# A HIGH SPEED VACUUM CENTRIFUGE SUITABLE FOR THE STUDY OF FILTERABLE VIRUSES

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The study of filterable disease-producing agents has been much handicapped by the lack of suitable apparatus. The microscope, the ordinary laboratory centrifuge, and culture medium, all of which are invaluable in the study of disease-producing bacteria, are of no assistance in the study of viruses. Viruses associated with disease in animal and man are also always associated with a large amount of extraneous protein matter originating from the host, and no satisfactory method has yet been developed by which these pathogenic agents can be induced into a pure and concentrated state so that their specific antigenic properties and physicochemical characteristics can be properly investigated. Recently Stanley (1) reported success in the preparation of the virus of tobacco mosaic in crystalline form by the extraction and salting-out process, but as yet this method has not found wide application in the study of animal and human viruses. As is well known, most of the viruses pathogenic to animal and man are rather labile and become easily inactivated by the processes involved in chemical and physical investigations, and since the presence of a virus in a given substance can be demonstrated only by its specific action in a susceptible animal, these methods have proven of little value.

The work of Craigie (2) and Parker and Rivers (3) on the vaccinia virus has shown that a centrifuge by which the elementary bodies can be not only separated from the associated protein matter but washed and concentrated as well, can be an exceedingly useful tool in the study of this virus. Similar application of the centrifuge to a considerable number of other viruses has not yielded equally satisfactory results. Within recent years, through the work of Elford (4) and Bauer and

Hughes (5), a method for the preparation of finely graded collodion membranes of uniform porosity has become available. By the use of these membranes the particle size of a number of viruses has been measured by various investigators. The results of these investigations are summarized in Table I. A glance at this table reveals a wide variation in their size. Assuming arbitrarily that virus particles are more or less spherical bodies with a density equal to that of most proteins, a calculation from the familiar Stokes' law would indicate that for a given length of time a centrifugal force several hundred times greater would be required to sediment the virus of foot and mouth disease than was successfully applied to vaccinia virus by the workers mentioned above. This will explain, at least in part, why most of the centrifugation experiments with various viruses in the past have not been successful. It is true that there are a number of commercial centrifuges available which give fairly high rotational speeds although most of them have a relatively short effective radius. But as they are generally designed to rotate in air, their speed is necessarily limited by the air resistance. Moreover, in such centrifuges, with the increase of speed a correspondingly greater amount of frictional heat is developed in the rotating head. This heating is not only responsible for convection currents which interfere with effective separation, but the temperature frequently rises to such a critical degree that denaturation and inactivation of the material during centrifugation may result.

Recently we reported (28) the construction of an air-driven centrifuge built on the principles described by Beams, Weed, and Pickels (29). This centrifuge was primarily intended for the separation and concentration of the yellow fever virus which, as shown in Table I, is one of the smallest viruses and which is of particular interest to us. Good separation was obtained in this centrifuge with some of the larger viruses, such as that of vesicular stomatitis, but the results with the yellow fever virus were entirely negative. Because this centrifuge rotates in open air and has a large surface area exposed to air drag, its speed is necessarily limited; and although an air pressure as high as 225 pounds per square inch was applied, a speed of 16,000 revolutions per minute could not be exceeded. This corresponds to a centrifugal force at the top portion of the fluid of only about 13,500

times gravity. Obviously this speed was insufficient, since it was estimated that a centrifugal force of at least 30,000 times gravity must be exerted before a definite sedimentation of yellow fever virus could be expected within a reasonable period of time. It was obvious also that any type of centrifuge designed to give the desired centrifugal force must be protected from air friction. In the centrifuge described

TABLE I

*Approximate Particle Size of Viruses as Determined by Filtration through Graded Collodion Membranes*

| Virus | Estimated particle size | Authority |
|---|---|---|
| | *millimicrons* | |
| Vaccinia | 125–175 | Elford and Andrewes (6) |
| Canary pox | 125–175 | Burnet (7) |
| Lymphogranuloma inguinale | 125–175 | Broom and Findlay (8) |
| Rous sarcoma 1 | 100–150 | Elford and Andrewes (9) |
| Ectromelia | 100–150 | Barnard and Elford (10) |
| Pseudorabies | 100–150 | Elford and Galloway (11) |
| Herpes | 100–150 | Elford, Perdrau, and Smith (12) |
| Borna disease | 85–125 | Elford, Galloway, and Barnard (13) |
| Influenza, swine and human | 80–120 | Elford, Andrewes, and Tang (14) |
| Vesicular stomatitis | 70–100 | Galloway and Elford (15); Bauer and Cox (16) |
| Fowl plague | 60–90 | Elford and Todd (17) |
| Rift Valley fever | 23–35 | Broom and Findlay (18) |
| Equine encephalomyelitis | 20–30 | Bauer, Cox, and Olitsky (19) |
| St. Louis encephalitis | 20–30 | Bauer, Fite, and Webster (20); Elford and Perdrau (21) |
| Yellow fever | 17–25 | Findlay and Broom (22); Bauer and Hughes (23) |
| Louping ill | 15–20 | Elford and Galloway (24) |
| Poliomyelitis | 10–15 | Theiler and Bauer (25); Elford, Galloway, and Perdrau (26) |
| Foot and mouth disease | 8–12 | Galloway and Elford (27) |

below the difficulties were entirely overcome when the rotor was placed in very high vacuum, and the results obtained with this machine have been satisfactory.

*Driving Mechanism.*—The driving mechanism is a modification of those described by Beams and Pickels (30) and later by Biscoe, Pickels, and Wyckoff (31). It is shown in Figs. 1, 2, 3, 4, and 5. Figs. 1 and 2 show vertical cross-sectional

views of the essential parts. The horizontal cross-sectional view in Fig. 3 gives further details of the turbine and driving jets. Fig. 4 is an actual photograph of the turbine and air-bearing units, and Fig. 5 pictures the complete driving assembly.

The rotating members are the turbine (*1*, Figs. 1, 3, 4), the drive shaft (*2*, Figs. 1, 4), and the rotor (*3*, Figs. 1, 5). The turbine is made of light phosphor bronze and the angle of its cone-shaped base is 90°. It is hollowed out to reduce its

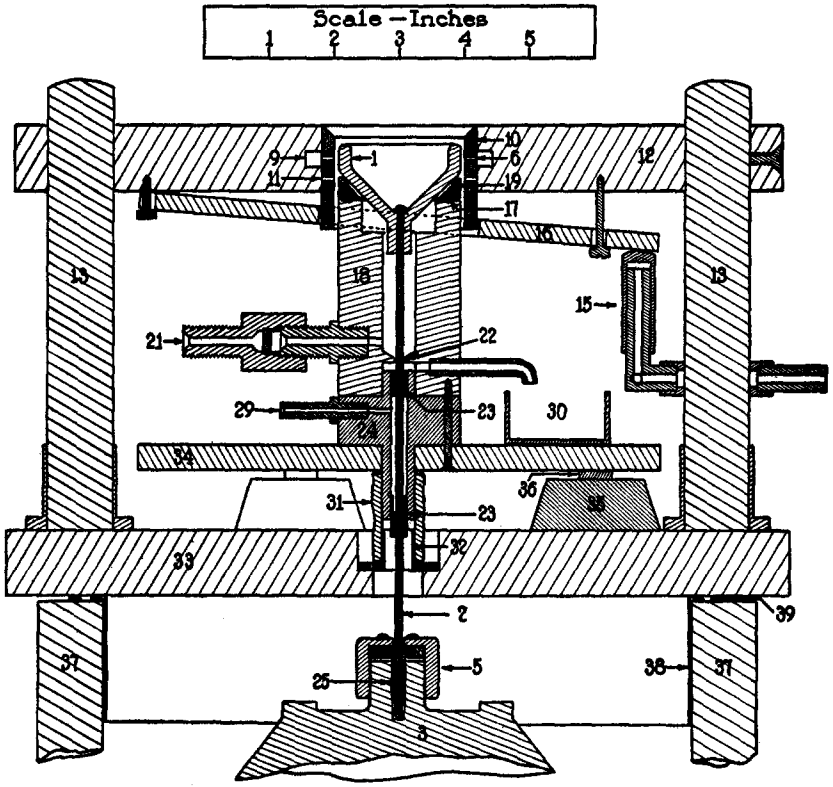


FIG. 1. Vertical cross-sectional view of the entire driving mechanism.

weight and is provided with nineteen flutings cut with a $\frac{3}{8}$ inch dovetail milling cutter to a depth (along the radius of the turbine) of about $\frac{3}{32}$ inch. The shaft is a straightened section of spring steel wire having a diameter of $\frac{1}{10}$ inch. Its upper end is snugly fitted and soldered into the turbine (care being taken not to overheat the wire and cause a loss of its temper) and its lower end is fastened to the rotor by a special chuck arrangement (*5*, Fig. 1). The driving power is supplied by air jets issuing from eight $\frac{1}{16}$ inch holes spaced and directed as illustrated (*6*, Figs. 1, 3).

The compressed air is conducted to these holes through the flexible pressure tubing (*7*, Fig. 5), the coupling (*8*, Fig. 3), and the distributor chamber (*9*, Figs. 1, 3). The brass sleeve (*10*, Figs. 1, 3) containing the forward jet holes is also fitted with eight reverse jet holes (*11*, Fig. 1), two of which are indicated in the background of Fig. 3 under *11*. Each jet hole in both sets is directed along a line which is $\frac{3}{4}$ inch from the axis of rotation. The clearance between the sleeve and turbine is $\frac{1}{16}$ inch.

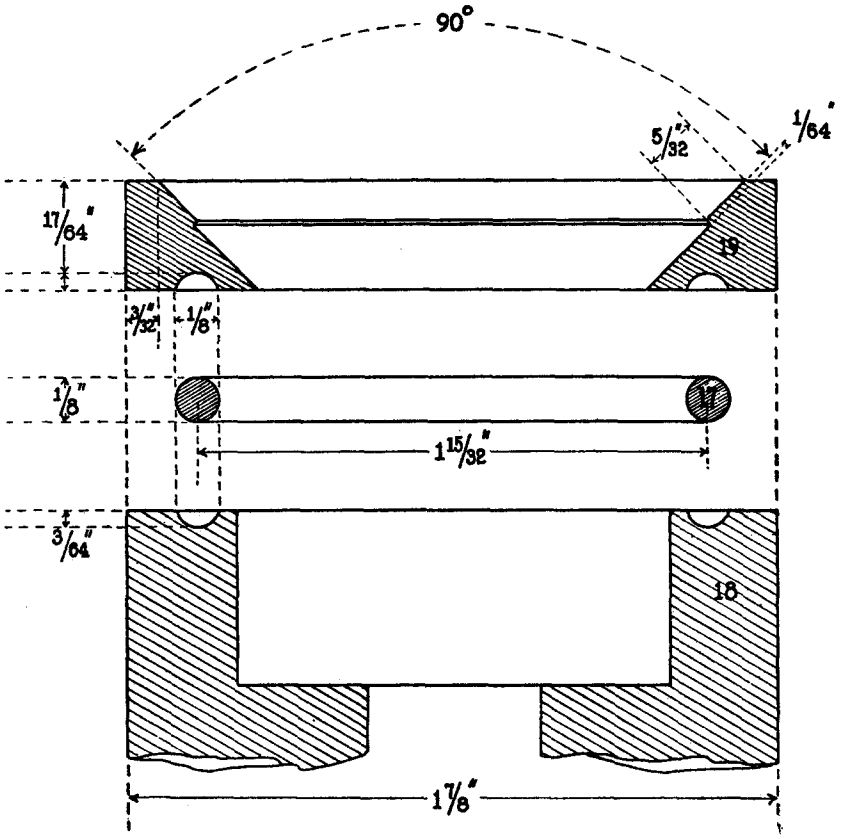


FIG. 2. Vertical cross-sectional view of the air-bearing units.

The sleeve can move up and down in the duralumin cross-arm (*12*, Figs. 1, 3, 5) which is supported by two steel rods (*13*, Figs. 1, 5). The reverse jets are put into operation by supplying compressed air through the rubber tubing (*14*, Fig. 5) to the air piston (*15*, Fig. 1). This raises the end of the brass plate (*16*, Figs. 1, 3) which in turn communicates the movement to the sleeve, *10*, through two pins, *17*, one of which is shown in Fig. 3. The other is directly opposite but has

been omitted to simplify the drawing. This action results in a shift of the reverse jet holes, *11*, to a position adjoining the distributor chamber, *9*, and a consequent shift of the forward jet holes to a shut-off position above the chamber. Under the action of gravity, the sleeve and plate drop back to their normal positions when the pressure is released.

The rotating elements are supported by the air-bearing arrangement shown as disassembled in Fig. 2, and as during operation in Fig. 1. A rubber ring (*17*, Figs. 1, 2, 4) is made by splicing together with rubber cement the ends of a length of $\frac{1}{8}$ inch round rubber belting. This ring rests in a circular groove of the brass support (*18*, Figs. 1, 2). The rubber supports and centers a bakelite ring (*19*, Figs. 1, 2, 4) as indicated. This ring is cut from ordinary black bakelite panel

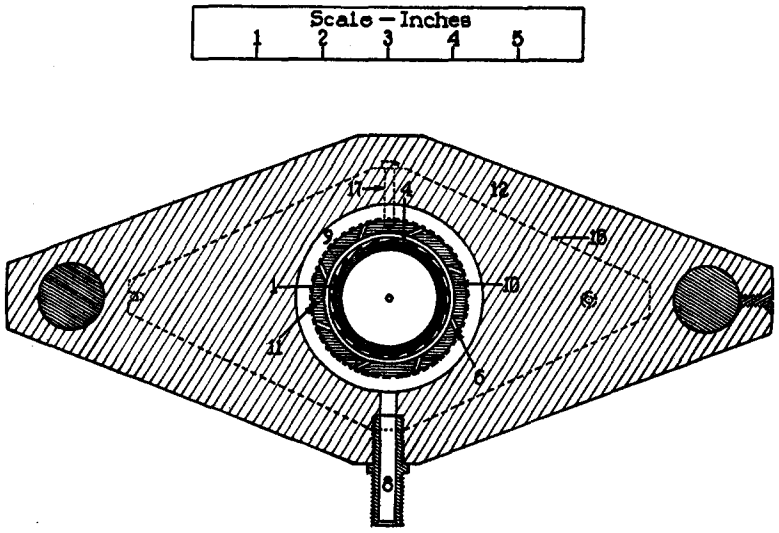


FIG. 3. Horizontal cross-sectional view of the turbine and driving jets.

board. The turbine actually rides on a thin film of air escaping between its conical surface and that of the bakelite ring. This air is supplied through the pressure tubing (*20*, Fig. 5) which is coupled to the fine wire screen filter (*21*, Fig. 1). The clearance (*22*, Fig. 1) about the wire just below the inlet hole from the coupling is made small enough to prevent any great loss of air, and yet sufficiently large to eliminate the possibility of the wire touching the edges of the hole. A $\frac{1}{8}$ inch hole was found to be satisfactory.

The shaft is guided by two phosphor bronze bushings (*23*, Fig. 1), each of which is prepared as follows: A bronze plug is machined approximately to size, and a central hole slightly smaller than the shaft is then drilled. The wire selected for the shaft is cut to a length several inches longer than actually needed for the apparatus With the help of an emery wheel, one end is ground to the shape of a triangular, slightly tapered reamer. The hole of the plug is reamed to a snug fit

and the bushing then fitted on an arbor and turned to the correct size for forcing into the brass gland (*24*, Fig. 1). The same method of reaming and finishing on an arbor is used in fitting the shaft into the turbine and into the chuck (*25*,

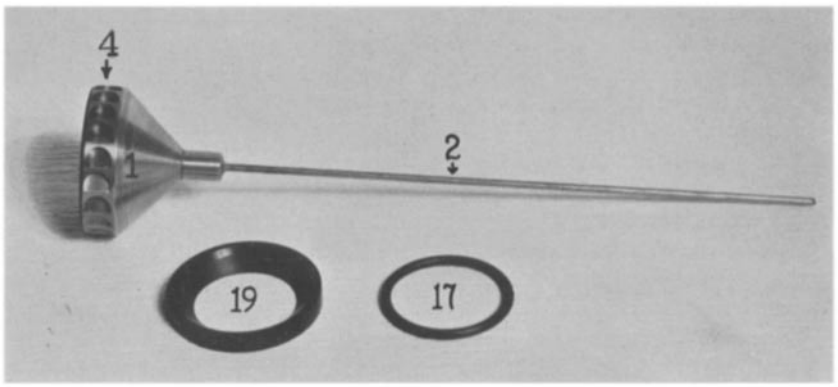


FIG. 4. Turbine and air-bearing units shown separately.

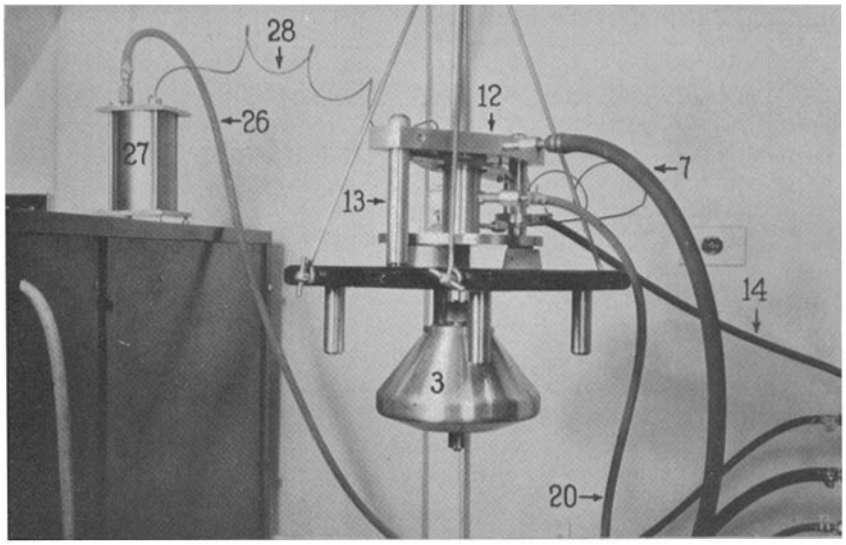


FIG. 5. Complete assembly of the driving mechanism and the rotor.

Fig. 1). After the bronze plugs are in place, a smooth bearing fit with the shaft is obtained by polishing the surfaces together with rouge. When finished, the fit should be such that the wire slips easily through the gland without excessive play.

Air pressure transmitted through the tubing (*26*, Fig. 5) forces lubricating oil from the container (*27*, Fig. 5) through the flexible copper tubing (*28*, Fig. 5) connecting to the coupling (*29*, Fig. 1) into the central cavity of the gland. The oil forms an air-tight seal about the shaft and also lubricates the two bearings. The lubricant escaping through the upper bearing (usually a fraction of a cubic centimeter per hour) is collected in a container (*30*, Fig. 1). The oil passing the lower bearing is allowed to fall into the vacuum chamber, although a collecting system such as described by Biscoe, Pickels, and Wyckoff (31) could be provided if necessary.

The vacuum seal is completed and the necessary flexibility provided by a section of soft, thick walled rubber tubing (*31*, Fig. 1) which joins the stem of the gland and a brass sleeve (*32*, Fig. 1) that is fastened and tightly sealed to the top plate (*33*, Fig. 1) of the chamber. Three screws clamp the air-bearing support and the oil gland to a circular brass plate (*34*, Fig. 1). This plate is supported by three soft rubber stoppers (*35*, Fig. 1) surmounted by small live rubber discs (*36*, Fig. 1).

*Rotor.*—The rotor which is shown in Figs. 6, 7, and 8 is so designed that while it rotates in a very high vacuum, the material which is being centrifuged is maintained under normal atmospheric pressure. It is machined from a solid block of duralumin alloy commercially known as ST 17. As shown in the illustrations (*47*, Figs. 6, 7, and 8), it is given a pear-shaped form having a maximum diameter of 8 inches and tapering off at the top to $3\frac{1}{2}$ inches. This form was chosen in order to reduce the weight of the rotor as much as possible without sacrificing its strength materially. At the top of the rotor there is a cavity (*50*, Fig. 6) cut to the depth of $1\frac{9}{16}$ inch. At the bottom edge of this cavity are drilled sixteen equally spaced holes, each $\frac{1}{2}$ inch in diameter and $2\frac{7}{8}$ inches deep, to accommodate the celluloid containers described below. The holes (*48*, Fig. 6) are inclined at an angle of 45° to the axis of rotation, and their bottoms are rounded to correspond to the shape of the containers. The distance from the inner edge of each hole to the axis of rotation is 3.8 cm. at the top, and 8.5 cm. at the bottom, where the outside supporting wall reaches a minimum thickness of $\frac{1}{4}$ inch. This wall is made considerably thicker at the top where the holes are close to each other and additional strength is needed. In Fig. 6, one container, *49*, is indicated as being in position. The sixteen containers, each holding conveniently 7 cc. of fluid, give the centrifuge a total capacity of 112 cc. which can be centrifuged in one run. At the base of the rotor there is machined out a stem 1 inch in diameter and in length, which is used for fastening the rotor in the milling machine chuck while the holes are being drilled.

The rotor head (*44*, Figs. 6, 7, and 8) is machined from a separate piece of duralumin and is made to fit snugly into the top portion of the rotor. The stem (*44a*, Fig. 6) fits into a hole in the center of the rotor and aids in properly centering the rotor head when the latter is placed in position. A rubber washer (*45*, Figs. 6, 7) made of round rubber belting, $\frac{1}{8}$ inch in diameter and smeared with lubriseal,

is inserted in the step joint between the rotor head and the rotor proper to furnish an air-tight seal (Fig. 6). The rotor head is fastened to the rotor with six equally spaced screws (*46*, Figs. 6, 7). Fig. 8 shows the rotor complete. When assembled it weighs, without containers, 6,460 gm.

The rotor is fastened to the drive shaft with a special chuck arrangement attached to the rotor head as shown in detail in Fig. 9. The essential units of this arrangement are the steel chuck, *40*, the duralumin screw cap, *41*, three screws, *42*, the steel ring, *43*, and the steel bushing, *41a*, which is forced into the screw cap. The chuck is closely fitted in the rotor head and is tapered and slotted (four cuts,

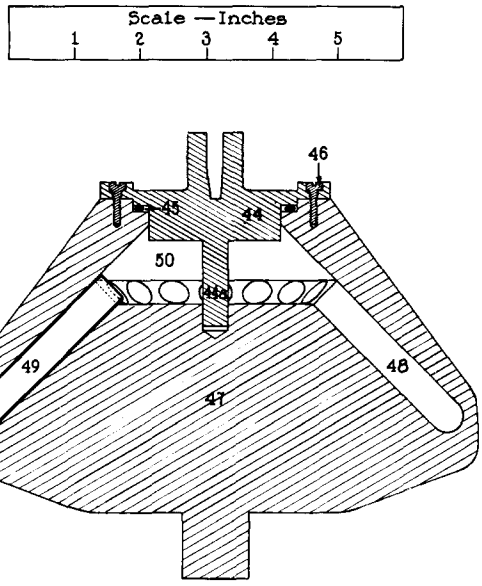


FIG. 6. Vertical cross-sectional view of the rotor.

*40a*, Fig. 9) at its lower end. The steel ring, *43*, automatically raises the chuck and releases its hold on the shaft when the cap is unscrewed.

*Containers.*—Glass containers cannot be used in this centrifuge, as glass would be crushed under the centrifugal force necessary to separate viruses. The containers[1] are made of a celluloid composition, are transparent and flexible and, as shown in Fig. 10, have the shape of ordinary Wassermann tubes. They are manufactured in various sizes, but those used by us have a length of 75 mm., outside diameter of 13.2 mm., and a wall thickness of 0.4 mm. They weigh 1.35 gm. each, and have a total capacity of 8.5 cc. when filled to the rim. For centrifuging

[1] The most satisfactory containers for this purpose were found to be those manufactured by the Lusteroid Container Co., South Orange, New Jersey.

purposes 7 cc. of fluid can be safely placed in each without danger of spilling. As mentioned above, the bottoms of the holes in the rotor are reamed to correspond to the shape of the bottoms of the containers, which fit into the holes fairly snugly, leaving a minimum amount of empty space. When fitted in this manner and filled with fluid, these containers will withstand very high centrifugal forces--over 95,000 times gravity at the bottom portion--without breaking or leaking. These containers, holding 7 cc. of fluid and run for several hours at a speed of 25,000 R.P.M., remained unaltered; but when the speed exceeded 27,000 R.P.M., the empty portion at the top became bent and folded over the surface of the fluid, sealing it completely (see *A*, Fig. 10). When used only half filled, the empty portion of the tube usually collapses. The one serious disadvantage of these containers is that they cannot be sterilized in an autoclave or in a hot air sterilizer. They can be sterilized, however, either by boiling or by soaking in 75 per cent alcohol and subsequently by rinsing in sterile distilled water. They cost a little over one cent apiece and after being used once are usually discarded. It is not necessary to balance the containers by accurate weighing. In our experience it was sufficient to measure accurately with a pipette an equal amount of fluid into any two of the containers and to place them in the rotor in positions opposite to each other.

*Vacuum Chamber.*—The vacuum chamber in which the centrifuge rotates is made up of a cylinder and two flat plates as shown in Figs. 11 and 12. The cylinder (*37*, Fig. 11) is of chrome nickel steel alloy, S.A.E. 3140.[2] Its dimensions are: height, 11 inches; inside diameter, 9 inches; and wall thickness, 1 inch. After forging, it is machined to the desired shape and then heat treated. The heat treatment renders the steel so hard that it cannot be cut with ordinary lathe tools. This type of steel, having a very high tensile strength, was chosen to furnish safety provision in case of a rotor explosion at high speed. The top and bottom of the chamber (*33*, *33a*, Fig. 11) are made of finished cold-rolled steel plate 12 inches square and 1 inch thick. At each of the four corners of these plates are inserted 1 inch stainless steel rods, each 3 inches long, to hold the cylinder in place. The sealing arrangement, which is the same at both the top and bottom of the cylinder, can be seen in Fig. 1. The two rubber washers (*39*, Figs. 1, 11) are made of round rubber belting $\frac{1}{8}$ inch in diameter, spliced and cemented together at the ends. When smeared with lubriseal, they furnish a satisfactory vacuum seal. A ring of sheet metal (*38*, Fig. 1) greatly facilitates the placing of the two washers in position and also removes the possibility of their slipping into the chamber. It is raised for placing the washers and is pushed into the indicated position (Fig. 1) by the top of the chamber. To the top plate is fastened the driving mechanism as described above. Through the bottom plate are bored two holes leading into the chamber. Into these are inserted brass sleeves to provide for rubber tubing connections, one of which leads to the vacuum pump, and the other to the vacuum gauge. The vacuum chamber is mounted on four rubber stoppers to absorb any vibration and rests on a concrete block 24 inches square and 6 inches thick, which

[2] Made for us by the Martin Forge Co. of Brooklyn.

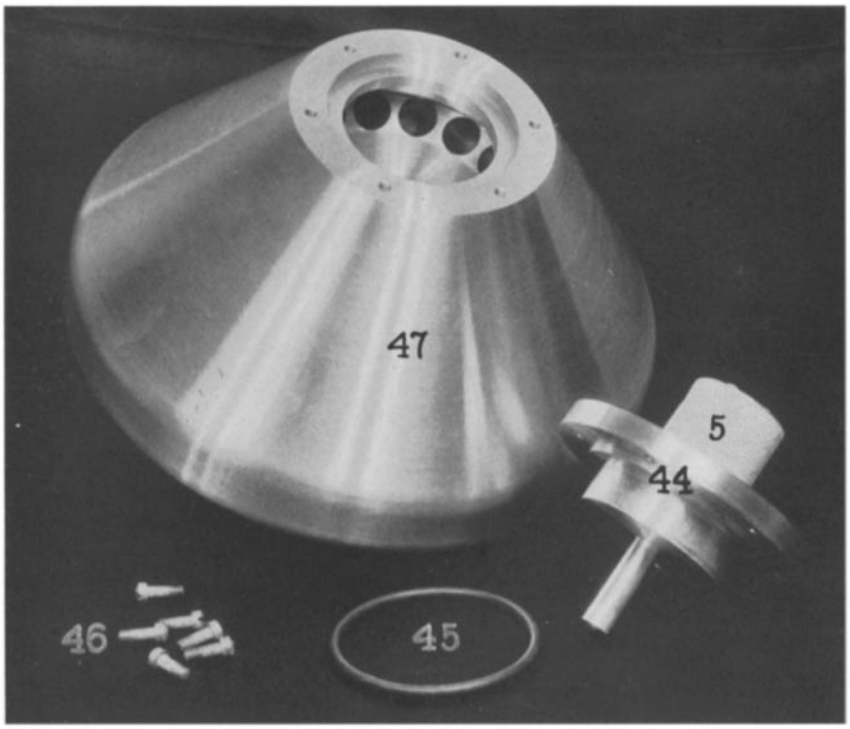


FIG. 7. Component parts of the rotor shown separately.


FIG. 8. Rotor shown assembled.

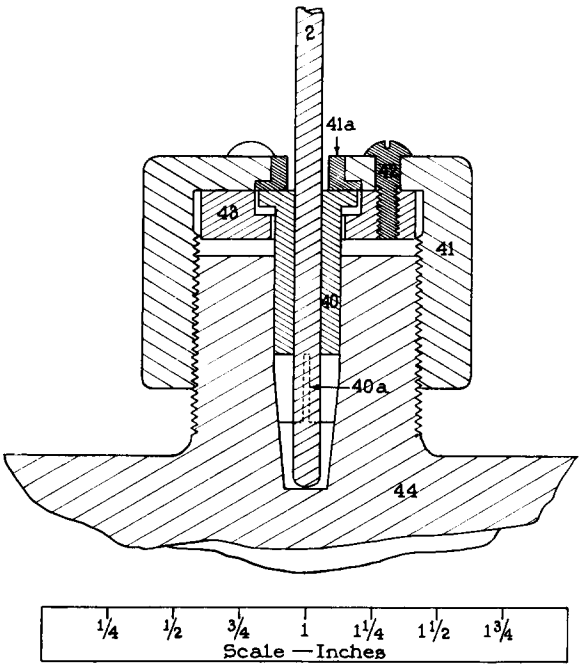


FIG. 9. Vertical cross-sectional view of the rotor chuck arrangement.

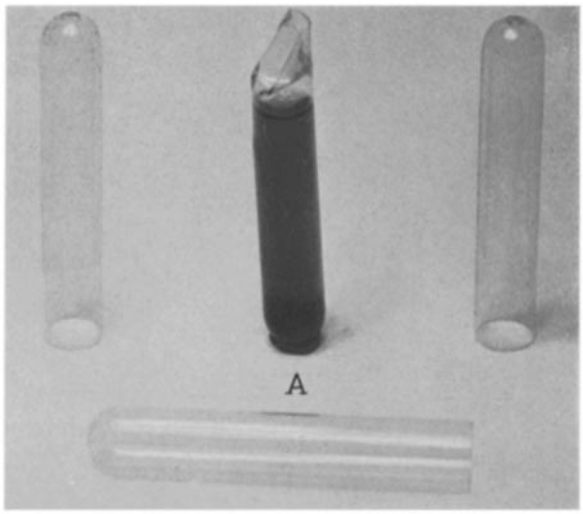


FIG. 10. Celluloid centrifuge tubes.

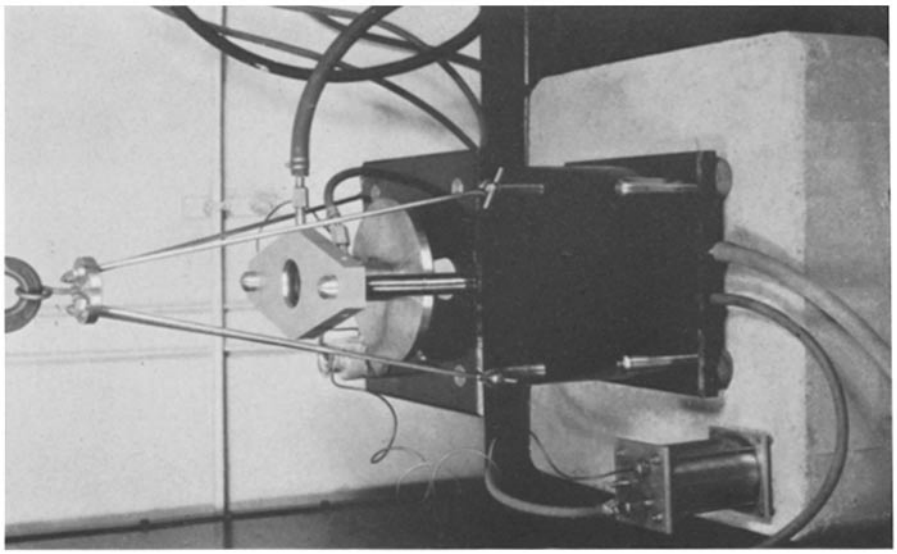

FIG. 12. Vacuum chamber closed.

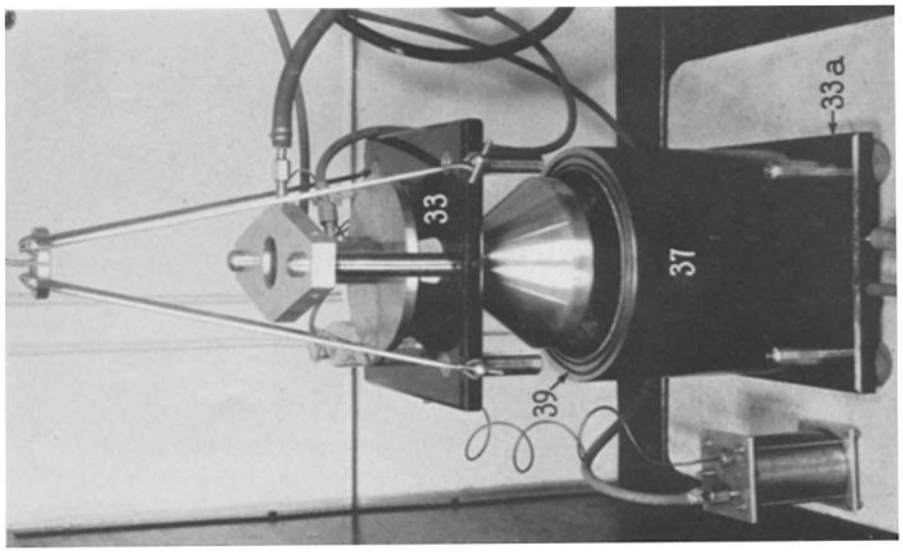


FIG. 11. Vacuum chamber open.

in turn rests on a layer of cork 3 inches thick. Fig. 12 shows the concrete block and the vacuum chamber closed.

*Operation.*—The accessories used in operating the centrifuge are pictured in Figs. 13 and 14. The air controls and the stroboscope for measuring speeds can be seen in Fig. 13. Fig. 14 shows, as they appear to the operator from the position of the controls and stroboscope: the hoist, *51*, used to raise and lower the top of the vacuum chamber and the driving mechanism; the mirror, *52*, used for viewing the turbine; the mercury manometer, *53*, for measuring the pressure in the vacuum chamber; the vacuum pump, *54*; and the barricade, *55*, which is intended to serve as an additional protection to the operator in the event of a rotor explosion. This

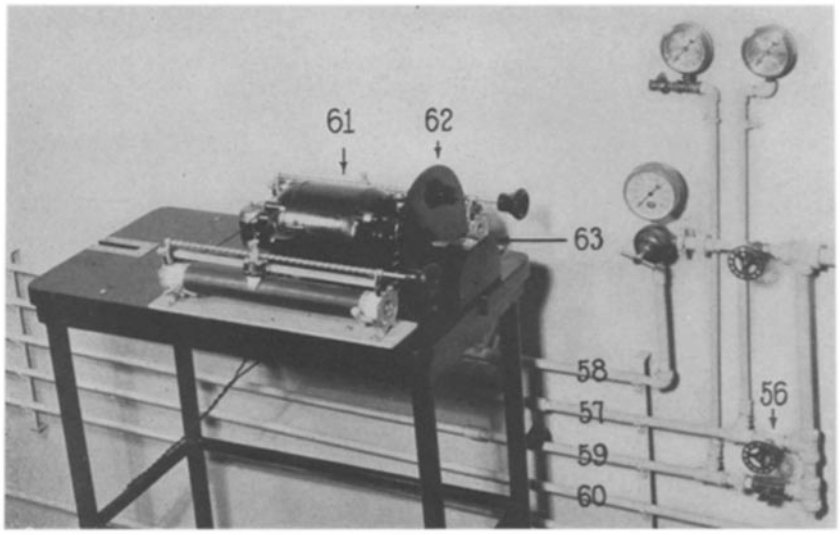


FIG. 13. Stroboscope and air controls.

barricade is a wooden box, 1 foot thick, which is filled with sand. Our vacuum pump is a high speed Cenco Megavac, but a smaller pump will serve the purpose, although the evacuation time preliminary to centrifuging is lengthened somewhat. A 60 watt lamp behind the barricade illuminates the turbine for stroboscoping.

Successful operation of the centrifuge depends upon having approximately constant pressures in all the air lines supplying the various parts of the driving mechanism. Since the head pressure in our laboratory is subject to considerable variation, the air is first passed through a two-stage reducing system of adjustable regulators. The first regulator drops the head pressure (150 to 225 pounds per square inch) to about 100 pounds per square inch. The second regulator reduces the pressure to 50 pounds per square inch and holds it constant at that value as long as the setting of the control valve (*56*, Fig. 13) for the driving line is not

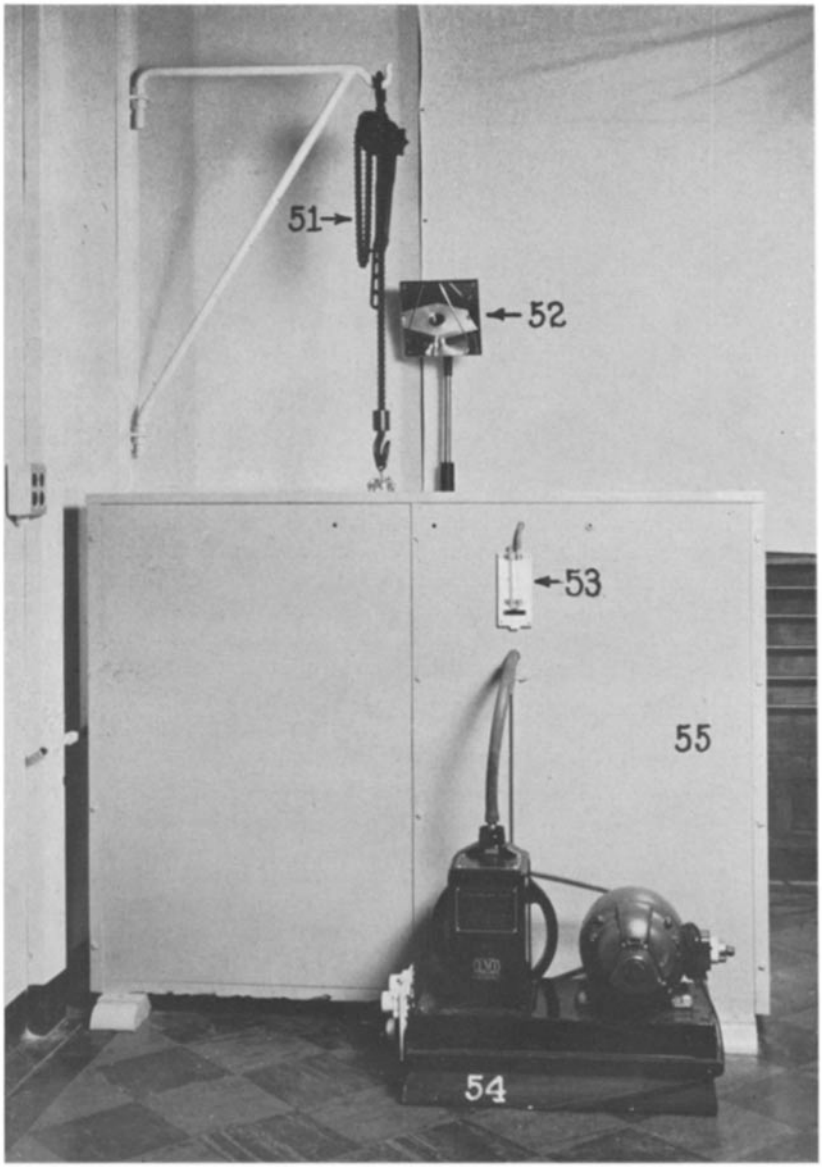


FIG. 14. Barricade, vacuum pump, gauge, hoist, and stroboscope mirror shown in position.

varied over too wide a range. Inexpensive regulators (Type 3Y)[3] have proved satisfactory.

In Fig. 13 can be seen the air lines which lead to the centrifuge and connect with the flexible pressure tubings previously described. Line *57* supplies the driving jets. An ordinary needle valve, *56*, gives sufficiently sensitive adjustment of the driving pressure. The gauge has a range of 0 to 60 pounds per square inch. Line *58* furnishes pressure for the oil container. This requires the least critical control and therefore either a small reducing valve or simply a needle valve and a small air leak serve very well. Any gauge which reads as high as 15 pounds per square inch is suitable. Air for the supporting bearing is supplied through line *59*. It requires very critical adjustment and only a small flow of air. Consequently, a fine needle valve[4] is used. The gauge range is 0 to 30 pounds per square inch. Air in line *60* operates the reversing mechanism and is controlled by a needle valve (not shown).

The speed attainable with the apparatus is limited only by the strength of the rotor, and since the explosion of a rotor run at excessive speed is both costly and dangerous, it is very important accurately to measure and control the speed. It is also important to know approximately what speeds can be regarded as safe for routine operations. The experiments of Biscoe, Pickels, and Wyckoff (32) on tensile strength and fatigue endurance of duralumin rotors furnish a practical basis for such approximation. It is estimated that the rotor described above would perhaps explode in the neighborhood of 40,000 R.P.M. Prolonged operation at a speed not exceeding 30,000 R.P.M., on the other hand, is considered comparatively safe if there are no serious flaws in the metal of which the rotor is made. In our experiments we have not exceeded this speed. The speed is regulated entirely by the adjustment of valve *56*, in Fig. 13, and is measured frequently with the stroboscope, *61*, which is of the simple motor-driven, slotted disc type. The operator views the spinning turbine, with the aid of the mirror mounted above the barricade, by sighting through the revolving slots, *62*. A disc with 25 slots is appropriate for use with this particular centrifuge. Ours contains 50 slots because it is also used for higher speed ranges with the smaller rotors employed for molecular weight determinations. The motor is a $\frac{1}{4}$ horse power, 110 volt, direct current type. Its speed is controlled by two rheostats and is measured with a tachometer, *63*, which is attached to the motor shaft with flexible rubber tubing. A stop watch is used for timing. It is highly desirable to have as steady a source of electric current as possible. To facilitate the synchronization of the centrifuge speed with the frequency of vision interruption, the turbine is first painted a dull black on its top surface and then has a white mark placed near the edge of this dark background. This mark appears to be stationary (although blurred) when synchronization is obtained. It should be remembered that such a single pattern can be obtained when the turbine speed is one, two, or three times the frequency of vision interrup-

[3] Supplied by the Foster Engineering Co. of Newark.

[4] Supplied by the Ashton Valve Co. of Boston.

tion. After practice, one can easily make distinctions by noting the amount of blurring. A variety of multiple patterns can also be obtained, and they serve as convenient checks on one another as well as on the single patterns. The double pattern, in which the turbine appears to have two opposite marks, is very convenient for measurement in the lower speed ranges because it is well defined and necessitates running the stroboscope motor at double speed and consequently more smoothly.

In the actual operation of the centrifuge the following procedure is generally used. After the containers with the material to be centrifuged are placed in the rotor, the rotor head is fastened in place and the assembled rotor fastened tightly to the drive shaft with a pin wrench. After the chamber top with the driving mechanism is lowered into place, the vacuum pump is started and is kept running throughout the experiment. The pressure in the oil container is set at approximately 10 pounds per square inch and the air-bearing pressure is raised until the turbine will coast freely when given a turn with the hand. The pressure required for this purpose with our present turbine and rotor is about 7½ pounds per square inch. As soon as the vacuum gauge registers only a small fraction of 1 mm., the driving pressure is set at 35 pounds per square inch and maintained at this level until the desired speed is reached. About 35 minutes of acceleration are required to attain a speed of 30,000 R.P.M. Most of our centrifugation experiments with the yellow fever virus were carried out at a speed of 27,300 R.P.M. and after initial acceleration a driving pressure of 13 pounds per square inch was sufficient to maintain this speed fairly steadily. At times, however, a very slight acceleration was noted and further adjustments in the driving pressure were necessary in order to maintain the centrifuge at a constant speed. It should be noted that the rotor has a rather high inertia and therefore responds very slowly to small changes in the driving pressure.

In our early experiments the air filter (*21*, Fig. 1) was not used. Occasionally difficulty was encountered in getting the centrifuge started, and at times sudden drops in the speed occurred. The source of both troubles was traced to the braking action of grit or other small particles which had become lodged in the seat of the bakelite supporting ring. However, after the installation of the filter this source of trouble was eliminated. Difficulty may also be encountered if the bakelite ring is warped or improperly machined; in either case an examination will reveal that the ring is polished in two or more places instead of evenly all around.

At the start the rotor passes through a short period of rather pronounced precessional motions, but as it accelerates it soon settles into rotation about a fixed axis. Vibrations are perceptible in the oil gland and its supporting plate until the speed reaches about 10,000 R.P.M. With further increase in speed the motion gradually becomes extremely smooth with hardly a trace of vibration in any part. There are various critical speeds in the lower range at which vibration is quite pronounced, but the turbine and the rotor accelerate through these without ill effects. Undue vibration or shaking may be caused by: (*a*) a drive shaft of improper length; (*b*) a greatly unbalanced or misaligned rotor or turbine; (*c*) too

loose a fit in the bearings; (*d*) a bent drive shaft; (*e*) an improperly functioning air float; or (*f*) a lack of sufficient flexibility and damping in the mountings of the driving mechanism.

The air-bearing pressure sometimes has to be lowered very slightly as the rotor reaches higher speeds. A little experience with the peculiarities of this bearing makes the critical adjustment of its pressure comparatively easy. One soon becomes accustomed to the sounds emitted by the centrifuge and can readily tell by ear whether or not the valve can be better adjusted. While the speed is about 15,000 R.P.M., the operator can lightly touch the supporting plate (*34*, Fig. 1) with comparative safety and discover in that way the effects of slight readjustments in the needle valve controlling the support. Lowering the pressure too much causes the turbine to drag and decelerate. Generally the correct adjustment at any speed can be made by gradually increasing the pressure until the needle of the pressure gauge (in line *59*, Fig. 13) begins to oscillate with a low period. At this stage the turbine may be seen to shift slightly from side to side in step with the needle oscillation. The pressure is then reduced just enough to eliminate completely these movements of the needle and the turbine. Runs of several hours' duration have been made without a single resetting of the air-bearing pressure.

A rapid stopping of the centrifuge is accomplished by opening the valve controlling the reversing mechanism and readjusting the driving pressure to the desired value; 35 pounds per square inch is sufficient to stop the rotor in about 20 minutes. The same vibrational periods occur during both deceleration and acceleration although the precessional motions are less pronounced in the slowing down.

If the reverse drive is not applied, and the rotor is allowed to coast until it comes to a standstill, it usually takes over 2 hours to decelerate from a speed of 30,000 R.P.M. to a full stop.

## EXPERIMENTAL

A series of experiments was carried out with a view to studying the operating characteristics of the centrifuge as well as the behavior of yellow fever virus in an intense centrifugal field. In most of these a neurotropic strain of the virus derived from mouse brain was used. This was chosen because of its greater virulence for mice and its presence in the material under study is therefore more easily determined than that of the viscerotropic form derived from the blood of infected monkeys. As the virus becomes rapidly inactivated when suspended either in distilled water or in physiological saline, all the virus suspensions were prepared either in normal monkey serum or in ascitic fluid; also all dilutions in connection with the titrations of the virus were made in a diluent of similar nature. In each instance the

material was rendered clear, either by filtration or by preliminary centrifugation at lower speeds prior to its final centrifugation in the vacuum centrifuge. Various dilutions of the supernatant fluid and of the sediment after final centrifugation were tested in groups of six mice by intracerebral inoculations. The following experiments are described in detail as illustrative of the results obtained.

*Experiment 1.*—Five infected mouse brains were finely ground in a sterile mortar and were suspended in 20 cc. of a diluent consisting of 25 per cent normal

TABLE II

*Experiment 1. Speed 25,000 Revolutions per Minute; Time 3 Hours*

| Material | Dilution | Mouse group | Results |
|---|---|---|---|
| Supernatant fluid | Undiluted | D 0429 | All died |
| | $10^{-1}$ | D 0430 | 3/5 " |
| | $10^{-2}$ | D 0431 | All lived |
| | $10^{-3}$ | D 0432 | " " |
| Sediment 1. Resuspended in 1 cc. of supernatant fluid | $10^{-3}$ | D 0433 | All died |
| | $10^{-4}$ | D 0434 | " " |
| | $10^{-5}$ | D 0435 | " " |
| | $10^{-6}$ | D 0436 | " " |
| | $10^{-7}$ | D 0437 | All lived |
| | $10^{-8}$ | D 0438 | " " |
| Sediment 2. Supernatant fluid poured off entirely and sediment suspended in 1.0 cc. of fresh diluents | $10^{-3}$ | D 0439 | All died |
| | $10^{-4}$ | D 0440 | " " |
| | $10^{-5}$ | D 0441 | " " |
| | $10^{-6}$ | D 0442 | 4/5 " |
| | $10^{-7}$ | D 0443 | All lived |
| | $10^{-8}$ | D 0444 | " " |

monkey serum in distilled water. This suspension contained approximately 7.5 per cent of the mouse-brain substance by weight. The mixture was first centrifuged for 30 minutes at a speed of about 3,000 R.P.M. and the supernatant fluid was then passed through a Seitz filter. The filtrate was placed in two sterile celluloid tubes, 7 cc. in each, and was centrifuged in the vacuum centrifuge for 3 hours at a speed of 25,000 R.P.M. (417 per second by stroboscopic measurement). From one of the two tubes 1.5 cc. of the topmost portion of the supernatant fluid was removed and was used for the titration of the virus content in mice; the middle portion was discarded and only 1 cc. was left at the bottom, in which the sediment was resuspended and designated as sediment 1. From the other tube the supernatant fluid was poured off entirely and the sediment which had been packed on

the bottom of the tube was resuspended in 1 cc. of a mixture consisting of 25 per cent ascitic fluid in distilled water. This was designated as sediment 2. Although the material before centrifugation was clear, both of the resuspended sediments were distinctly turbid. Serial tenfold dilutions were made from the portion of the supernatant fluid mentioned above as well as from both of the sediments, and groups of six mice were inoculated intracerebrally with each dilution. All dilutions were made in 25 per cent ascitic fluid and distilled water. The results which are shown in Table II indicate that effective separation of the virus had been obtained and that there was very little virus left in the supernatant fluid. The results further indicate that the virus apparently was packed on the bottom of the tubes to the extent that the supernatant fluid could be poured off and replaced with a new diluent without much loss of activity.

In order to determine the effect of the viscosity of the diluent on the rate of separation of the virus, the above experiment was repeated with a virus suspension which contained a much higher concentration of protein, as follows:

*Experiment 2.*—A 20 per cent mouse-brain suspension was prepared by grinding finely fifteen mouse brains in a sterile mortar and suspending them in 25 cc. of undiluted normal monkey serum. The suspension was centrifuged for 30 minutes at a speed of 3,000 R.P.M. The supernatant fluid was pipetted off and then was centrifuged for 1 hour at a speed of 13,000 R.P.M. After the second centrifugation the supernatant fluid was placed in two celluloid tubes, 7 cc. in each, and centrifuged for 3½ hours at a speed of 27,300 R.P.M. (455 per second by stroboscopic measurement) in the vacuum centrifuge. Along with the tubes containing the virus, two additional tubes containing water were placed in the centrifuge. At the end of the run the temperature of the water was measured with a thermometer and was found to be 1.2°C. higher than room temperature. After centrifugation the tubes containing the virus showed a definite whitish sediment at the bottom, and while most of the supernatant fluid was clear, the topmost layer of the column, about 5 mm. thick, showed a distinct grayish turbidity and apparently contained much finely dispersed fat. The supernatant fluid and the sediments were tested in mice in exactly the same manner as in the preceding experiment and the results are summarized in Table III. Although the material was centrifuged at a higher speed for a longer time than in the preceding experiment, the separation of the virus was less satisfactory. In fact, the supernatant fluid in this experiment proved still infective in a dilution of 1:10,000, while in the preceding test a 1:100 dilution gave entirely negative results.

*Experiment 3.*—It was difficult in Experiment 2 to understand the reasons for the relatively poor separation of the virus, which could not be explained by the higher viscosity of the diluent alone. Therefore it was decided to repeat the experiment in every detail with the exception that the material be centrifuged for a

longer period of time. Accordingly fifteen mouse brains were finely ground in a mortar and suspended in 25 cc. of normal monkey serum. After the suspension had been centrifuged for 30 minutes at 3,000 R.P.M. and for 1 hour at 13,000 R.P.M., the clear virus suspension was centrifuged for 5½ hours at a speed of 27,300 R.P.M. After the completion of the run there was again present a layer of fat on the surface of the fluid similar to that observed in the preceding experiment. In addition, while most of the column of the fluid was reddish yellow in color due to the presence of hemoglobin, the topmost portion of the fluid had become perfectly

TABLE III

*Experiment 2. Speed 27,300 Revolutions per Minute; Time 3½ Hours*

| Material | Dilution | Mouse group | Results |
|---|---|---|---|
| Supernatant fluid | Undiluted | D 0703 | All died |
| | $10^{-1}$ | D 0704 | " " |
| | $10^{-2}$ | D 0705 | " " |
| | $10^{-3}$ | D 0706 | 2/6 " |
| | $10^{-4}$ | D 0707 | 2/6 " |
| Sediment 1. Resuspended in 1.0 cc. of supernatant fluid | $10^{-3}$ | D 0708 | All died |
| | $10^{-4}$ | D 0709 | " " |
| | $10^{-5}$ | D 0710 | " " |
| | $10^{-6}$ | D 0711 | " " |
| | $10^{-7}$ | D 0712 | 5/6 " |
| | $10^{-8}$ | D 0713 | 3/6 " |
| Sediment 2. Supernatant fluid poured off entirely and sediment suspended in 1.0 cc. of fresh diluent | $10^{-3}$ | D 0714 | All died |
| | $10^{-4}$ | D 0715 | " " |
| | $10^{-5}$ | D 0716 | " " |
| | $10^{-6}$ | D 0717 | " " |
| | $10^{-7}$ | D 0718 | 3/5 " |
| | $10^{-8}$ | D 0719 | All lived |

colorless to a depth of about 1 cm., indicating a separation of hemoglobin from that portion. The supernatant fluid and the sediments were tested in animals precisely as in the preceding experiments. The sediment in both tubes was packed at the bottom so firmly that it was resuspended only with considerable difficulty; in fact, it was found impossible to break up some of the lumps. The results of the tests in mice are shown in Table IV. As seen from this table the effective separation of the virus was not much better than was observed in Experiment 2, although the centrifugation time had been prolonged 2 hours. The temperature of the rotor at the end of the run was not actually recorded, but it did not feel much warmer than room temperature. It was considered that the slight rise in the temperature that had taken place during centrifugation must

have been very gradual, and not sudden enough to set up temperature gradients and consequent convection currents which would have interfered with effective sedimentation of the virus. Moreover, there was evidence to indicate that separation of hemoglobin had taken place, which hardly could be expected had convection currents been present. It was felt, therefore, that if yellow fever virus particles are more or less of uniform size and have a density approximately that of protein, it was not the lack of sufficient centrifugal force which prevented complete separation of the virus, but the presence of other factors not clearly understood.

TABLE IV

*Experiment 3. Speed 27,300 Revolutions per Minute; Time 5½ Hours*

| Material | Dilution | Mouse group | Results |
|---|---|---|---|
| Supernatant fluid | Undiluted | D 0895 | All died |
| | $10^{-1}$ | D 0896 | " " |
| | $10^{-2}$ | D 0897 | 5/6 " |
| | $10^{-3}$ | D 0898 | 4/6 " |
| | $10^{-4}$ | D 0899 | All lived |
| Sediment 1. Resuspended in 1.0 cc. of supernatant fluid | $10^{-3}$ | D 0900 | All died |
| | $10^{-4}$ | D 0901 | " " |
| | $10^{-5}$ | D 0902 | " " |
| | $10^{-6}$ | D 0903 | " " |
| | $10^{-7}$ | D 0904 | 2/6 " |
| | $10^{-8}$ | D 0905 | 3/5 " |
| Sediment 2. Supernatant fluid poured off entirely and sediment suspended in 1.0 cc. of fresh diluent | $10^{-3}$ | D 0906 | All died |
| | $10^{-4}$ | D 0907 | " " |
| | $10^{-5}$ | D 0908 | " " |
| | $10^{-6}$ | D 0909 | 5/6 " |
| | $10^{-7}$ | D 0910 | 4/6 " |
| | $10^{-8}$ | D 0911 | All lived |

As mentioned above, in the last two experiments in which 20 per cent mouse-brain suspensions in undiluted monkey serum were used, there was in each instance a layer of finely dispersed fat present in the topmost portion of the fluid after centrifugation. This fat undoubtedly was derived from the brain tissue, although a small amount might have been present in the monkey serum. In all the experiments described above, the upper portion of the supernatant fluid, which was pipetted off and tested in mice for the presence of virus, contained most of the fat. It was considered a possibility that some of the virus particles might have been encapsulated in the fat globules and brought to the surface instead of being sedimented down under the centrifugal force. In order to determine this

possibility, the following experiment was carried out in which the fat from the mouse brains was extracted prior to use for centrifugation.

*Experiment 4.*—Dr. T. P. Hughes of this laboratory in his chemical studies on the virus found that the fat from infected mouse brains can be removed by extraction with ether without a material loss of the virus activity. He very kindly furnished us with 25 brains which had been dried *in vacuo* in the frozen state and from which the fat had been extracted in a Soxhlet apparatus with ether and petroleum ether. These brains were finely ground with quartz sand in a sterile mortar and suspended in 25 cc. of a diluent made up of equal parts of ascitic fluid and distilled water. As in the previous experiments, the material was rendered

TABLE V

*Experiment 4. Speed 27,300 Revolutions per Minute; Time 3 Hours*

| Material | Dilution | Mouse group | Results |
|---|---|---|---|
| Supernatant fluid | Undiluted | D 1086 | All died |
| | $10^{-1}$ | D 1087 | 5/6 " |
| | $10^{-2}$ | D 1088 | 4/5 " |
| | $10^{-3}$ | D 1089 | 3/6 " |
| Sediment 1. Resuspended in 1.0 cc. of supernatant fluid | $10^{-2}$ | D 1090 | All died |
| | $10^{-3}$ | D 1091 | " " |
| | $10^{-4}$ | D 1092 | " " |
| | $10^{-5}$ | D 1093 | " " |
| | $10^{-6}$ | D 1094 | " " |
| | $10^{-7}$ | D 1095 | All lived |
| Sediment 2. Supernatant fluid poured off entirely and sediment suspended in 1.0 cc. of fresh diluent | $10^{-2}$ | D 1096 | All died |
| | $10^{-3}$ | D 1097 | " " |
| | $10^{-4}$ | D 1098 | " " |
| | $10^{-5}$ | D 1099 | " " |
| | $10^{-6}$ | D 1100 | " " |
| | $10^{-7}$ | D 1101 | 1/6 " |

clear by centrifuging first for 30 minutes at 3,000 R.P.M. and afterward for 1 hour at 13,000 R.P.M. The clear material was then centrifuged in the vacuum centrifuge for 3 hours at a speed of 27,300 R.P.M. The virus content in the supernatant fluid and sediments was tested in exactly the same manner as in the preceding experiments. The results are shown in Table V. As seen from this table there was still a considerable amount of virus present in the supernatant fluid, and in the titrations which were carried to a dilution of 1:1,000 the negative limit had not been reached. It is obvious from these results that factors other than the presence of fat are responsible for the failure to obtain a complete separation of the virus in the centrifuge.

In all these experiments the centrifuge was stopped by the application of the reverse air jets, and it usually required 20 minutes to decelerate from full speed to a full stop. It was considered another possibility that during the rapid deceleration a stirring and mixing may occur in the tubes, although the sharp boundary of the separated hemoglobin in Experiment 3 indicated against this. In order to obtain information on this point, another experiment was carried out in which fifteen mouse brains were finely ground with quartz sand and suspended in 25 cc. of a diluent containing 20 per cent normal monkey serum. After clarification by centrifuging at lower speeds, as in the preceding experiments, the virus mixture was again centrifuged for 3 hours in vacuum at a speed of 27,300 R.P.M. At the end of this period the driving pressure was cut off and the centrifuge was allowed to coast until it came to a standstill, which required slightly over 2 hours. As in Experiment 3, the top portion of the fluid showed a definite separation of the hemoglobin with a well defined boundary at about 1 cm. below the surface of the column of fluid. There was also a layer of fat accumulated on the surface, as observed in the preceding experiments. Titrations of the virus content of the supernatant fluid and of the sediment in mice gave similar results as observed in previous tests. Although the centrifuge was decelerated very slowly and there was evidence to indicate that a separation of hemoglobin in the top portion of the supernatant fluid had taken place, there was still a considerable amount of virus present in that portion of the fluid.

## DISCUSSION

The centrifuge described above was successfully applied for the separation and concentration of yellow fever virus. The major portion of the virus was sedimented out of the suspension when centrifuged for 3 hours at a speed of 25,000 R.P.M.; in fact, it became so firmly packed to the bottom that the supernatant fluid could be poured off and the sediment resuspended in fresh diluent without appreciable loss of the virus activity. This would seem to indicate that the centrifuge can be applied for the washing and purification of the virus. At the speed of 25,000 R.P.M. the centrifugal force equals approximately 34,800 times gravity at the top of the fluid column and 66,000 times gravity at the bottom. However, a complete sedimentation of the virus was not obtained even when greater centrifugal forces were applied over a considerably longer period. A certain small proportion of the virus remained persistently in the supernatant fluid even when hemoglobin which was present in the virus suspension had been effectively separated. We are unable as yet to find a satisfactory explanation for the failure to secure a complete separation of

the virus under the conditions described, although we feel that this failure was not caused by convection currents or by stirring during deceleration. Investigations intended to determine the nature of this phenomenon are at present under way, but the results are not yet available.

Although we have not had an opportunity to study the behavior of other viruses in this centrifuge, we feel that it should prove a useful instrument in the study of filterable disease-producing agents, including the smallest known. It can be operated with comparative safety at speeds up to 30,000 R.P.M., giving a centrifugal force of approximately 50,000 times gravity at the top of the fluid columns and 95,000 times gravity at the bottom. Although the maximum hydrostatic pressure exerted by the fluid at this speed equals more than 600 atmospheres, the celluloid tubes have withstood this pressure well. The construction of the centrifuge is relatively simple and it could be built by any first class machinist. The cost of construction of the centrifuge itself, exclusive of the vacuum pump and other accessories, should not exceed the price of a standard laboratory centrifuge. In view of the fact that its air consumption is small and high pressures are not needed even for rapid initial acceleration, it could be operated in any laboratory where there is a supply of compressed air available.

## SUMMARY

1. A high speed centrifuge is described in which the speed is limited only by the strength of the material of which the rotor is made. It carries sixteen tubes, each of which conveniently accommodates 7 cc. of fluid.

2. The centrifuge operates in a very high vacuum and therefore requires only a small amount of driving energy. The arrangement has been found to eliminate the possibility of producing injurious frictional heat.

3. The rotating parts are supported by an air-bearing and are driven by compressed air.

4. The centrifuge has been successfully operated at a speed of 30,000 revolutions per minute, representing a maximum centrifugal force in the fluid of 95,000 times gravity.

5. Celluloid tubes used for centrifugation of fluid at high speeds are described.

6. Experiments are described in which good sedimentation of the yellow fever virus was obtained.

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