

tured protein spread on the surface of the water should have a side-chain spacing of about 10 A.U. No spacing of this order of magnitude could be detected on the present x-ray patterns. On the other hand, a long spacing of the order found may well be in accord with the theory of protein structure which pictures

them as polymerized cyclols, propounded by Dr. Wrinch.⁶

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A TUBULAR VACUUM TYPE CENTRIFUGE

THE air-driven vacuum type ultracentrifuge¹ has been found to be an efficient apparatus for purifying many different organic and inorganic materials. It has proven useful especially in the separation and purification of biological substances such as the so-called "macromolecular proteins."^{2,3} In this work it is standard practice to centrifuge the liquid in a rotor the diameter of which is greater or approximately equal to its length. The rotor is supported in a vacuum chamber by a single flexible shaft which passes out of the chamber through a vacuum-tight gland and connects with an air-supported air-driven turbine above the chamber. The rotor is constructed to hold several test-tube containers for liquid and is equipped with a vacuum-tight cover.² Consequently, in making a single separation it is necessary for the operator not only to start and stop the centrifuge but to evacuate the chamber containing the rotor as well. Since the largest rotors^{2,3} employed so far hold only from 120 to 150 cc, this procedure may become time-consuming if large quantities of material are to be centrifuged. Clearly, if the apparatus could be modified so that the materials could be passed continuously (or intermittently) through the centrifuge and the lighter and heavier fractions separately collected (in the manner of the familiar cream separator), without stopping the centrifuge or without lowering the efficiency of separation appreciably, the quantity centrifuged would be much increased.

In connection with a problem on the separation of gases (isotopes), we have been spinning long cylindrical tubes in such a manner that the gas enters at the top and is collected in two fractions at the bottom. Recently the same type of apparatus has been applied to the separation of liquids and has proven effective enough to warrant possibly a short description.

In Fig. 1 the cylindrical rotor (centrifuge) C is supported inside the vacuum chamber V by a stainless steel tube A (gauge 12).⁴ A passes through the vacuum-tight gland G₁ and is attached to the air-supported air-driven turbine T. The construction and operation of this type of turbine and vacuum gland

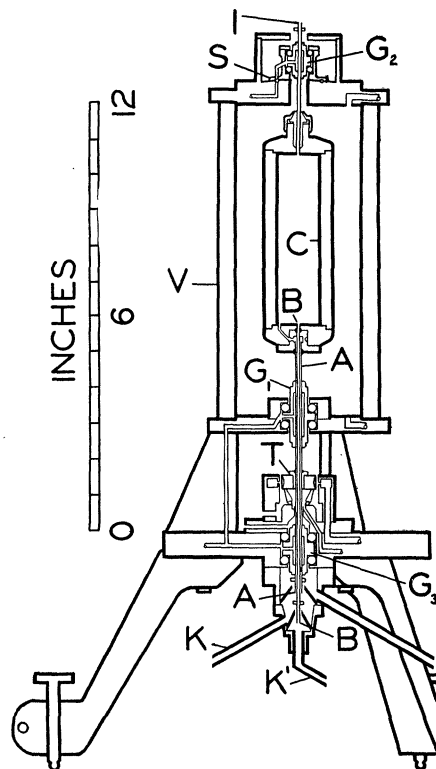


FIG. 1.

have been previously described.^{1,5} A second stainless steel tube B (gauge 14 or 15) is mounted inside and coaxial with A in such a manner that A communicates with the periphery, while B connects with the axis of C. A third stainless steel tube I (gauge 15 or greater) is mounted above and in the axis of C as shown. I passes out of the vacuum chamber through the vacuum-tight oil gland G₂, which is mounted in a special self-aligning vacuum-tight bearing S. The bearing S consists of two carefully ground or lapped surfaces separated by a thin film of vacuum pump oil, continuously supplied under pressure, requiring a few cc per hour. Although this bearing is almost ideal for the purpose, it may be dispensed with by mounting G₂ in, for example, flexible rubber.

To operate the centrifuge, first, the chamber V is evacuated and the centrifuge accelerated to the desired

¹ Beams and Pickels, R. S. I., 6: 299, 1935.

² Bauer and Pickels, *Jour. Exp. Med.*, 64: 503, 1936; 65: 565, 1937.

³ Wyckoff, *SCIENCE*, 86: 92, 1937.

⁴ Obtained from the Jensen-Salsbery Inc., Kansas City.

⁶ Dr. Dorothy Wrinch, *Proc. Roy. Soc.*, A 160, 59, 1937.

⁵ Beams and Linke, R. S. I., 8: 160, 1937.

rotational speed. Next, the liquid to be centrifuged is injected (by a hypodermic syringe) into I until C is filled, that is, until a slight amount of liquid overflows into the tubes A and B. Following this, the centrifuge is run at a constant speed until the required separation has taken place. More liquid is then injected into the tube I and the resultant forces push the heavier fraction out of A into the collector K and the lighter fraction out of B into a second collector K'. Immediately upon entering C, the liquid mixture begins to separate so that as the successive amounts are introduced into I the separated fractions are collected in K and K'. Upon inspection of Fig. 1 it might appear that all the liquid would flow out of A, since the diameter of A is greater than that of B. However, in practice this is not the case because the material emerging through A has a slightly increased density, the diameter of I is less than that of B, and the liquid mixture is introduced rapidly for short times instead of continuously. The amount of material introduced at one time in this manner must not be great enough to alter appreciably the rotational speed of C. If it is desired to introduce the liquid mixture continuously at I and at the same time accurately control the amount of heavy to light fractions, the vacuum or liquid-tight gland G_4 may be added as shown in Fig. 2. This arrangement of Fig. 2 is used in the separation of gases.

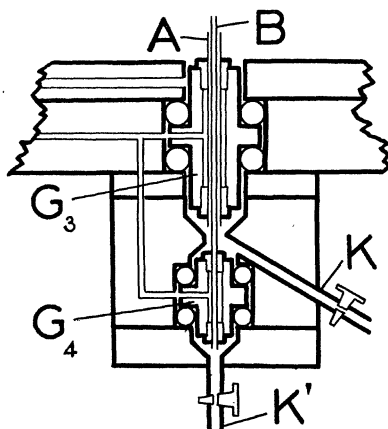


Fig. 2.

In the above apparatus, the quantity of liquid which can be separated in a given interval of time is directly proportional to the length of the rotating cylinder C. Furthermore, it can be shown that the rate of separation is greater the smaller the diameter of the tube C, provided C is spun to almost its bursting speed. Since the maximum speed to which C may be spun is set only by the bursting strength of C, it is advantageous to employ long small-diameter tubes for the centrifuge. Two-inch cylinders two feet in length have been spun successfully.

With the apparatus of Fig. 1, we obtained an easily observable separation of hemoglobin (kindly prepared by Professor Alfred Chanutin) from the solution when it was introduced at the rate of from 20 to 30 cc per hour with C spinning only 1000 r.p.s.

Unfortunately, the tubular rotor is not as strong as certain other shapes. However, by employing high strength alloy steels and special construction, it is probable that tubular rotors may be built, the bursting speed of which can be made to approach the best rotors of the same effective diameter that have been in practical use. Also the efficiency of C might be increased by mounting properly designed plates or discs inside it.

It is of course possible to replace the tube C in Fig. 1 by other shapes (the angle centrifuge, for example) obtaining, with a slight change in internal construction, the advantage of continuous quantity separation. Further, the use of three or more hollow, concentric flexible shafts and vacuum-tight glands similar to those in Fig. 2 would make it possible to adapt the ordinary type of air-driven ultracentrifuge to continuous operation.

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