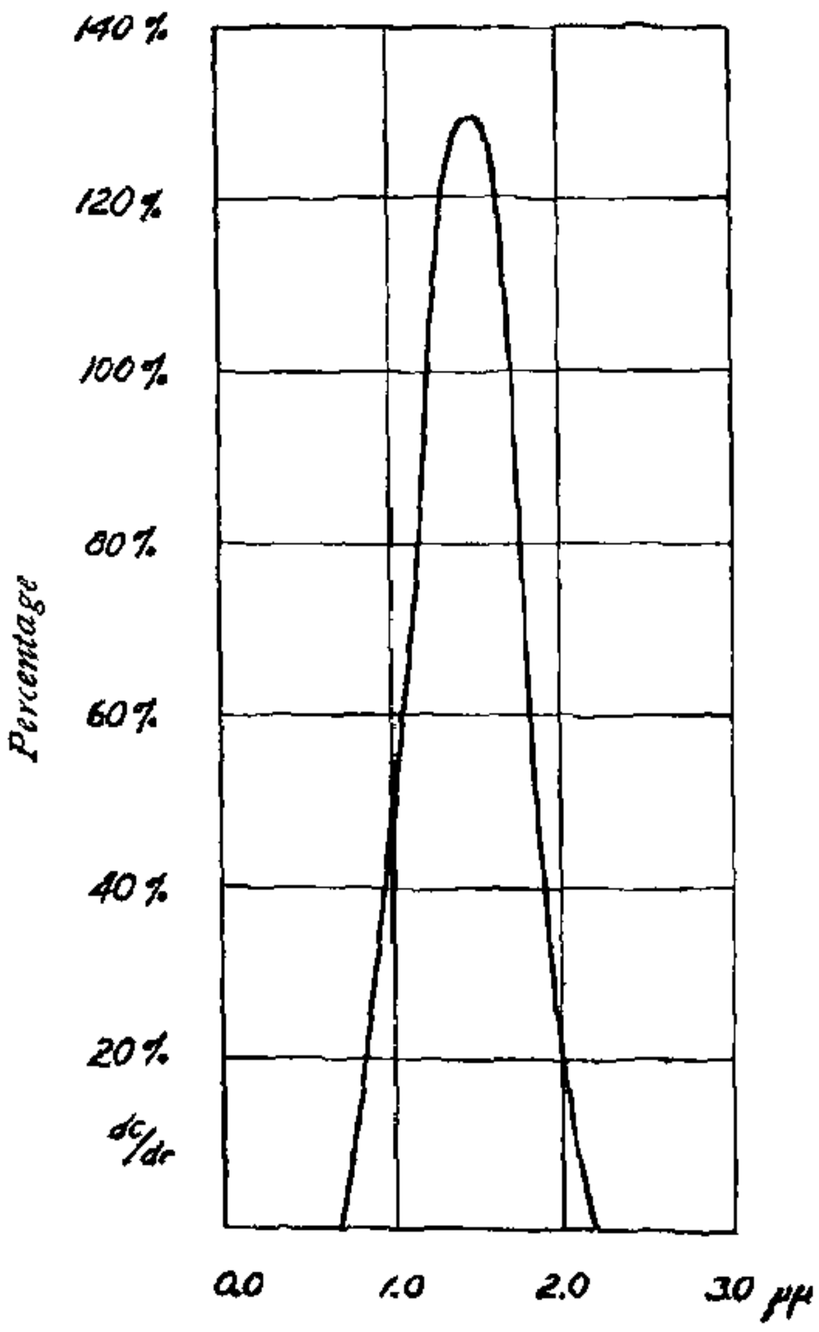

Fig. 12.
Size-distribution in a gold colloid.
(H. Rinde)

The molecular homogeneity of a protein remains unchanged when the pH of the solution is changed within certain limits. At well-defined pH-values changes in the shape or in the weight of the protein molecule take place. Among the hemocyanins a number of reversible dissociation-association reactions have been observed. As an example the behaviour of Helix-hemocyanin at the alkaline stability border may be given. Fig. 9 (K. O. Pedersen) shows the presence of two dissociation products of mass ½ and ⅛ together with unchanged molecules. A closer study of this phenomenon reveals the fact that the hemocyanin-molecule is at first split into halves and some of these halves into four parts (Inga-Britta Eriksson-Quensel).

In some cases the association state of a protein is dependent on the dilution and on the presence of other proteins. Recent investigations on serum have shown (A. S. McFarlane) that the globulin may appear in molecules of different mass according to the concentration of the albumin present in the serum. Pathological states of the organism bring about characteristic changes in the sedimentation diagram of the serum proteins (Fig. 10), a fact that suggests the use of the ultracentrifuge as a possible instrument for diagnostic purposes.

An ultracentrifugal study of solutions of starch (O. Lamm) has given the following result. Depending on the previous treatment the particle size varies, but always in a continuous way. No distinct molecular species were found. Preparations treated with acid show two maxima corresponding to amylose and amylopectin (Fig. 11). A detailed investigation of the solutions of poly-styrols in various organic solvents has been carried out (R. Signer). The molecules were found to be very elongated and free movement was observed only in very dilute solutions. The viscosity increases with molecular weight. Gold colloids were among the first objects of ultracentrifugal investigations. Fig. 12 shows the size-distribution curve of a very fine-grained gold sol (H. Rinde).

iii.

The utilisation of the ultracentrifuge for the study of high molecular compounds is only at its beginning. As research goes on new problems present themselves for treatment with this new tool. So far the main interest of applications has been in the field of biology and medicine because of the various kinds of new information which the ultracentrifuge has made available with regard to the behaviour of the proteins—those substances of paramount importance to all living beings. But there are also the vast fields of the carbohydrates, the hydrocarbons and the synthetic organic high-molecular compounds. A number of important chemical industries are handling materials belonging to one or the other of these classes of substances. The research laboratories connected with such industries are beginning to realise that the ultracentrifuge may be able to render services of great value in elucidating the properties of the molecules and particles which are the building-stones of cellulose, artificial silk, varnishes, rubber, dyes and many other products,