AN AIR-DRIVEN ULTRACENTRIFUGE FOR MOLECULAR SEDIMENTATION

By J. BISCOE, E. G. PICKELS, Ph.D., and RALPH W. G. WYCKOFF, Ph.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, and the Laboratories of the International Health Division of the Rockefeller Foundation, New York)

Plates 2 to 4

(Received for publication, April 14, 1936)

Centrifuging at very high speeds is one of the most fruitful methods for studying large molecules in solution. Apparatus for carrying out such studies has been developed by Svedberg[1] over the last 12 years and has been used by him and his students in the investigation of a large number of macromolecular systems. In this equipment high rotational speeds are obtained by driving a large steel rotor with a turbine using oil under several atmospheres pressure. Such a machine is so elaborate and costly both to build and to maintain that as yet no duplicates of it have been put in operation.

The development of usable air-driven turbines for high rotational speeds[2] has opened up the possibility of making comparatively inexpensive ultracentrifuges. One such air-driven machine for molecular sedimentation[3] has already been described; it has the disadvantage with respect to Svedberg's that it uses much smaller rotors and is accordingly incapable of yielding results of equal accuracy. The apparatus described in the present paper is one that we have been developing to provide data needed in studying viruses and crystalline proteins. It has been built in our own shop and the materials necessary for its construction, including the lenses, have cost less than one thousand dollars. In designing this ultracentrifuge we have sought

[1] See Svedberg, T., *Naturwissenschaften*, 1934, **22**, 225 for bibliography.

[2] See Beams, J. W., and Pickels, E. G., *Rev. Scient. Instruments*, 1935, **6**, 299 for bibliography.

[3] McBain, J. W., and O'Sullivan, C. M., *J. Am. Chem. Soc.*, 1935, **57**, 2631.

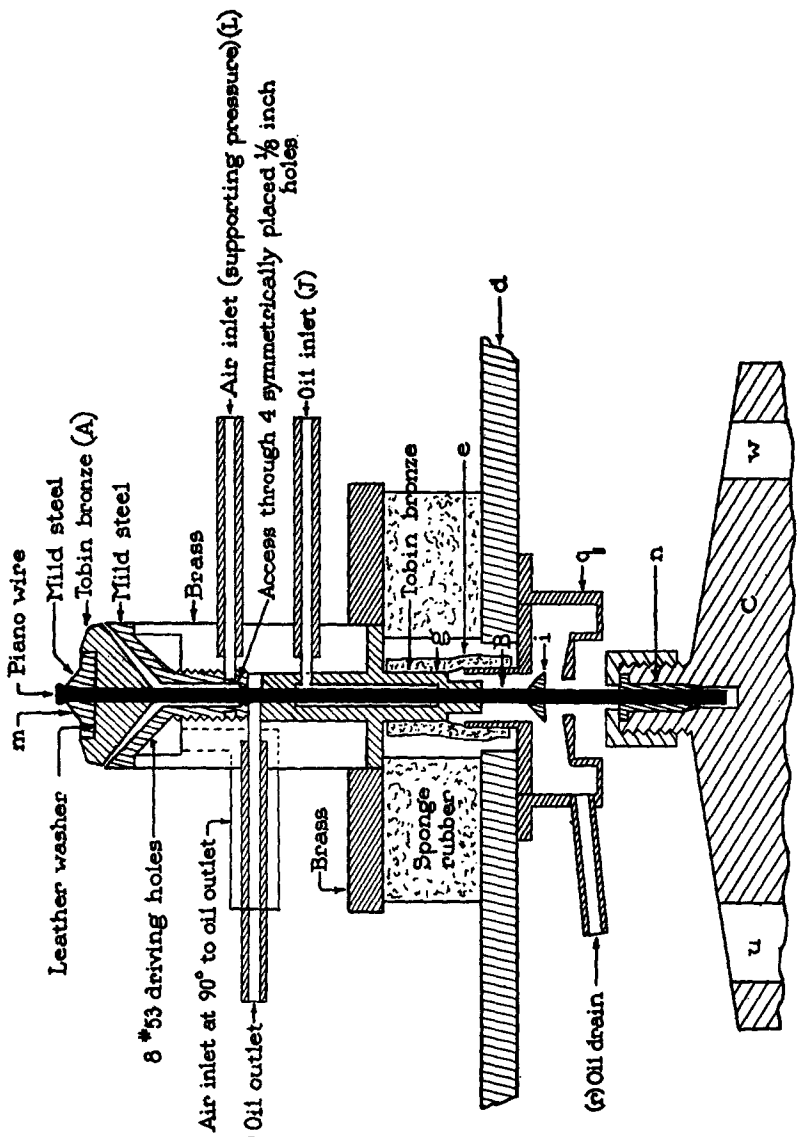
to utilize, to the greatest possible extent, the experience embodied in the publications of Svedberg. We have therefore copied his optical system directly and have employed large rotors capable of giving the 6.5 cm. distance between cell center and rotation axis which he has adopted for his most accurate measurements.

In all forms of the ultracentrifuge for the sedimentation of molecules in solution, a small volume of this solution is enclosed in a cell that can be rotated at high speeds. If the dissolved substance has a density different from that of the solvent, there will be a gradual separation of the two kinds of molecules due to the intense "gravitational" field produced by the rotation. The extent of this separation can be measured by photographing through the moving cell. Two methods for carrying out this photography have been devised, one based on changes in absorption, the other on variations in refractive index. We are using the former in which the chosen light is such that it is transmitted by the solvent but absorbed by the solute. By measuring in this way the rate or the extent of the separation of solvent and solute data are obtained that give an indication of the weight and shape of the dissolved molecules. From this outline it can be seen that the essentials of an ultracentrifuge consist of (1) a rotor containing the cell and its solution so mounted that it can turn at high speeds, (2) a driving mechanism for the rotor and (3) a camera and suitable light source for obtaining pictures through the rotating solution.

General Description

The general arrangement of these essential parts in our apparatus, similarly lettered in the drawing and the pictures, is shown in Text-fig. 1 and Figs. 1–4. The driving mechanism, patterned after that of Beams and Pickels,[2] is a small bronze air turbine (*A*). Attached to this turbine is a steel shaft (*B*) that, passing through an oil-sealed bearing (*g*), supports the large duralumin rotor (*C*) turning in the vacuum chamber (*D*). In obtaining photographs of the rotating solution, suitably filtered light from a mercury vapor arc (*E*) passes into the vacuum chamber through a bottom quartz window, then intermittently through the solution cell in the turning rotor, out through another quartz window (*a*) and along the camera tube (*F*) to be registered on a plate in the holder (*G*).

A cross section through the driving mechanism is shown in Text-fig. 1. Details of construction not shown in this figure, such as the nature of the flutings to be milled in the turbine and the positions of the air ports in the conical driver, are to be found in the paper by Beams and Pickels. The necessary air-tight but



TEXT-FIG. 1. A cross sectional drawing of the rotating system of the ultracentrifuge. Parts lettered in this and the plates are described in the text.

flexible connection between the driver and the top (*d*) of the vacuum chamber is provided by a short length of rubber pressure tubing (*e*). If the rotor is to turn smoothly the bearings must be carefully and accurately reamed to fit closely the chosen shaft; furthermore this shaft should be straight and precisely centered in the turbine clutch (*m*). It is equally important that the rotor clutch (*n*) be exactly in the axis of rotation and that the dummy cell (*w*) be of such a weight that when loaded with its cell the rotor is well balanced about the axis.

The speed of the turbine and rotor is determined by observing with a stroboscope a spot of paint on the top of the turbine. The stroboscope consists of a slotted disc (*Q*) mounted, together with a small magneto, on the shaft of a storage battery driven motor. Speeds can thus be read directly in terms of the voltage developed by the magneto. The necessary compressed air is drawn from the house high pressure lines. A very small flow (a few cubic centimeters per hour) at 5–15 lb. pressure is used to force oil from the tank (*H*) through the inlet (*J*) into the pocket between the bearings. Oil passing the upper bearing drips from the outlet (*K*); that passing the lower bearing is thrown clear of the shaft by a conical deflector (*i*), falls into a well (*q*) and drips through (*r*) into a collector attached to the vacuum chamber. At low speeds it is desirable to use a supporting air column for the rotating system. For this purpose air at 30–40 lb. pressure is introduced through the inlet (*L*); at higher speeds, above *ca.* 200 R.P.S., this air stream is not of advantage. The driving air, at pressures up to *ca.* 100 lb./sq. in., is fed into the inlet (*M*). The pressure on the usual compressed air line is variable so that some kind of regulation is needed to drive the rotor at constant speed. For studies of sedimentation velocity it is possible to obtain a sufficiently good manual control using a well made gate valve as a reducer. It is, however, better to install an automatic pressure regulating reducer for sedimentation rate determination, and one is essential for the long equilibrium runs. Several such reducing valves capable of handling a sufficiently large air flow are made commercially; one is shown at (*N*). At low speeds this regulator results in rotor speeds that are constant within 1 per cent even when the line pressure varies by 40–50 lb./sq. in.; at the highest speeds the regulator has given constancy within *ca.* 2 per cent but this can be improved either by manual help or by having a steadier line pressure. It is very important that the incoming air be clean, both for the proper functioning of such a regulator as (*N*) and to prevent clogging of the driving ports. One of the numerous commercially available compressed air filters should therefore be installed close to the regulator, and brass and rubber rather than iron piping should be used between the filter and the turbine.

The optimum diameter of the steel driving shaft will depend on the speed at which the ultracentrifuge is to operate. For higher speeds (*ca.* 300–900 R.P.S.) piano wire or drill rod 0.100–0.110 inch thick is satisfactory. It is essential that the shaft be strictly uniform in diameter, and straight. While a shaft of this size can be used for speeds below *ca.* 300 R.P.S. the resulting system is liable to show several regions of mechanical vibration when turning slowly. These vibrations disappear if the shaft is of 3/16 inch drill rod. This larger shaft of course presents

a greater bearing surface, necessitating a greater driving air pressure and a somewhat increased flow of oil.

The rotors for this ultracentrifuge have thus far been of duralumin. A study[4] has been made to establish a suitable rotor shape and to determine the maximum speed that can safely be attained with the commercially available alloys. A rotor of the chosen shape is shown at (*C*). It is 7 inches in diameter and of a tapering thickness that is 1 inch at the edge and 2 inches near the axis; the outside edge of the cell hole is half an inch from the periphery. The maximum permissible speed for such a rotor made of ordinary duralumin (17 *ST*) is slightly over 800 R.P.S.; if alloy 14 ST is used, 900 R.P.S. are possible. At 800 R.P.S. the field at the center of the cell is 180,000 times gravity; at 900 R.P.S. it is 225,000 gravity. These experiments on rotors have also indicated the amount of shielding that must be provided against bursting rotors. The top and bottom plates (*d*) of the vacuum chamber are of half inch boiler plate, the cylinder (*D*) is of 1½ inch thick heat-treated chrom-vanadium steel. Protection from a broken turbine is furnished by the three sided wooden barricade (*P*) filled with sand.

When in operation a vacuum of less than 1 mm. of mercury is maintained in the chamber (*D*) by a fast oil pump operating through the port (*k*). Residual air pressure is indicated by a gauge (*p*) connecting with (*t*). The cylinder (*D*) and the top and bottom plates (*d*) are sealed by soft rubber rings.

The liquid to be centrifuged is contained in a cell (*u*) that is essentially the same as Svedberg's. It differs mainly in that, being fillable from the end during assembly, an oil seal is unnecessary.

The optical system for absorption measurements of the extent of sedimentation is indicated in Figs. 1, 3, and 4. In our work we are dealing with proteins, all of which show strong absorption for ultraviolet wave lengths shorter than *ca.* 2750 A. Combined chlorine and bromine filters of appropriate thickness (8 cm. of chlorine gas, 2.5 cm. of bromine vapor at room temperature) absorb all wave lengths between the green and *ca.* 2700 A. Distribution of protein within the cell can therefore be recorded on photographic plates sensitive only to the blue (and ultraviolet) by employing the light from a quartz mercury lamp after passage through these filters. In Fig. 4 light from the mercury arc in (*E*) is rendered roughly parallel by the quartz condensing lens (*R*) and, passing through the bromine (*S*) and chlorine (*T*) filters, is reflected by the aluminum-sputtered mirror (*V*) through quartz windows into the vacuum chamber (*D*). The light through the cell emerges through a second window (*a*) in the top of the chamber, is reflected by a second aluminum mirror in (*W*) and is focused by the 100 cm. focal length quartz-fluorite lens at (*X*) upon the plate at (*G*). The total distance from the rotor *C* to the plate *G* is 4½ meters; the lens (*X*) can move through 12 cm. about a point in (*F*) 3 meters distant from (*G*). When in operation exposures

[4] Biscoe, J., Pickels, E. G. and Wyckoff, R. W. G., *Rev. Scient. Instruments*, 1936, in press.

are made with an electromagnetically operated timing shutter placed between the condensing lens (*R*) and the filter (*S*). A rotating shutter of variable aperture (not shown) is used to lengthen the exposure when making the variously timed exposures required for plate calibration. The exposure varies from 1–10 seconds depending on the type of photographic plate used and the voltage drop across the lamp. With a camera as long as that shown, precautions must be taken to prevent vibration. This has been done by mounting the tube on Lally columns (Y_1, Y_2, Y_3). The bases of these columns, as well as the posts (*Z*) that support the ultracentrifuge, are all imbedded in blocks of cement themselves supported on half inch thick rubber pads.

Provision has been made for simultaneous measurements by the absorption and refractive index methods. To do this a second set of windows (*b*) and a second, and longer, camera tube (*f*) have been placed at right angles to those for absorption.

In order to have accurate measurements of sedimentation it is necessary to know the temperature of the sample during the experiment. There are two ways in which the rotating system could become heated. One is through friction developed in the shaft bearings (*g*), the other is through frictional interaction between the rotor and the small amount of gas remaining in the evacuated chamber. We have made a number of experiments to estimate the amount of this heating. In one set a thermocouple was inserted into the lower bearing (*g*) and a second couple of very low heat capacity was mounted within the chamber along the axis of the turning rotor, and close to it. In other tests the ultracentrifuge was operated at high speed for many hours, stopped as quickly as possible and the rotor temperature compared with that of the chamber walls. Having also determined the rate of cooling of a stationary rotor it was possible to estimate the maximum temperature of the turning rotor. These trials have shown that under our conditions of operation the maximum rise in temperature for runs at 800 R.P.S. is of the order of 2°C.; at lower speeds it is even less. The switchboard used for handling these thermocouple measurements during runs is shown at (*h*).

In order to check the operation of the ultracentrifuge before using it upon proteins of unknown molecular weights, a series of determinations was made of the sedimentation constant of horse hemoglobin that had been repeatedly recrystallized according to the methods of Heidelberger.[5] One of these photographs made with the rotor turning at 800 R.P.S. is shown in Fig. 5. Sedimentation constants from individual experiments differed from the mean by 0.1×10^{-13} cm./sec. Several systems consisting of viruses and of crystallizable proteins are now being analyzed with this instrument. Results obtained with

[5] Heidelberger, M., *J. Biol. Chem.*, 1922, **53**, 31.

various strains of the crystalline proteins[6] of the tobacco mosaic disease will shortly be published; one of the photographs from this study illustrating the sedimentation of a large molecule at low speeds is reproduced in Fig. 6.

We are indebted to Mr. J. B. Lagsdin for much help in connection with the design and building of this apparatus.

SUMMARY

Details of construction are given for an air-driven ultracentrifuge for molecular sedimentation. This instrument, like the standard oil-driven machine of Svedberg, uses rotors giving a 6.50 cm. radius of rotation and has cameras of great depth of focus.

EXPLANATION OF PLATES

PLATE 2

FIG. 1. A view of the control panel and the absorption camera of the ultracentrifuge.

FIG. 2. A photograph of the rotating system removed from the vacuum chamber. *Cf.* Text-fig. 1.

PLATE 3

FIG. 3. A view of the centrifuge and its mountings.

PLATE 4

FIG. 4. A view of the optical system used for the ultraviolet photography of proteins by the absorption method.

FIG. 5. A series of pictures showing the course of sedimentation of hemoglobin molecules. Light source: visible light from incandescent lamp. Exposure time: 2 seconds. Speed: 800 R.P.S. Interval between exposures: 10 minutes.

FIG. 6. A similar picture showing sedimentation of the very heavy crystalline protein[6] of the tobacco mosaic disease. Light source: ultraviolet light after filtration through chlorine and bromine cells. Exposure time: 4 seconds. Speed: 140 R.P.S. Intervals between exposures: 5 minutes.

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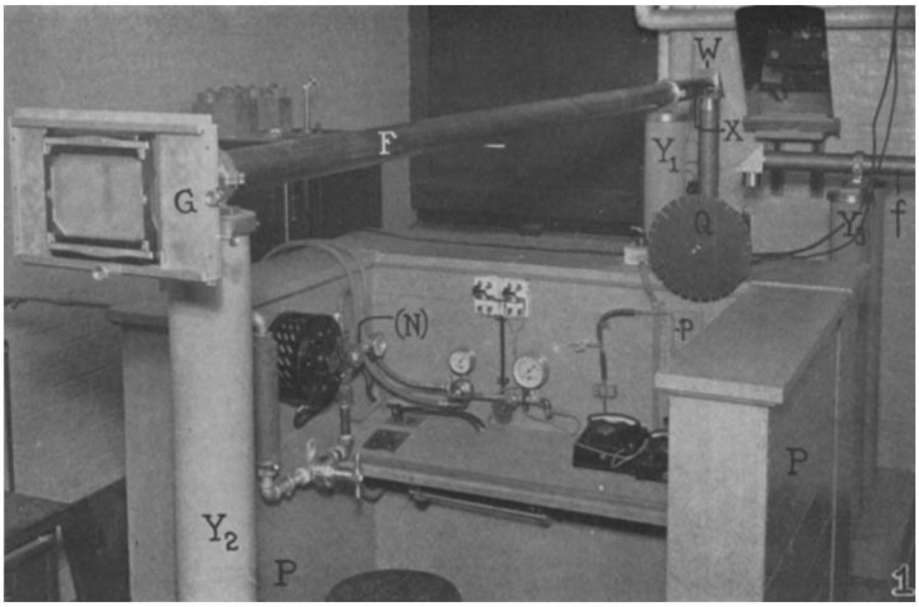
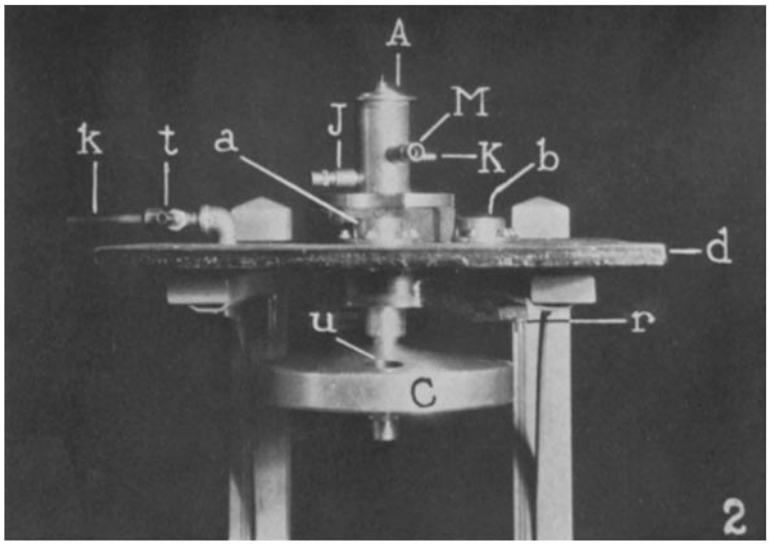
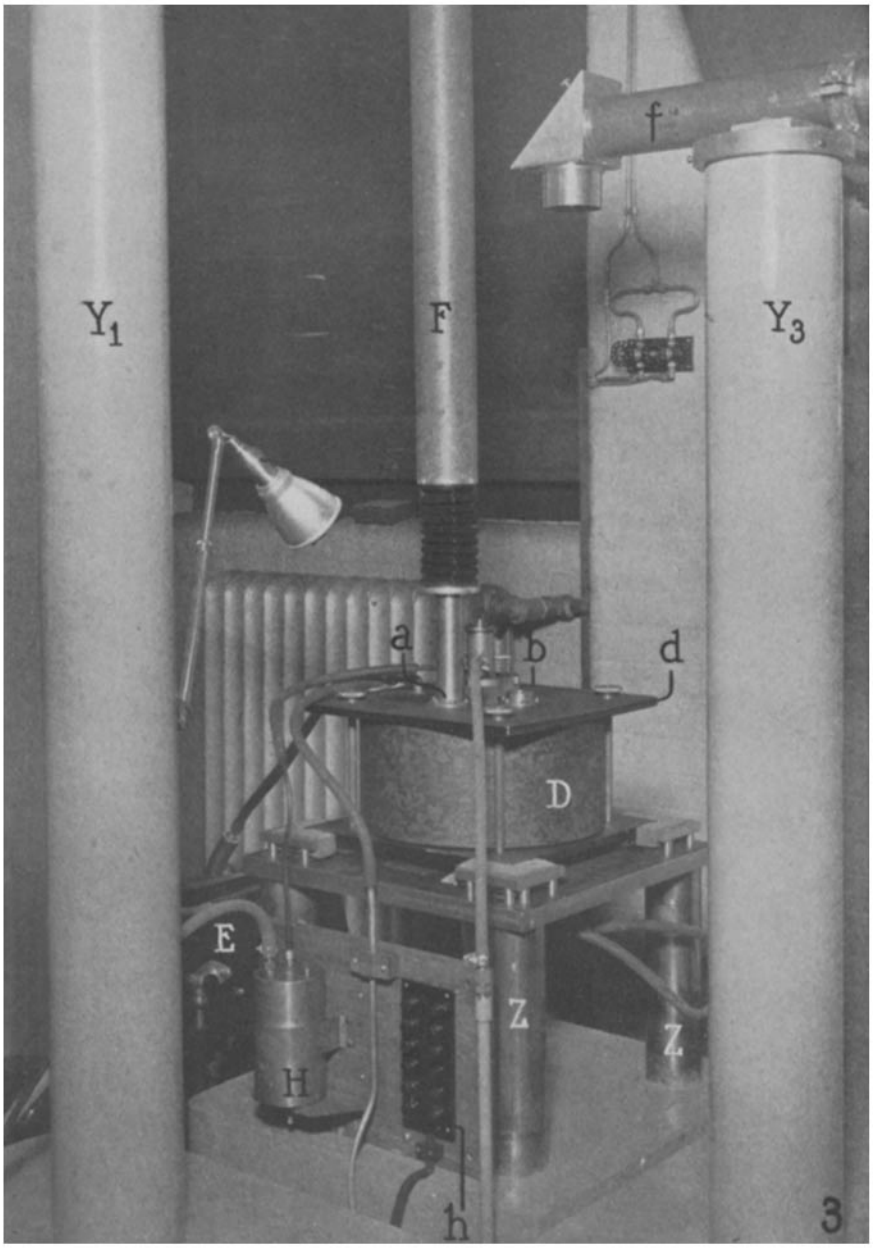
[6] Stanley, W. M., *Science*, 1935, **81**, 644.





(Biscoe *et al.*: Molecular ultracentrifuge)



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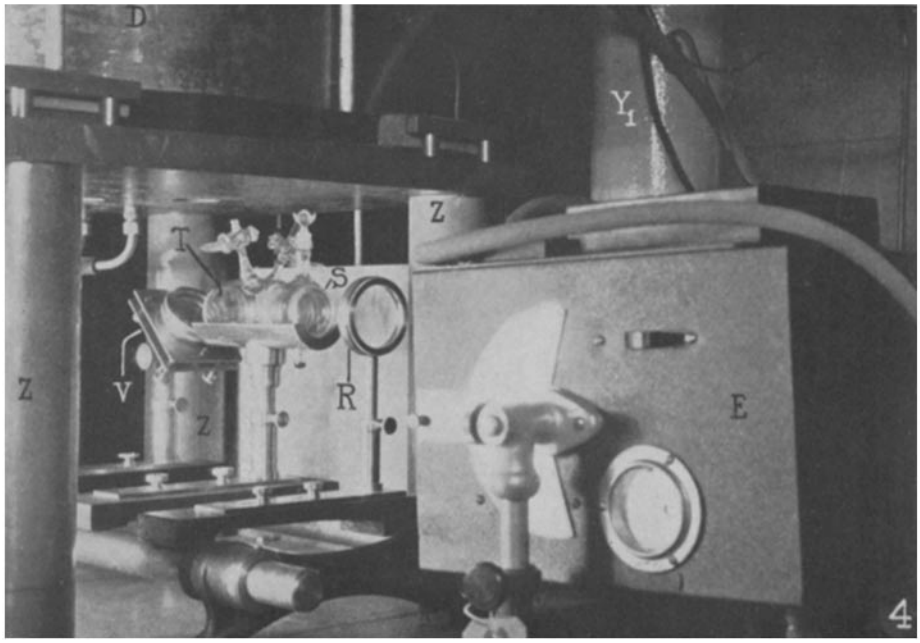

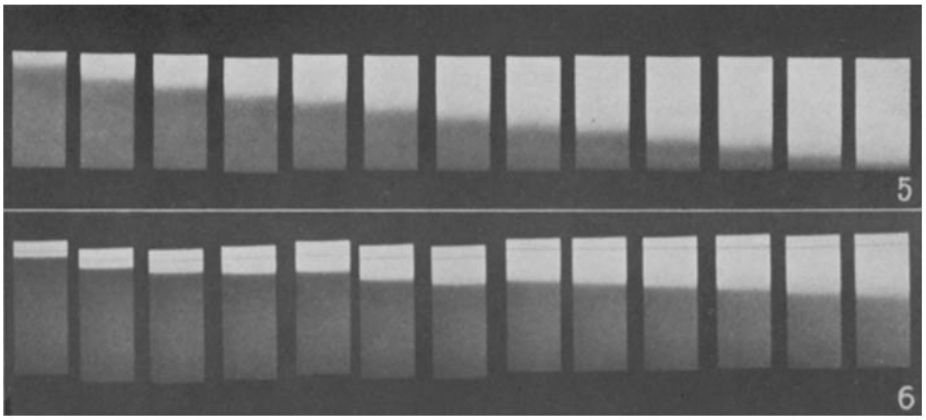

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