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THE DEVELOPMENT OF SVEDBERG'S ULTRACENTRIFUGE

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The first attempt to use centrifuges for the determination of particle sizes was made by Dumansky and co-workers in 1913 [1]. However, their results were unsatisfactory, probably because of non-ideal sedimentation, chiefly due to convection in the cylindrical centrifuge tubes.

In 1922 Svedberg and Rinde had studied the sedimentation of various metal sols in a gravitational field [2]. They had also developed a method for the determination of the distribution of size of particles which depended on the variation of concentration with time and height in a sedimenting system. The concentration was measured by means of a light absorption method worked out by Rinde. However, this sedimentation method could only be used for particles down to a certain size. For smaller particles the rate of setting was too slow to be measured with any accuracy. As Svedberg and Rinde were especially interested in the very small

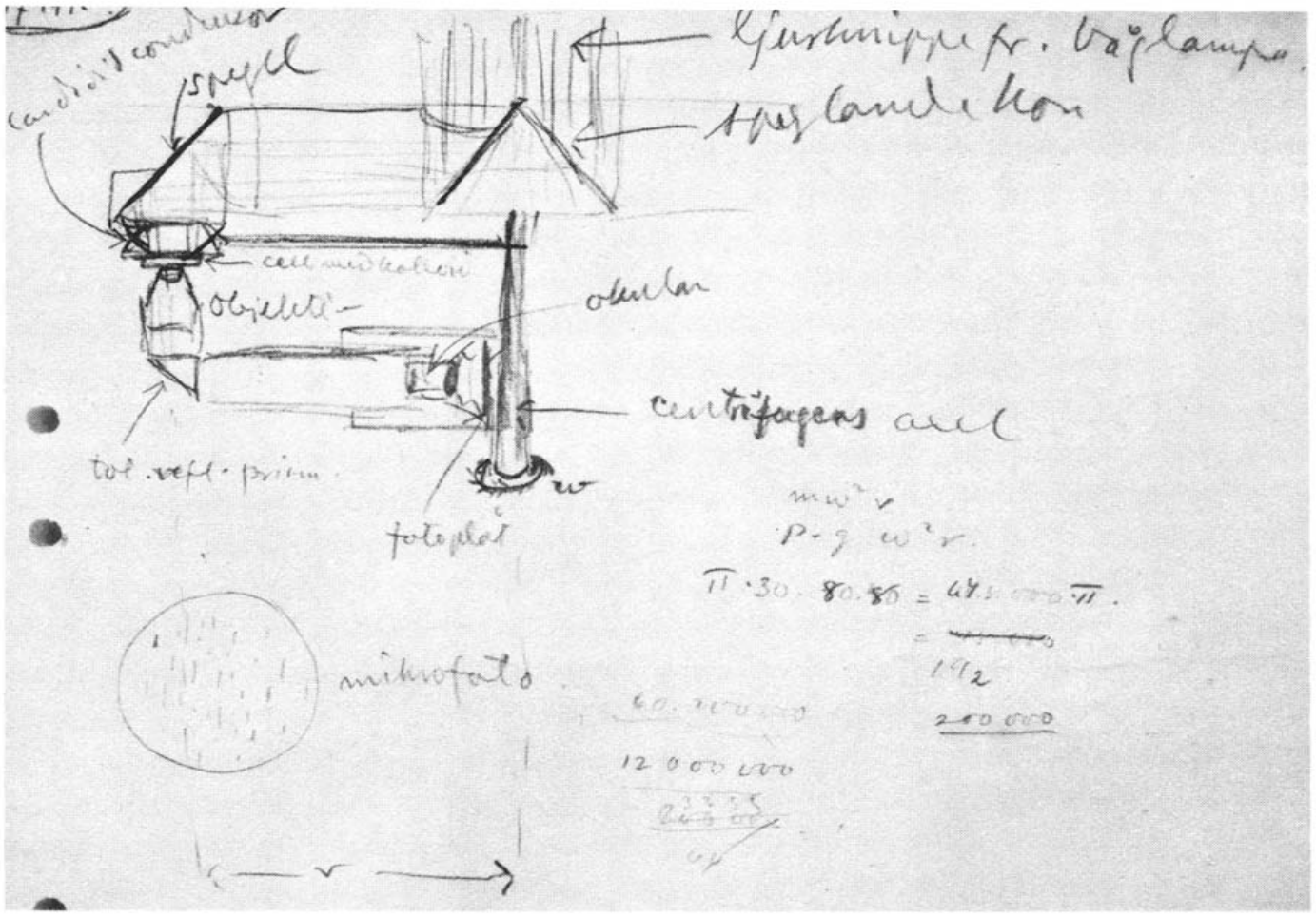

Fig. 1. Svedberg's first rough sketch of the optical arrangement in a centrifuge to be used in the study of amicroscopic colloids making use of ultramicroscopic photography with darkfield illumination by means of a cardioid condenser (31 July 1922). The light beam from an arc-lamp is reflected from a cone on top of the centrifuge axis onto a mirror placed above the cardioid condenser. The cell with the colloid is placed just below the condenser. By means of the microscope objective, a total reflection prism, and the ocular, an image of the reflections from the sedimenting metal particles is formed on the photographic plate sitting on the axis of the centrifuge.

particles in connection with studies on the formation and growth of particles in metal sols, it would be important if a centrifugal method could be used. In their 1923 paper these authors therefore also discussed the modifications necessary in this method when used in connection with sedimentation in a centrifugal field.

In July 1922 Svedberg made a first rough draft of a system for following the sedimentation in a centrifuge. It was based on ultramicroscopic observation during the centrifugation by means of dark-field illumination using a cardioid condenser. The idea remained on paper and so did other proposals drafted during the fall of 1922.

The first practical result came in the spring of 1923, when Svedberg was a visiting professor at the University of Wisconsin at Madison. Here Svedberg and Nichols constructed an optical centrifuge, where the settling of the particles could be continuously observed or photographed during the run [3]. In this centrifuge, where the solution was enclosed in a cylindrical tube, the particles were carried down by sedimentation as well as by convection along the walls of the tube. No simple and exact calculation of the rate of sedimentation could therefore be made. Svedberg was convinced, however, that this problem could be solved, and on the boat trip across the Atlantic in August 1923, he made the first drafts of the rotor for a new type of centrifuge.

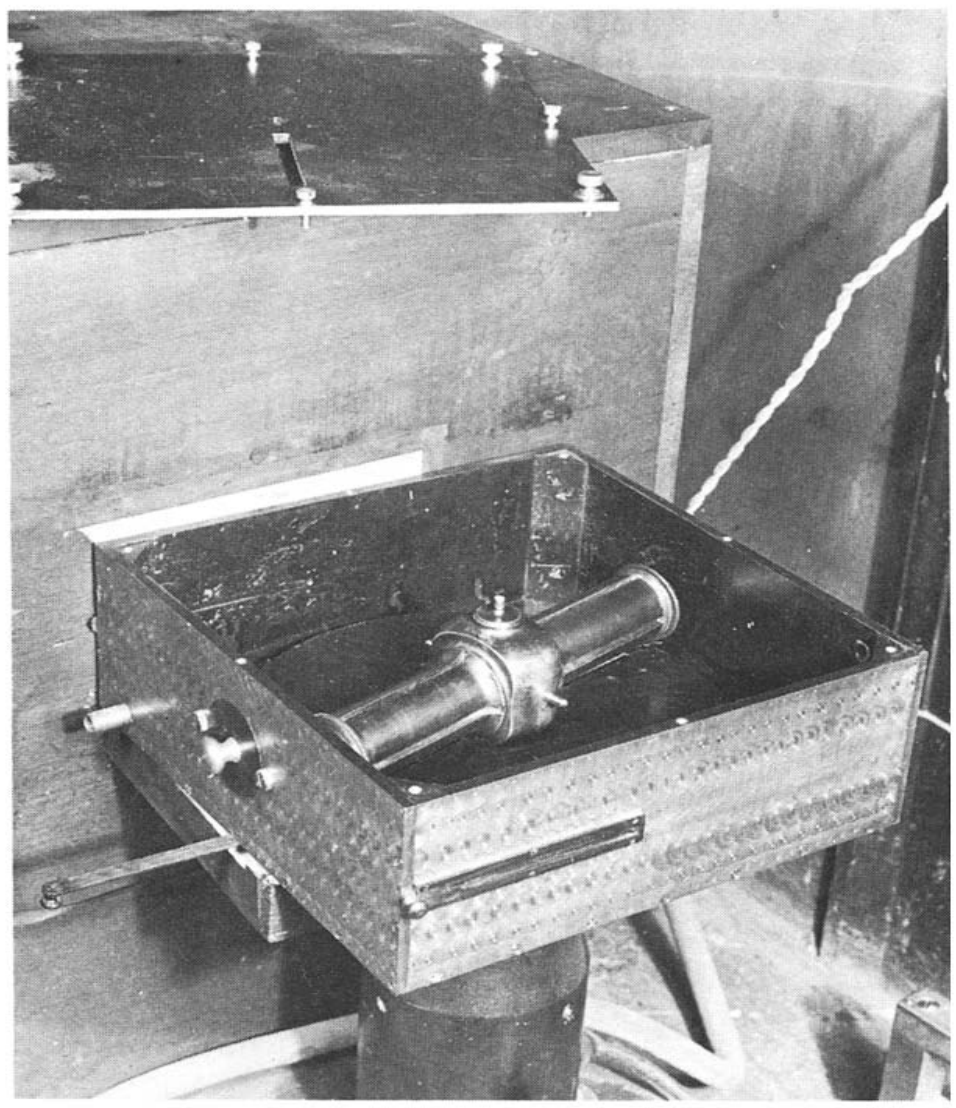

Fig. 2. Svedberg's and Nichols' optical centrifuge (Madison 1923).

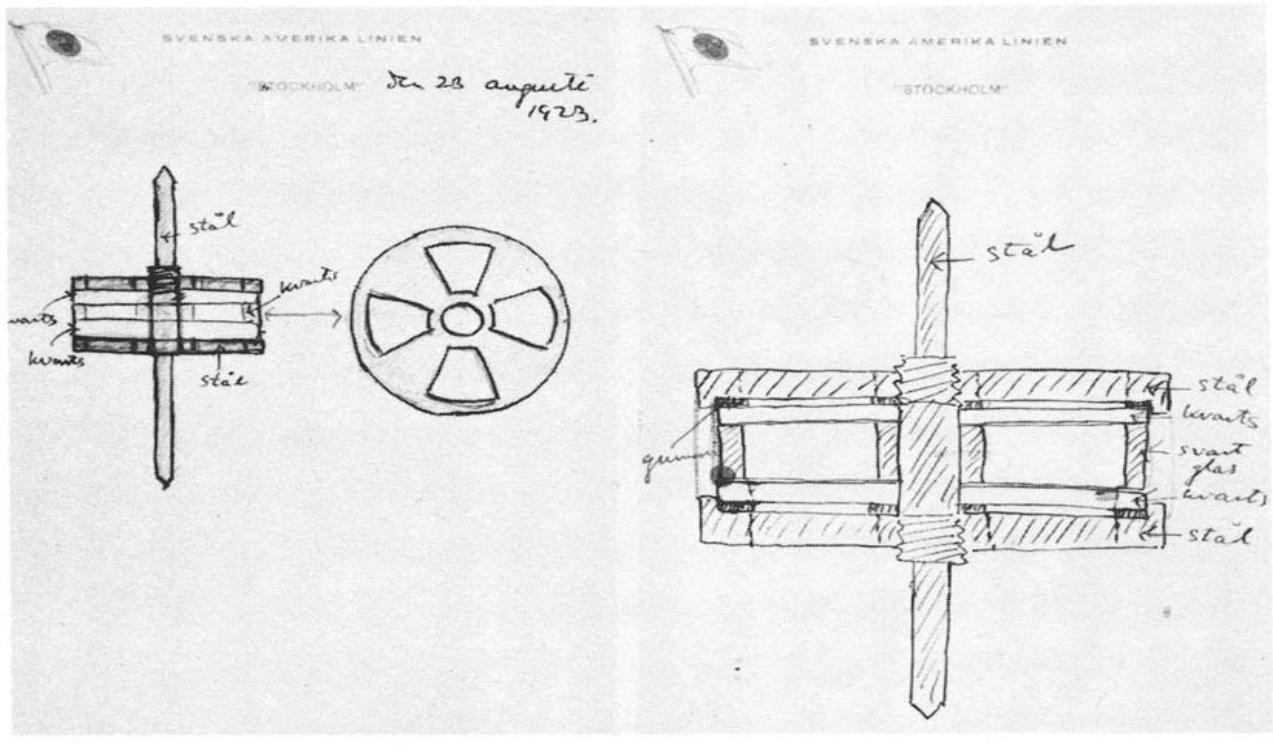

Fig. 3. The first drawing of an ultracentrifuge rotor made by Svedberg on the Atlantic on his way home from the United States (August 1923).

The eight months at Madison had been very stimulating for Svedberg, and he returned to Uppsala with his head teeming with ideas, not only on centrifuges, but also on problems in colloid chemistry, on electrophoresis, and on diffusion. In a lecture at the First Colloid Symposium held in June 1923 at Madison, he had said [4]: "It is possible to build up a method for recording distribution curves from observations of diffusion" and a little further on "Experiments of this kind are planned in my laboratory. They are of importance because *we are here dealing with one of the few possible means for studying the distribution of sizes in protein sols*". From Svedberg's laboratory records and notes it is evident that his mind was working on the problem of calculating distribution curves from diffusion and from sedimentation measurements. A rough sketch of a centrifuge rotor with cells is marked *centrifugal-analysis* of proteins 23 November 1923. This may be a first indication that Svedberg planned sedimentation equilibrium measurements on proteins.

Svedberg and Rinde were already working on some ideas for a new centrifuge to be used in the study of amicroscopic colloids. The new centrifuge therefore had to give a stronger centrifugal field than the optical centrifuge, and it also had to overcome the latter's trouble with convections, vibrations, etc. One of the main steps towards the realization of a working centrifuge was the introduction of a cell for the sedimenting solution which was sector-shaped and for which they derived the important square dilution law. With the sector-shaped cell convection-free sedimentation becomes possible. The cell was placed in a heavy rotor resting on the axis of a modified cream separator. The top part of the separator stand had been reconstructed in such a way that the rotor was enclosed in a gas-tight case where it could rotate in a controlled atmosphere. At the beginning they had great trouble with convection. Rinde thought that this was caused by vibrations, whereas Svedberg was more inclined to attribute it to temperature disturbances. They then tried to let the rotor spin in a hydrogen atmosphere, and this procedure made it possible to get convection-free sedimentation.

On 15 February 1924, the centrifuge was for the

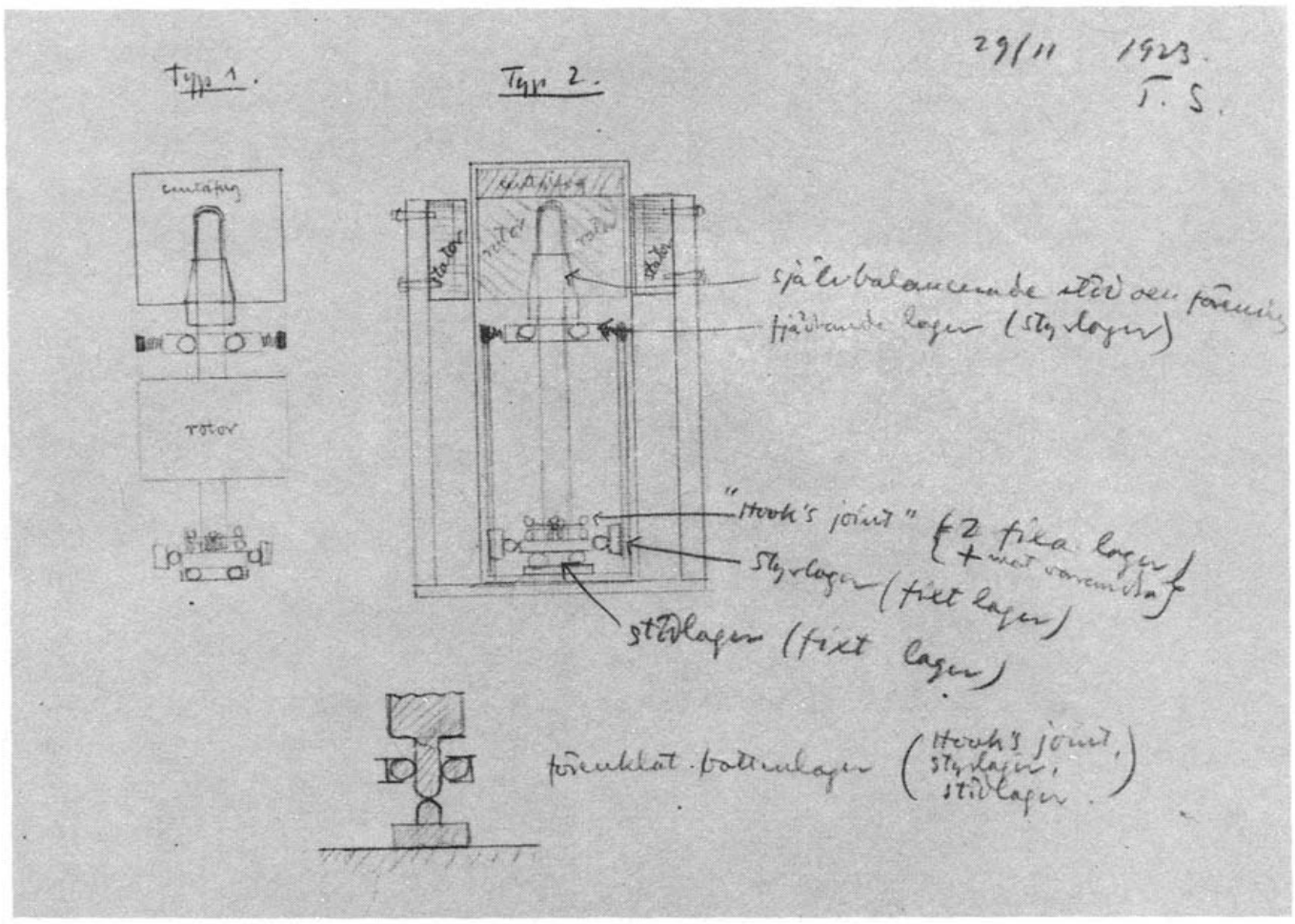

Fig. 4. An early sketch (29 November 1923) of two types of electrically driven centrifuges for sedimentation equilibrium measurements.

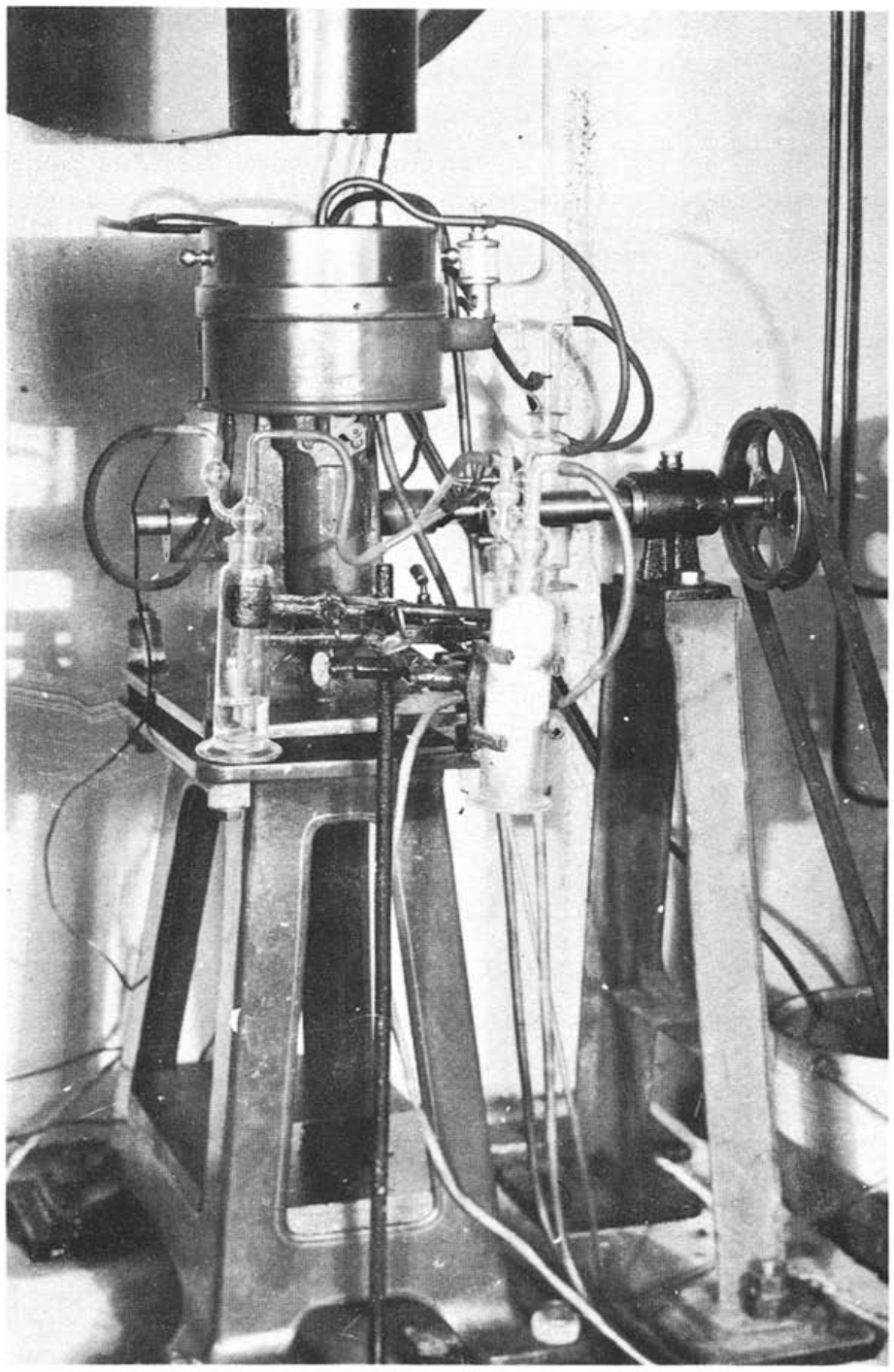

Fig. 5. Svedberg's and Rinde's first ultracentrifuge (Uppsala 1924).

first time called the *ultra-centrifuge* in Svedberg's laboratory record. Then followed a period of adjustments and tests. After 5 to 6 weeks, the first sedimentation measurements on a colloid were noted in the record and here the name *ultracentrifuge* without a hyphen appeared for the first time. In the following three months, Svedberg and Rinde made a number of experiments with gold sols, and in July 1924 the first paper dealing with ultracentrifuge experiments was sent to the Journal of the American Chemical Society [5]. In this paper the authors stress the importance of this new instrument. As to the name they say: "The new centrifuge constructed by us, allows the determination of particles that cannot be made visible in the ultra-microscope. In analogy with the naming of the ultra-microscope and ultra-filtration apparatus we propose the name *ultra-centrifuge* for this apparatus."

In September 1924 Robin Fåhraeus, who was assistant professor of pathology, came to work with Svedberg. They started to study native casein from milk, and they found that it showed a very broad frequency distribution with coarse particles of the order of 10 to 70 nm. Svedberg had been interested in studying proteins with his ultracentrifuge. As a colloid chemist he assumed that they were polydisperse, and he was interested in studying the size distribution in their sols. However, some experiments with egg albumin had been disappointing. Now Fåhraeus proposed that they should try with hemoglobin.

The first run with hemoglobin was made 16 October 1924 at a speed of 8400 rpm. After two hours a very distinct change in the color of the solution could easily be observed near the meniscus. There was no doubt that the hemoglobin had sedimented. However, long before any measurements could be made, a crack developed at the bottom of the cell and the solution leaked out. After a reconstruction of the cell, the first successful sedimentation equilibrium experiment was carried out on 12 to 14 November 1924. Another successful equilibrium experiment was made two weeks later; within the experimental error of the method the molecular weight was found to be constant all through the cell. This was the first indication that protein molecules had uniform size. It came as a great surprise to Svedberg. Could it be that proteins had a well defined molecular weight? How to test this? Sedimentation equilibrium measurements might give some indication of the uniformity of the particles. It was difficult, however, to get more detailed information about the homogeneity of a dissolved protein by this method. On the other hand, if the sedimentation velocity method could be used, an analysis of the shape of the boundary would reveal the presence of inhomogeneous material. However, this would demand a considerably increased centrifugal field; about 70 000 to 100 000 *g* would be necessary in order to give this method a reasonable sensitivity. This meant a 15 to 20 fold increase of the centrifugal force available at that time. An entirely new type of centrifuge had to be constructed, and a number of new

problems, concerning technique and safety, had to be discussed and solved.

Parallel to the work with the ultracentrifuge, Svedberg had started to study the diffusion of proteins and amino acids making use of fluorescence and of light absorption to follow the blurring of the boundary between solution and solvent in diffusion experiments.

He had also written a paper [6] for the *"Zsigmondy Festschrift"* on "Centrifugation, diffusion, and sedimentation equilibrium for colloids and high molecular substances". For the first time he indicated that these methods might be used to determine the molecular weights for high molecular weight substances. He derived the well-known Svedberg formula

$$M = \frac{RT\ln(x_2/x_1)}{D\omega^2(1-V\rho)(t_2-t_1)} = \frac{RTs}{D(1-V\rho)}$$

and he showed how the charge on colloidal electrolytes should influence their diffusion, sedimentation and sedimentation equilibrium.

Svedberg's interest was now mainly directed towards the study of proteins and the development of the ultracentrifuge technique. Rinde continued his very careful and extensive studies of the gold sols with the low speed ultracentrifuge leading to his inaugural dissertation [7] in 1928 "The distribution of the sizes of particles in gold sols prepared according to the nuclear method".

In April 1925 the hemoglobin studies were taken up again, this time with better cells and in a modified centrifuge rotor. During the following months a number of sedimentation equilibrium experiments were carried out under different experimental conditions. They all showed that hemoglobin was a monodisperse protein with molecular weight of about 67 000. In the paper Svedberg and Fåhraeus submitted for publication in July 1925, they say in the introduction [8]: "The lack of a reliable method for the determination of the molecular weights of substances possessing a very complicated structure has been a serious obstacle in the progress of our knowledge of the chemistry of the proteins. In the present paper such a method will be proposed and its use will be illustrated by a few preliminary measurements on hemoglobin". Later on in a footnote they say: "These measurements should be regarded more as an illustration of the method than as a precision determination of the molecular weight of hemoglobin. A more refined technique of measurement will, we hope, enable us to communicate such determinations later on."

Half a year earlier, after the first successful experiments with hemoglobin, Svedberg had already started to work on the problem of constructing a centrifuge that could yield a centrifugal field of 100 000 *g* in the solution cell. Svedberg approached Mr. F. Ljungström, of the Ljungström Steamturbine Co., Stockholm, who proposed the use of oil-turbines for driving the rotor, which would simplify the lubrication problem. He also made other valuable suggestions and let one of his young engineers, A. Lysholm, assist Svedberg in the construction and testing of the ultracentrifuge which was built at the workshop of the Ljungström Steamturbine Co. in Stockholm during the spring and summer of 1925. The installation of the centrifuge and all the necessary equipment was not completed until the beginning of 1926. On 10 January 1926 the first test run with the new high-speed oil-turbine ultracentrifuge could be made in Svedberg's laboratory. It was disappointing: instead of the 40 000 rpm aimed at, only 19 000 rpm were reached. By letting the rotor spin in a hydrogen atmosphere at low pressure (10–20 mm Hg) an increase of 10–15% in the speed could be attained. After a number of changes on the turbines and turbine chambers, improvements on the oil system, and introduction of a new type of bearings giving better lubrication, a speed of 40100 rpm was attained on 7 April 1926. The ultracentrifuge [9] could finally be run routinely at the speed originally aimed at, and Svedberg could start to test it in various ways.

The main question about the uniformity of protein molecules was taken up by Svedberg and Nichols in September 1926. A number of experiments with the sedimentation velocity method were carried out on CO-hemoglobin. The experimentally obtained sedimentation curves were compared with the theoretical curves for the combined sedimentation and diffusion. In his laboratory record for an experiment made 17 September 1926, Svedberg writes: "Not the slightest indication of the presence of differently sized molecules could be noticed". The uniformity of the hemoglobin had thus been confirmed with the new, more sensitive sedimentation velocity method.

Svedberg has decribed the different methods for studying substances of high molecular weight in various papers published during 1926–27 [10–13].

Arne Tiselius became Svedberg's research assistant in 1925 to study the electrophoresis of proteins. During his summer vacation in 1926, he started to study how the thermodynamic properties of colloidal solutions were influenced by the centrifugal field [14]. In a letter to Nichols on 16 July 1926, Svedberg writes: "It is quite funny that Tiselius is now starting a career as a theoretical physicist or chemist! He is trying to go through the main chemical laws and give their form in a centrifugal field. We think that the fact, that now actually the experimental facilities for a "centrifugal chemistry" is existing, gives the right to make some theory also".

In the fall of 1926, Svedberg was awarded the 1926 Nobel Prize in chemistry "for his work on disperse systems". The Nobel Prize was a great stimulation and facilitated the possibility for him to obtain funds for his research activities. He could now plan for a further development of his two types of ultracentrifuges.

In order to get rid of some mechanical and thermal weaknesses of the first low speed centrifuge, an improved type of ultracentrifuge with gearing drive was designed and built in the workshop of Svedberg's laboratory, using parts of a "Baltic" cream separator. Mechanically it was superior to the first centrifuge and its design was much simpler. With a new and lighter rotor construction it was also possible to have the rotor chamber enclosed in a thermostated water-bath, thus giving better control of the cell temperature than before [15].

Svedberg was not quite content with his oil-turbine centrifuge, and he wanted to increase the centrifugal field. In the spring of 1927 he started discussions with Lysholm. He had the idea that maybe one should try to drive the rotor by means of air turbines, considering all the troubles caused by the oil turbines. He made a sketch of a rotor with air turbines and went to Stockholm. Lysholm, with whom he discussed the matter, believed that some of the new types of steel might make it possible to spin a rotor of the same diameter as the first one at a speed of 80 000 rpm, provided the thickness of the rotor got smaller towards the periphery.

Svedberg had made plans to study various types of proteins. As some of these might have very little light absorption even in ultraviolet light, he had been looking for a convenient refractometric method. A couple of times he had indicated in his notes that it should be possible to make use of the bending of the light rays when passed through a concentration gradient. However, it was not until Tiselius proposed the intro-

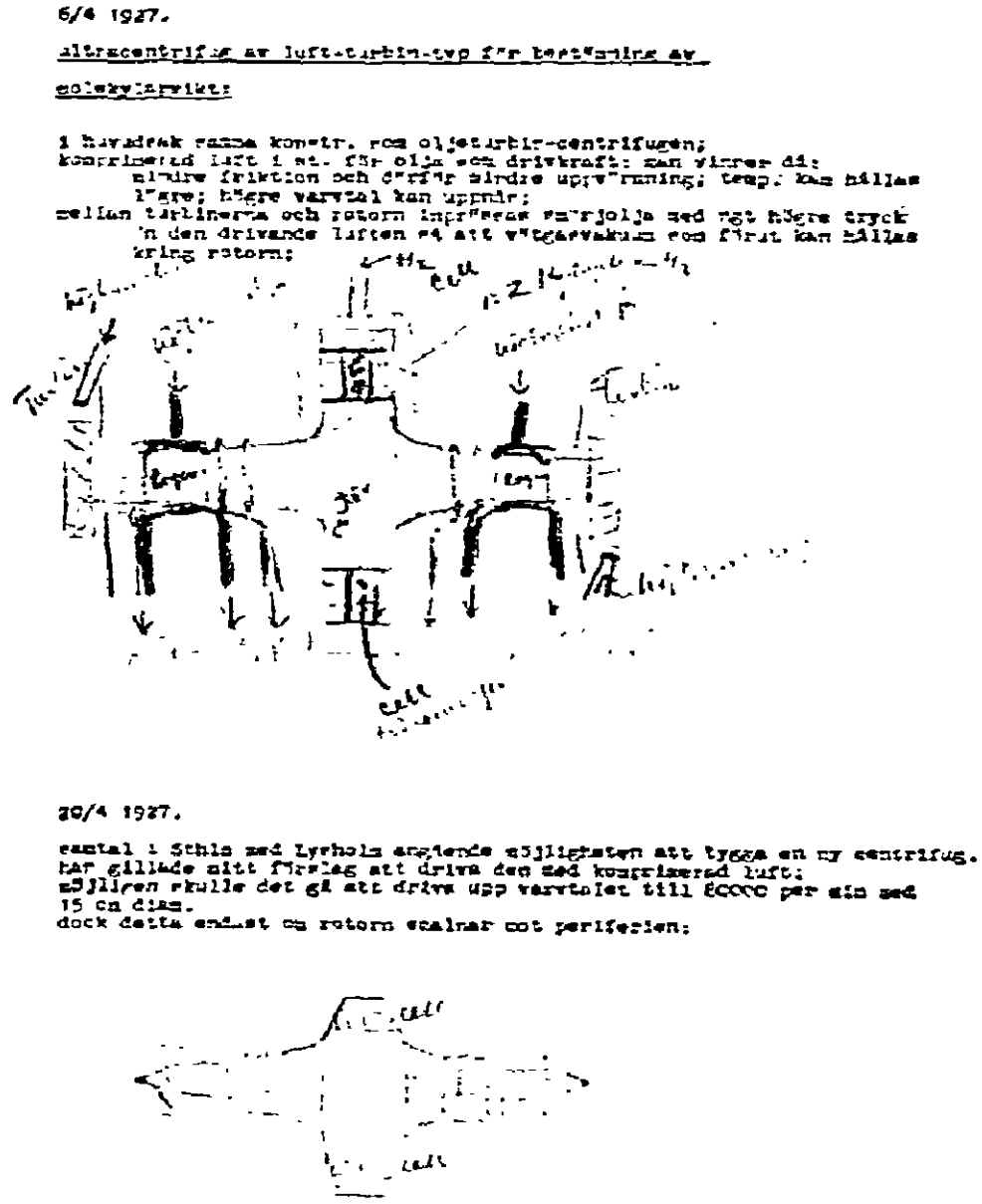

Fig. 6. Svedberg's proposal for an ultracentrifuge driven by air turbines (6 April 1927). After discussions with Lysholm on 20 April 1927, the shape of the rotor was changed. Translation of Svedberg's comments: "Ultracentrifuge of air turbine type for determination of molecular weight". In principle the same construction as the oil-turbine centrifuge. Compressed air instead of oil as motive power. The gain is: less friction and thus less heating; the temperature may be kept lower; higher speed may be attained. Lubricating oil, having a slightly higher pressure than the compressed air, is pressed through (the bearings) between the turbines and the rotor, so that a hydrogen vacuum, just as before, may be kept around the rotor. 20 April 1927 dialogue in Stockholm with Lysholm concerning the possibility of building a new centrifuge. He approved of my proposal to drive it with compressed air: it should perhaps be possible to raise the speed to 80 000 rpm with a (rotor) diameter of 15 cm; however, this only if the rotor gets narrower towards the periphery."

duction of a scale method, that a practical solution of the problem could be accomplished. Ole Lamm worked out the theory for the use of the scale method in sedimentation and diffusion experiments [16]. He was also much interested in the theory of sedimentation as well as diffusion [17], and he derived the partial differential equation of the ultracentrifuge [18] for which an approximate solution was given by Faxén [19].

Svedberg was now heavily engaged in studying different types of proteins. Several foreign students came to Uppsala to study these with the ultracentrifuge. Some of the chromoproteins studied sedimented faster than the hemoglobin and were also monodisperse.

The greatest sensation came in 1927 when Svedberg and Chirnoaga [20] studied *Helix pomatia* hemocyanin in the equilibrium ultracentrifuge. According to its copper content it should have a minimum particle weight of 15 000 to 17 000. At equilibrium it should thus show only little change in the concentration between the top and the bottom of the cell. Instead it was found that all the protein had sedimented down to the bottom of the cell, leaving a sharp boundary between protein and solvent. An estimation of the particle size showed that it had to be in the millions. Later it was found that the particles all had the same size. This was the first time such uniformly sized giant molecules had been observed. Furthermore it could also be demonstrated that the hemocyanin showed a constant molecular weight in buffer solutions from pH 4.5 to 7.4, whereas the particle size decreased strongly outside this region [15]. For many years different types of hemocyanins were the favorite proteins studied by Svedberg with the ultracentrifuge.

In September 1927 Lysholm proposed to Svedberg to try a new rotor construction. With some of the new steel alloys and an oval shaped rotor it was thought possible to obtain 200 000 *g* if the diameter of the rotor was increased from 15 cm to 26 cm. Except for the rotor and turbine housing very little change had to be made in the rest of the equipment. There are no direct comments in Svedberg's notes until 15 March 1928, when he writes to Lysholm to tell him that he had new funds for developing the ultracentrifuge. After a number of tests and changes made on the ultracentrifuge during the last six months, Svedberg had now come to the conclusion that they should stick to the oil turbines and not try the air turbine drive. However, a more tenacious steel should be used for the rotor which should be cylindrical with a diameter of 15 cm. The increase in the centrifugal field could be obtained by spinning the rotor at a higher speed.

Before being able to start the calculation for a higher speed rotor, Lysholm needed more information from the old centrifuge on friction in the bearings and in the gas. Results from these measurements were sent to him in the middle of May 1928. At this time, however, Lysholm became chief engineer at Ljungström's Steamturbine Co., Stockholm, and he delegated the further work with the centrifuge to one of his younger engineers, Gustaf Boestad. Incidentally, they both were professors at the University of Technology in Stockholm in the 1940's to 1960's. When in Stockholm, Svedberg often spent some time at Boestad's office. In between they exchanged long letters. In Svedberg's correspondence, the letters to and from Boestad number in the hundreds. Boestad brought new ideas, and he proposed various modifications for the bearings, one of them being a distinct improvement. The old rotor could now easily be run at 45 000 rpm, and 48 000 rpm was even reached once. He could now start the design and calculation of a rotor made with the new steel material.

His first attempt showed that if the rotor was cylindrical with a diameter of 15 cm, it could not stand any higher speed than about 55 000 rpm. He therefore tried Lysholm's proposal of an oval shaped rotor, but with a diameter of only 15 cm. The calculations showed that such a rotor should be able to withstand a speed of up to 60 000 rpm. With the equipment available at Uppsala it would probably be possible to reach a maximum of 55 000 rpm. Boestad and Lysholm recommended that Svedberg try such an oval rotor in the old apparatus. After much reluctance Svedberg agreed, and the material from the steelwork could then be ordered.

Funds for building an Institute of Physical Chemistry had now been granted by the Swedish Parliament, and Svedberg could start planning laboratories specially adapted to the ultracentrifuges. The experience gained with the first ultracentrifuges could be utilized for a complete reconstruction of all of the equipment.

Owing to difficulties with the hardening of the steel at the steel work, the oval rotor was not ready for testing before the end of July 1929. After the first test

run with this rotor, Svedberg writes to Boestad that they had obtained 55 000 rpm without the rotor flying into bits! He expects that when the bearings and journals are run in, they may reach 60 000 rpm, provided the rotor does not explode! Five days later he writes again to Boestad. He is very disappointed. It had been quite hopeless with very bad convection currents. Svedberg wonders if he had been very fortunate with his first oil turbine centrifuge, where the difficulties with convection currents had been less serious. However, no matter how the situation with the oval rotor develops, they must continue their preparation for the new centrifuge. In any event they had to build a new and convenient centrifuge, easier to handle than the old one, even if the centrifugal field was not as strong as hoped by Svedberg. In a very long letter (4 pages) Boestad tries to explain the cause for the convection and proposes certain measures. In the following six weeks they exchange no less than 18 letters concerning a series of new experiments with the oval rotor. Svedberg was still sceptical with regard to this rotor. Finally on September 14 he says it is safer to make the calculations for a cylindrical rotor for the new centrifuge. As a secondary matter one could of course do calculations for an oval rotor as well.

During the next few days Svedberg had great troubles with strong vibrations in the centrifuge. He wondered whether the rotor could be made self-balancing, or one could make some special damping bearings.

After some further tests with the oval rotor during the fall of 1929, it was put aside for a little more than two years. On January 8, 1932 a tiny crack was discovered at the periphery of the old cylindrical rotor. The oval rotor was then put back into the old centrifuge, where it was successfully used for five months before the entire old centrifuge with all its equipment was dismounted and replaced by a second new type centrifuge.

While all the preparations for the construction of the future ultracentrifuges were going on, Svedberg and his students had continued their study of new proteins, and they had found that these were also monodisperse. Very early it appeared to Svedberg that the molecular weight of the proteins, apart from the hemocyanins, could be divided into four subgroups. The molecular masses characteristic of the three higher subgroups are, as a first approximation, derived from the molecular mass of the first subgroup by multiplying by the integers two, three, and six. This hypothesis of the multiples was first presented in a letter to *Nature* on 8 June 1929 [21].

At this time the second low speed centrifuge with gearing drive was supplemented with a new type of low speed centrifuge. Although the former was superior to the first low speed ultracentrifuge, it sometimes showed certain mechanical deficiencies. It was therefore decided to develop a low speed ultracentrifuge with direct motor drive [22]. It was hoped that a symmetrical transfer of the energy of rotation would cause less disturbance, and that it would be possible to select the desired speed easily by varying the frequency of the current feeding the electrical motor. These low speed centrifuges worked satisfactorily, and four of them were eventually installed in Svedberg's new institute. Later on a total of six were made, mainly for laboratories abroad.

During the spring and summer of 1931 Svedberg moved to his new laboratory and test runs were started with the new completely reconstructed ultracentrifuge

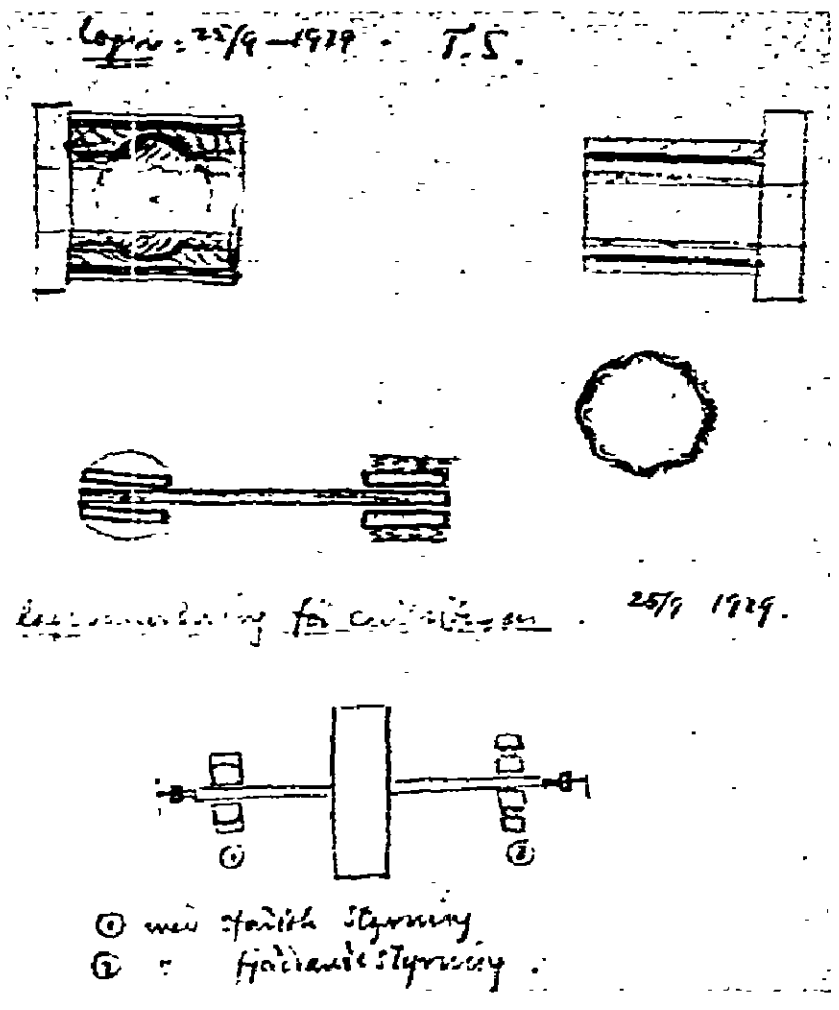

Fig. 7. Svedberg's sketches for some types of damping bearings for reducing or preventing vibrations. (1) by spherical adjustment, (2) by elastic adjustment.

[23]. However, a number of difficulties turned up: difficulty in getting the new cylindrical rotor, designated Rotor I, up to the desired speed of 55 000 rpm, too long accceleration time and too much heating of the rotor. A number of test runs were carried out with various shapes of turbines and turbine chambers, and the bearings were reconstructed to give better lubrication. It was not until June, four months after Rotor I was first tested in the new centrifuge, that it could be run up to 50 000 rpm. When finally a new driving oil with much lower viscosity had been introduced in September, the speed could be increased to 55 000 rpm.

Rotor I was now used by Svedberg for a systematic study of convection currents in the centrifuge cell. He studied how they started and how to avoid them; which precautions were necessary for obtaining convection free sedimentation, and which ones could be omitted. Most of these runs were made with the coloured protein phycocyan. He often emphasized how important it was at the beginning that most of the investigations in the ultracentrifuge were first carried out with coloured metal sols and later with coloured proteins. In this way the convection currents could often be discovered as soon as they started and their development could be followed in detail and related to the experimental conditions, such as temperature distribution and cooling of the system. The effect of changed cooling conditions could often be investigated immediately in an already spoiled experiment.

Only a few routine runs were carried out with Rotor I before it exploded on 4 November 1931, probably due to fatigue. According to Boestad the mistake was that the testing of Rotor I had not been carried out properly. The rotor should have been run at overspeed at an early stage in order to remove the stress peaks in the material, as is the usual practice with steam turbines. In general the highest speed should be attained in the third or fourth run. The maximum test speed was first put 5% higher than the highest permissible speed for routine runs. Later on, after several rotor explosions, it was increased to 10%. For the normal sized rotors the speed of the first test run was taken so low that no permanent stretching of the principal diameter would take place, usually at 50 000 or 60 000 rpm. After the rotor had been up to the maximum speed, the permanent stretching generally amounted to a little less than 0.1 mm.

Immediately plans were made for the construction of a new rotor; some modifications based on the experience gained from Rotor I were introduced in Rotor II, and exactly 3 months after the explosion of Rotor I, the new rotor was tested. It worked satisfactorily and could be used for routine runs almost from the beginning [24], see table 1.

In connection with the protein studies, Svedberg was anxious to increase the "resolving power" of the ultracentrifuge. He had long discussions with Boestad about the possibility of new rotor shapes, and Boestad calculated the advantages of the different designs. The first result of this was an oval Rotor III which was ready for test runs two months after the starting of routine runs with Rotor II. However, there was such a demand for "centrifuge time" on the new ultracentrifuge that we had to wait six weeks before Rotor III could be tested. Finally from 22 to 25 May 1932 it was tested in the following succession: 50 500, 60 000, 65 000, and 70 000 rpm. Rotor III was now ready for routine runs up to 63 000 rpm, and it replaced Rotor II in the new ultracentrifuge, named A. The success with Rotor III resulted in the total abandonment of the cylindrical rotor shape for all the following high speed rotors.

It was important to test different types of steel in order to find the ones best suited for high-speed centrifuge rotors. Since at that time very little was known about the behaviour of metals at such high rotational speeds, the results from the test runs helped in the development of a sounder base for the calculations of rotors of different design.

In the summer of 1932, the first oil turbine ultracentrifuge installation (from 1926) was dismounted, and the laboratory was completely reconstructed, enlarged and made to fit the new type of oil turbine ultracentrifuge. The reconstructed centrifuge laboratory had the great advantage of having the ultracentrifuge (B) itself separated from the observation and control room by a very heavy wall. This gave much better and safer facilities for testing all the new rotor types being planned.

On 3 November 1932 a Rotor IV could be tested in the new B-ultracentrifuge. It was of the same design as Rotor III, the steel was, however, somewhat less hard. It was tested successfully at a maximum speed of 65 000 rpm. Two months later an identical rotor, V, was tested, this time up to 70 000 rpm. It was used for routine runs in the B-centrifuge for almost 3 months

Table 1
Rotors tested 1926 to 1933

Rotor	Diam. (mm)	Shape	Cell hole (mm)	R (mm)	Steel	Maxim. (rpm)	Date	Explod. (rpm)	Date	Cell weight (g)
	150	Cylind.	34	52		43 000 48 000	21 May 1928	[Tiny crack 8 Jan. 1932]		220 177
	150	Oval	34	50		56 400	15 Oct. 1929			169
I	180	Cylind. A	34	65	LLL49 400 B	56 400	[illegible]	50 000	4 Nov. 1931	194 125
III	180	Oval. A	30	65	LLL49 400 B	70 000	25 May 1932	[50 000	1 Jul. 1940]	79
IV	180	Oval. A	30	65	CN 2 400 B	65 000	3 Nov. 1932			79
V	180	Oval. A	30	65	CN 2 400 B	70 000	6 Jan. 1933			79
VI	180	Oval. B	30	65	LLL49 400 B		30 Mar. 1933	58 000	30 Mar. 1933	57
VII	180	Oval. B	30	65	CN 2 400 B	78 000	5 May 1933			57

A detailed description of the individual rotors is given in ref. [36].

until a new differently shaped oval rotor was ready to be tested. This Rotor VI was made of a hard steel similar to rotor III. It exploded in the first test run at 58 000 rpm. The reason for the failure proved to be a so-called "pipe" in the centre. Such "pipes" are sometimes found in the upper end of an ingot, especially in its central part. All the following materials for the rotors were therefore taken from the periphery of the lower end of the ingot.

Just over a month later the next rotor, VII, was ready to test. It was of exactly the same design as the exploded Rotor VI, but Rotor VII was made of the same steel as Rotor IV and V. It was successfully tested at 78 000 rpm and has since been run several times at 75 000 rpm (400 000 g) [25]. Most frequently it was run at 60 000 rpm. There was a problem with vibrations with this rotor; they set in somewhere above 30 000 rpm and disappeared after 44 000, from where the centrifuge ran smoothly. On deceleration strong vibrations set in around 44 000 rpm and disappeared suddenly at 40 000 rpm.

Svedberg was now interested in seeing how much he could increase the centrifugal field above 400 000 g and still make meaningful sedimentation studies. It was quite clear that an appreciable increase in the centrifugal field beyond 400 000 g was not possible with rotors having a diameter of 18 cm as in all the new rotors (Rotor I to VII) and where the distance from the centre of the cell hole to the axis of rotation was set at 65 mm. It was therefore decided to make a small rotor capable of doubling the centrifugal field produced by the normal sized rotors.

Rotor VIII was made of the same steel as used in Rotor VII. It had a diameter of 102 mm and R = 36 mm. At the final test run on 15 December 1933 135 000 rpm was reached [26]. One week later, on the fourth test run with hemoglobin, it exploded right along the axis of rotation at 125 000 rpm. After determination of the hardness of various parts of the exploded rotor, Boestad made new calculations of Rotor VIII. As a result he recommended that the following two small rotors planned be made of a much harder steel than used for the exploded Rotor VIII (see table 2).

Rotor IX was of an entirely different design, having a diameter of 104 mm and R = 36 mm. On 7 June 1934 it was tested at 133 000 rpm. According to the plan it should have been taken up to 160 000 rpm. After a number of tests and changes on the turbines and bearings, etc., it became possible to attain

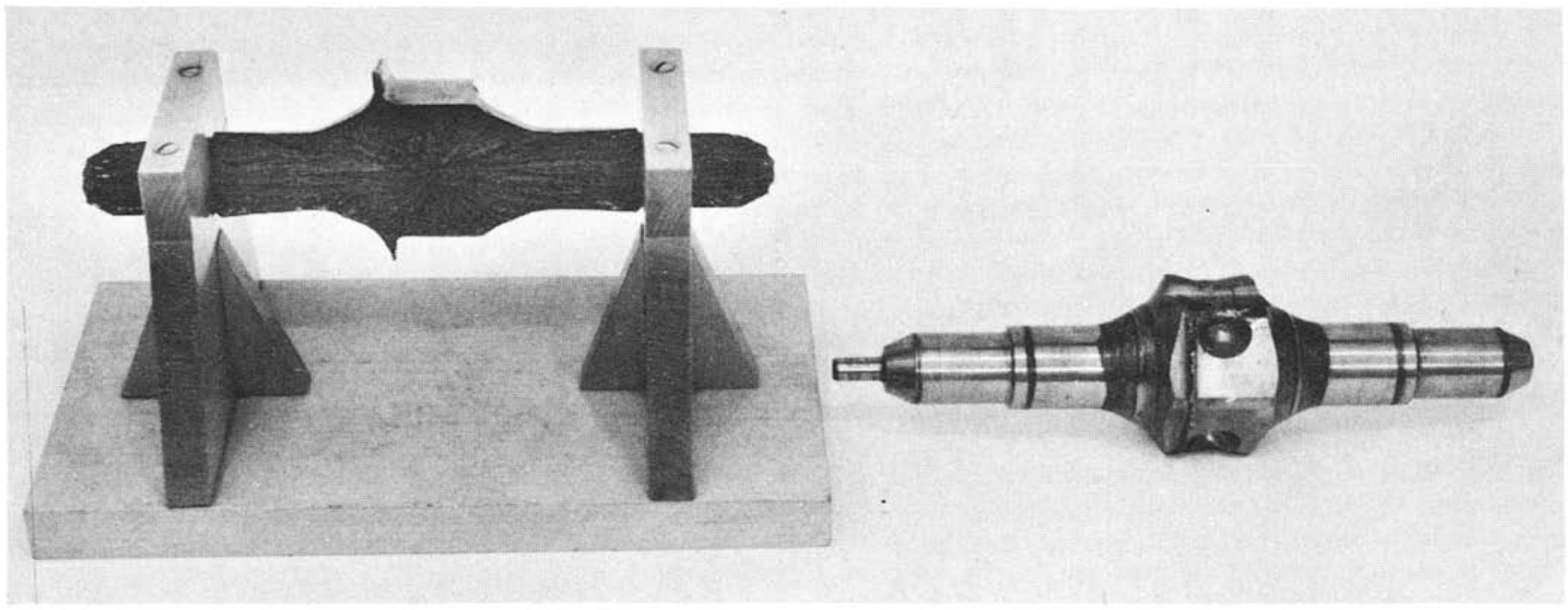

Fig. 8. Rotor VIII (left) after explosion at 125 000 rpm and rotor IX (right) after explosion at 144 000 rpm.

Table 2
Rotors tested 1933 to 1935

Rotor	Diam. (mm.)	Shape	Cell hole (mm)	*R* (mm)	Steel	Maxim. (rpm)	Date	Explod. (rpm)	Date	Cell weight (g)
VIII	102	Oval. C	16	36	CN 2 400 B	135 000	12 Dec. 1933	120 000	20 Dec. 1933	12
IX	104	Oval. D	16	36	LLL 49 470 B	160 000	15 Jun. 1934	145 000	19 Jun. 1934	12
X	104	Oval. E	14	32.5	LLL 49 470 B	155 000	28 Sept. 1934			12
XI	178	Oval. F	19.6	65	LLL 49 470 B	65 000	29 Nov. 1934	50 000	29 Nov. 1934	
XII	178	Oval. F	19.6	65	LLL 49 470 B	80 000	2 Apr. 1935	84 000	3 Apr. 1935	
XIII	178	Oval. F	19.6	65	KRO 866 430 B		23 Jul. 1935	60 000	23 Jul. 1935	

A detailed description of the individual rotors is given in ref. [36].

160 000 rpm on 15 June [27, 28]. However, on the fourth test run with hemoglobin the rotor bursted around the cell holes at 144 000 rpm.

The last small rotor, Rotor X, was of the same steel material as in Rotor IX, but the design was quite different. It also had a diameter of 104 mm and R = 32.5 mm. It was successfully tested in a series of runs at the end of September 1934, where finally 155 000 rpm was attained. A number of runs were carried out to see if these centrifuges could be used for sedimentation velocity runs. Some runs were made at the maximum permissible speed of 140 000 rpm on one of the erythrocruorins with s_{20} below 2S. As could be foreseen from the calculated value for the resolving power of that ultracentrifuge, the experiments were worse than those obtained with the standard sized rotors having R = 65 mm. The tiny cells were also difficult to seal tight and gradually became deformed.

In case of equilibrium determinations, the more intense centrifugal field (up to 700 000 g) obtainable with small rotors could be an advantage, and the low column of solution together with the short distance from the axis of rotation to the centre of the cell should not cause difficulty. A number of sedimentation

equilibrium measurements were carried out at 120 000 rpm (525 000 *g*) on NaCl, LiCl and glycocol and gave satisfactory results [29]. The measurements were complicated by a gradual deformation of the cell. For this reason it was necessary to use an interpolated reference scale obtained from runs before and after the actual equilibrium run.

While the small rotors were of no value for the development of ultracentrifugation technique, the results from the exploded rotors gave valuable information to Boestad for his further work on the design of new rotors.

From time to time the runs in the centrifuge were disturbed by weak vibrations. At the beginning it was usually taken as an indication that the bearings were worn out and had to be renewed and that the journals of the rotor had to be repolished. Usually this helped for some time. As, however, the speed of the centrifuge was increased and the oval type rotors came into use, the problem became more treacherous and serious. The centrifuges could run without trouble for some time and then suddenly one day we would get frightful vibrations and have to stop the centrifuge. Svedberg was very much concerned about the situation. Some of us were suspicious about the balancing, especially the dynamic. Could it be that our balancing machine did not measure a dynamic unbalance properly? Finally in the fall of 1934 it was found that the balancing machine had to be reconstructed so that it could register not only the static, but also the dynamic balance. After the reconstruction it was found that the dynamic balance was much more important for a smooth running of the centrifuge than the static. With the new machine a dynamic unbalance of as little as 10 mg near the cell holes could easily be discovered.

The rotors usually got deformed during the test runs, and some grams of material had to be removed in order to get the rotor back into perfect balance. The cell holes got deformed as well, and their diameters were therefore kept 0.1 mm smaller than necessary for the cells in the routine runs. After the test runs they were ground to the proper size.

The first rotors all became deformed by use, making the cell holes slightly oval. Boestad therefore tried to improve the design of the rotors around the cell holes in such a way that even minor permanent deformations could be prevented during the life time of the rotors. In a first attempt, a series of normally sized rotors was designed in similar way as the small Rotor X, all exactly alike. Two different types of very hard steel were used. The maximum test speed was set to 84 000 rpm. They all failed and exploded through the centre (table 2), Rotor XI in the second run at 50 000 rpm, rotor XII at 84 000 rpm in the fourth test run. Rotor XIII in the first test run at 50 000 rpm. A careful metallographic examination of the pieces from the broken rotors failed to bring out any imperfections in the material. There was no doubt: the design was a mistake.

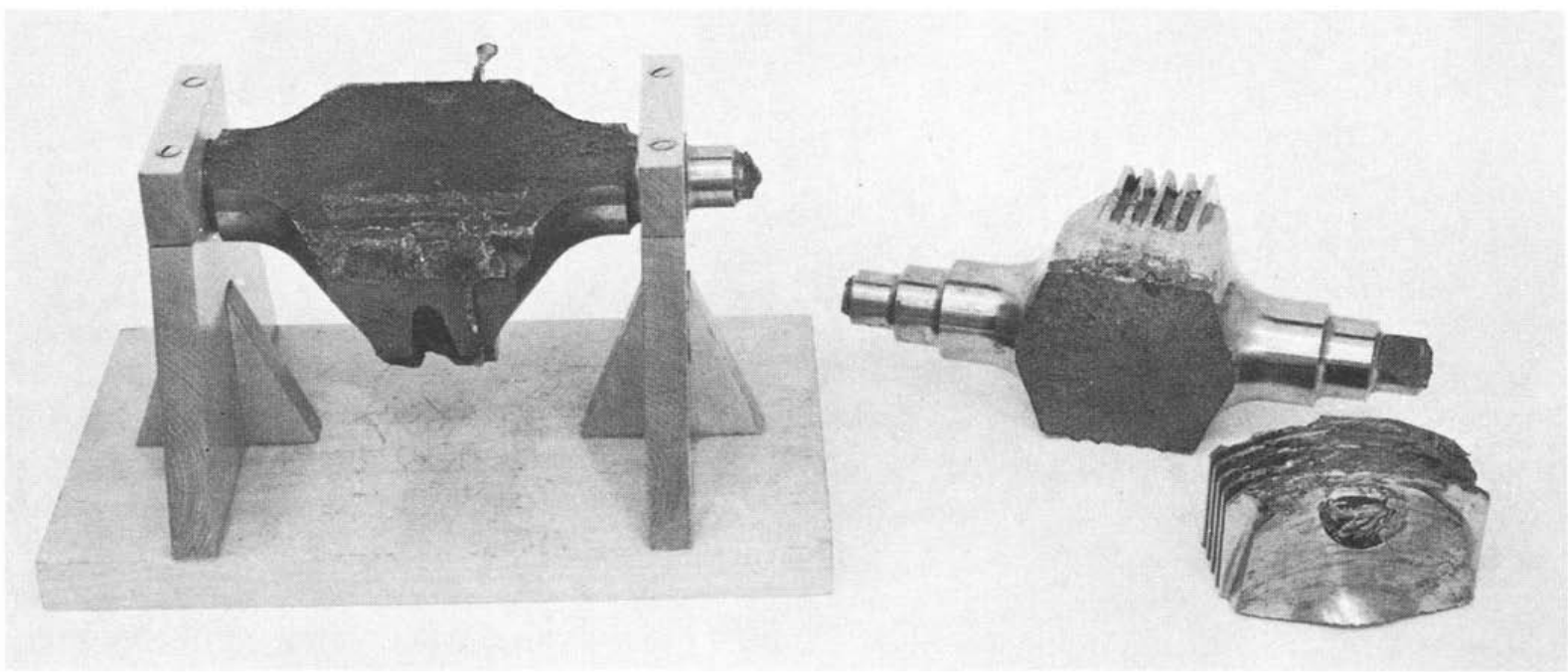

Fig. 9. Rotor XII (left) after explosion at 84 000 rpm and rotor XX after explosion at 61 000 rpm.

Table 3
Rotors tested 1935 to 1945

Rotor	Diam. (mm)	Shape	Cell hole (mm)	*R* (mm)	Steel	Maxim. (rpm)	Date	Explod. (rpm)	Date	Cell weight (g)
XIV	184	Oval G_1	20.4	65	CN 2 400 B	84 000	22 Oct. 1935			23
XV	184	Oval G_2	24.5	65	CN 2 400 B	80 000	27 Jan. 1936			34
XVI	188	Oval G_3	27	65	CN 2 400 B	73 000	14 Mar. 1936			42
XVII	188	Oval G_3	27	65	CN 2 400 B	73 000	9 Jun. 1936			42
XVIII	188	Oval H	27	65	CN 2 400 B		2 Nov. 1936	53 200	2 Nov. 1936	
XIX	188	Oval J_1	27	65	CN 2 400 B	73 000	1 Feb. 1937			42
XX	188	Oval J_2 no vane	27	65	CN 2 400 B	70 000	8 Dec. 1937	61 000	8 Dec. 1937	42
XXI	188	Oval K	27	65	CN 2 400 B	75 000	5 Jan. 1939			42
XXII	188	Oval K	27	65	CN 2 400 B	75 000	30 Sep. 1940			44 (55)
XXIII	188	Oval K	27	65	CN 2 400 B	75 200	14 Dec. 1944			44
XXIV	188	Oval K	27	65	CN 2 400 B	75 000	15 Dec. 1945			44

A detailed description of the individual rotors is given in ref. [36].

Boestad now designed a new series of rotors, this time with maximum diameters of 184 and 188 mm and with three different cell hole diameters. As seen from table 3, all four rotors (XIV to XVII) were successfully tested and have since been used for hundreds of runs. The same is true for rotor XIX which is identical with XVI and XVII except for small twin turbines and thinner journals (10 mm instead of the usual 18 mm).

For special purposes Svedberg was interested in having some extra long cells available. Boestad designed a rotor that would allow a cell thickness of 24 mm as compared with the usual 12 mm. The length of the cell hole was increased from 40 mm to 50 mm. This rotor XVIII exploded at 53 200 rpm during the first test run, probably due to a flaw in the material.

Now the question was: had we reached a practical limit, as regards resolving power, with the rotors XVI, XVII and XIX? Boestad thought that an increase of about 10 percent could be gained if the vanes on the rotor could be omitted. The blank for the rotor could then get a better pretreatment. The omission of the vanes meant at that time a considerable complication with regard to the observation technique. For this purpose an arrangement for synchronization of illumination and exposure was actually worked out by Björnståhl [30, 31]. The rotor XX was made, and for the first time Svedberg was not present at the test runs. He was in bed with influenza. The first two test runs gave the expected small stretching; in the third test run, however, rotor XX exploded. When I called Svedberg on the telephone, I had a definite impression that he felt it as a relief. He had developed his ultracentrifuge to the practical limit of efficiency that was attainable with the materials available at that time. Even if it would have been possible to construct a rotor that could stand a routine speed of 80 000 to 85 000 rpm, we should have had to face several new problems: cracked quartz windows (this was before the availability of sapphire windows), leaking and deformed cells, more complicated technique for the synchronizing of the illumination and exposures.

At one point the rotor design could be improved according to Boestad. Even though the long-time deformation of the cell holes had already been much reduced in the rotors XVI, XVII and XIX, it might have been possible to redesign the surrounding of the cell holes in such a way that they would remain cylindrical for the life time of the rotors. In the final set of rotors, from rotor XXI onwards, all material was removed which was not necessary for carrying a cell weighing, say 44 g, at the maximum speed. If the cell weight had been increased to perhaps 50 g, the rotor would have burst around the cell holes as rotor IX did. All the rotors from rotor XXI have been tested without any mishap. Some of these have been used for thousands of runs. The bearings and the thin journals have worked perfectly and very little overhaul was necessary. Even for running 8 to 10 hours every day there could be 2 to 3 years before the journals had to be reground and polished and the bearings had to be recast. This should be compared with the much shorter time intervals in the early days, when bearings and journals had to be corrected with an interval of a few weeks. Likewise no changes in the dimensions of the cell holes could now be observed even after decades of use.

With the introduction of this last series of rotors, we can say that Svedberg had developed his rotors to the limit possible with the materials available at that time. It does not mean that improvements could not be introduced into the cells and in the ultracentrifugation techniques.

So far I have said very little about the cells. They have given rise to many problems: it was difficult to get them leak proof, the rock-crystal windows often cracked, the cells got deformed, and some cells were more apt to give convection current than others.

As mentioned earlier, Svedberg spent much time studying the problems with convection. He found that the solution in the cell should be well insulated from the metal part of the cell. It is also important that the sedimentation does not start before the centrifuge has reached full speed. There should therefore be *strong convection and mixing* of the cell content during the acceleration of the rotor. By postponing the introduction of the hydrogen until just before the rotor reached full speed, and by controlling the cooling of the turbine oil according to a certain scheme, this problem was solved.

The difficulties with leaking cells at the top speeds were not really solved until the problem with the deformation of the cells had been solved. In the first ultracentrifuge cells the centerpiece with the sector was made of ebonite; for the low speed ultracentrifuges this worked fairly well. As the centrifugal field was increased to 200 000 *g* and to 300 000 *g* this became a serious obstacle. The introduction of bakelite and other synthetic resins was an improvement. Still better results were obtained with a British made reinforced synthetic resin, Tufnol, which was introduced at the end of the thirties. The final solution came more than a decade later, when the sector piece was cast in Araldite around a special frame work of stainless steel wires. Such cells have kept their shape for very long times.

As almost all of the substances studied in the early days of the ultracentrifuge were coloured or had light absorption in the long or in the short wave ultraviolet, it was very convenient to use the light absorption method. At the end of the twenties a small number of runs were carried out with the Lamm scale method. The trouble with this method was at that time that one had to decide upon the scale distance to be used, before the run was started, which limited the use of the scale method, as one would like to have a short scale distance at the beginning of the run, when the refractive index gradient is very steep, and one wants a long scale distance at the end of the run. This is especially true for solutions containing many components such as serum. The breakthrough came when Lamm proposed the use of a scale projecting system, which was used by McFarlane in an extended study of normal and of pathological sera [32].

Svedberg had hoped, already when Fåhraeus worked with him, that the ultracentrifuge would turn up to be of diagnostic value in the study of pathological sera. In some cases new components were observed, usually it was just a variation in the proportions of the different components. As a whole this was a disappointment to Svedberg.

For some years in the 1940's the criteria for Waldenströms macroglobulinea could only be definitely settled by the ultracentrifuge. In 1944 a new serum protein, fetuin was discovered first in serum from newborn calves and later in relative large amounts in other fetal sera [33]. Nowadays the medical people have renamed it α-fetoprotein.

In 1937 we tried to introduce the Toepler "Schlieren" method in the ultracentrifuge, but it never became a success [34]. Likewise the later Philpot–Svensson diagonal slit method was never introduced into the ultracentrifugation technique at Uppsala.

In 1937 we found it necessary to develop a separation cell where we could follow the sedimentation optically during the run as well as analytically after the conclusion of the run. In this way we could ascertain whether an activity actually sedimented at the same rate as the optically observed component [35].

In this paper I have dealt mainly with the technical side of the development of Svedberg's ultracentrifuge. It would, however, not be correct if I did not mention a little more about the proteins.

The first runs on hemoglobin started a new epoch in protein chemistry and most of our knowledge about the molecular weight of proteins has been gained from sedimentation measurements carried out during the last 50 years. On the other hand the results obtained with the proteins greatly stimulated Svedberg to work on the development of the ultracentrifuge. I wonder what would have happened, if Svedberg, instead of the proteins, had started to investigate cellulose or some other macromolecular substances with his first ultracentrifuge. I doubt very much whether he would then have had enough enthusiasm to develop his ultracentrifuges as far as he did. In 1934, when we had all the troubles with vibrations and explosions, he was about to drop any further development. Had it not been for the proteins and his overwhelming interest in the multiple system for the molecular weight of the proteins, he might have stopped further work on the ultracentrifuge.

To sum up, I would say that the introduction of the ultracentrifuge led to the discovery that the proteins are well defined chemical substances, having definite molecular weights. The sedimentation velocity ultracentrifugation, and later also the Tiselius electrophoretic technique, made it possible in a much more direct way to visualize how far the isolation and purification of an individual protein had been successful. It led Svedberg to advance his hypothesis of the multiple system for their molecular weights. Although this hypothesis was not of such general nature as Svedberg first assumed, it meant much for the development of protein chemistry, especially in the 1930's and at the beginning of the 1940's. It initiated greater interest in this group of substances. Several chemists and physicists discovered that the proteins no longer had to be considered ill-defined lyophilic colloids, but rather well-defined exceedingly interesting and important substances, which were well worth studying. The first steps were taken in the new science of molecular biology.

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