Evaluation of a Class III Biological Safety Cabinet for Enclosure of an Ultracentrifuge

M. A. CHATIGNY,[1] S. DUNN,[1] K. ISHIMARU,[2] J. A. EAGLESON,[3] AND S. B. PRUSINER[4]

Naval Biosciences Laboratory, University of California Naval Supply Center, Oakland, California 94625[1]; Beckman Instrument Company, Palo Alto, California 94304[2]; Baker Company, Sanford, Maine 04073[3]; and Howard Hughes Medical Laboratory and Departments of Neurology and Biochemistry and Biophysics, University of California, San Francisco, California 94143[4]

An evaluation of a special safety cabinet housing a high-speed centrifuge was made. The cabinet enclosed both the top access port and the drive and pumping machinery of the centrifuge. A titanium rotor was loaded with tubes containing a bacterial culture, weakened, and driven until rotor rupture occurred. There were several bent and broken components in the centrifuge, and bacteria leaked from the vacuum chamber. Although the forces were sufficient to displace the cabinet, none of the test bacteria were found outside the cabinet.

Centrifugation of microbial suspensions is a widely used method of concentration. By preparative ultracentrifugation, virus cultures may be brought to concentrations as high as 10^{14} to 10^{15} viral units per ml. Since most of the centrifuges used for this type of concentration achieve very high speed, they have been examined closely for leakage and aerosol generation during use (1, 2). Such high-speed units require that the rotating centrifuge head be enclosed in a chamber which is maintained under vacuum while in use. In general, the probability of leakage through the seals of the rotor drive mechanism and the vacuum system and of distortion of the heavy steel protective housing is not high, but the possibility of rupture of the rotor and subsequent release of the contents as massive aerosols must be considered. The consequences of such rupture could be serious. For work with moderately to highly hazardous materials, several designs for class III cabinets have been proposed. Most of these have been designed to fit over the loading top of the centrifuge and have been considered as additions to the basic machines. The cabinet used in the tests reported here provides a full enclosure for an ultracentrifuge with access to the loading area via rubber gloves installed into fixed ports and material access through air locks. The objective of the tests was to run a rotor (loaded with a bacterial suspension) to failure by rupture and to observe the mechanical damage and consequent leakage of the tracer bacterium.

MATERIALS AND METHODS

Biological safety cabinet. The safety cabinet used was a special hood model 403, class III biocon-

tainment cabinet (Baker Co., Sanford, Me.). The top and bottom portions of the cabinet are separated by the centrifuge top, and are ventilated separately, through HEPA type filters, as shown in Fig. 1. The cabinet was connected to auxiliary exhaust blowers which exhausted 300 ft³/min of air through HEPA filters from the top and bottom sections of the cabinet which are isolated one from the other. Negative pressure of 0.8 inches of water (ca. 200 Pa) was maintained in the upper section and 0.3 inches of water (ca. 75 Pa) in the lower section housing the centrifuge mechanism.

Centrifuge and rotor. The centrifuge tested was a Beckman model L5-50 centrifuge using a Beckman type 50.2 Ti titanium rotor. Under test the rotor was loaded with 12 screw-capped bottles (20-ml capacity), each containing 20 ml of a *Flavobacterium* suspension diluted to ca. 10^9 viable cells per ml.

Experimental setting. The cabinet, exhaust ventilators, and associated sampling devices were located in a safety test cubicle at the Beckman Instruments, Inc., Spinco Division Plant, Palo Alto, Calif. The test cubicle was approximately 8 feet wide by 10 feet deep by 10 feet high (ca. 2.44 m by 3.04 m by 3.04 m). It had solid sides with a heavy-gauge steel mesh top and was provided with closed-circuit video viewing facilities and remote control of electrical equipment in the space. During the time of the rupture tests, the steel mesh ceiling was covered over with heavy kraft paper to provide a full enclosure and minimize any loss of aerosols.

Tracer organism. The *Flavobacterium* sp. (4) was grown in liquid medium to a final colony-forming unit concentration of 1.2×10^{10}/ml. Nutrient agar, with no additives, was used for assay of colony-forming units from both liquid suspensions and from aerosols in settling plates and air samplers. All plates were incubated at 37°C for 48 h before counting.

Aerosol sampling. Two types of aerosol samplers were used. (i) Anderson six-stage samplers (Anderson 2000 Inc., Atlanta, Ga.) used in conjunction with Gast
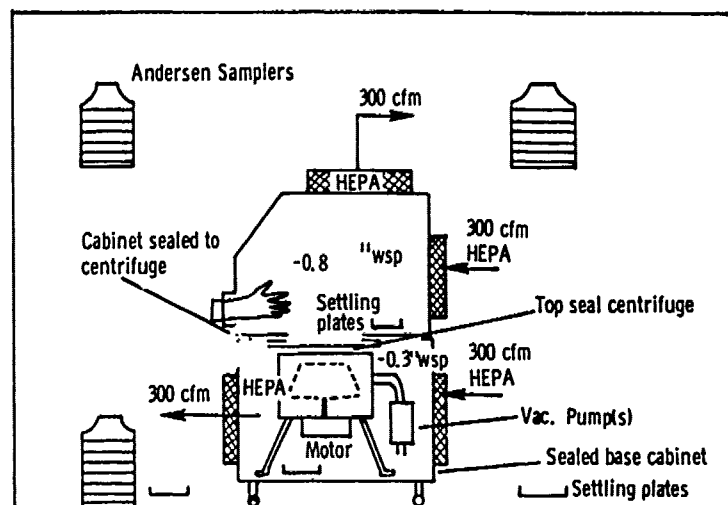
FIG. 1. *Setup for test of Beckman L5 centrifuge rotor breakup in Baker class III cabinet. The test cell was ca. 8 ft by 10 ft by 10 ft (ca. 2.44 m by 3.04 m by 3.04 m).*

one-cfm vacuum pumps (Gast Manufacturing Co., Benton Harbor, Mich.) were used for aerosol sampling in the test cubicle. (ii) Settling plates were used in locations supplementing the Anderson samplers in the test cubicle and also both lower and upper portions of the safety cabinet.

The samplers were numbered by sampling station and placed in the locations shown in Fig. 2.

Test procedures. The fully loaded rotor was set in the centrifuge, the unit was closed, and an appropriate vacuum was drawn on the rotor chamber. The rotor speed was then taken to 50,000 rpm (held for 5 min), reduced to 45,000 rpm (held for 5 min), and then advanced to 50,000 rpm (held for 5 min). This sequence was repeated three times (usually). In the absence of rotor rupture, the machine was turned off, the rotor was removed, and 0.5-inch (2.54-cm) holes were drilled through the bottom of the rotor into a cavity (or through the rotor). The hole axis was in a plan through the center of the rotor. The above sequence of machine operation was repeated 14 times, resulting in a progressive weakening of the rotor. When there was practically no more material to be removed by drilling, the decision was made to further stress this weakened plane by the addition of 100 g of lead to each side of the rotor orthogonal to this weakened plane.

Settling plates P-8 and P-9, in the bottom of the cabinet, were sealed into the cabinet and left open for up to 8 h, as were settling plates P-1, P-2, P-3, and P-4. Plates P-5, P-6, and P-7 were uncovered only after rupture of the rotor had occurred. Anderson sampler vacuum pumps were switched on only after the rupture of the rotor had occurred, and the Anderson samplers were run for two successive 20-min periods after the rupture. The test was constrained by the availability of only a single rotor to test to rupture. The settling plates were covered each time it was necessary to enter the test cubicle and the cabinet to remove the rotor and redrill it, and every effort was
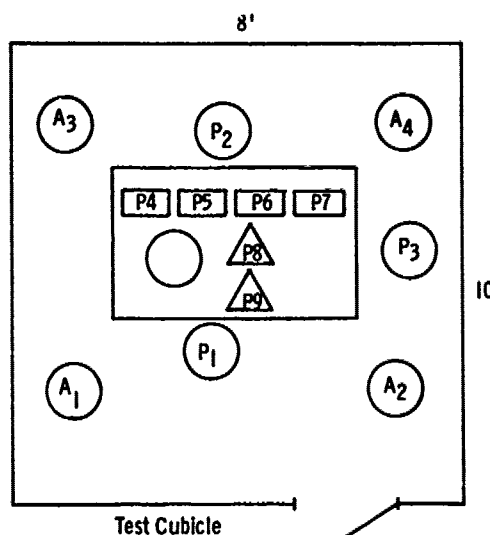

Test Cubicle

FIG. 2. *Location of aerosol sampling stations in centrifuge test cell and in safety cabinet. Abbreviations and symbols: A, Anderson samplers; P, settling plates; ⊕, outside cabinet; ⊞, inside upper cabinet on work surface; ▲, inside base cabinet, at bottom.*

made to minimize activity within the centrifuge cabinet and the test cubicle. All sampler plates, both Anderson and settling, were exchanged daily with fresh plates put in place at the start of the workday. The Anderson samplers were run for "background" tests in midmorning, and the plates were changed thereafter.

RESULTS

After almost 3 days of repeated weakening,

the rotor ruptured at 53,000 rpm. There was an immediate and violent dislocation of the centrifuge within the cabinet, some distortion of the rotor chamber and the sliding cover, and a rupture of the line leading from the rotor chamber to the vacuum pump (Fig. 3). The Baker cabinet moved about 3 inches (ca. 7.62 cm) (Fig. 4A). The centrifuge remained within the cabinet and did no damage to the cabinet or to its biological integrity (Fig. 4B). The sliding top closure plate for the centrifuge vacuum chamber was bent sufficiently so that it did not open normally, and it required dismantling the rails for inspection of the ruptured rotor.

Settling plates P-1, P-2, P-3, and P-4 were covered and removed 20 min after the rupture. Settling plates P-5, P-6, and P-7 were opened approximately 30 min after rupture and held open for 20 min. Approximately 20 to 30 min after rupture, settling plates P-8 and P-9 were removed from the interior of the safety enclosure.

All Anderson samplers in the test cubicle were switched on immediately at the time of rotor failure and run for 20 min. They were then reloaded, and a second set of samples was collected (20-min exposure). The second set of samples was collected after the bottom enclosure was opened, and settling plates P-8 and P-9 were removed from the interior of the safety enclosure. At that time, examination of the mechanical portion of the centrifuge indicated that the safety cabinet had not been violated but that the pipe line to the vacuum pump was broken, as were some electrical components in the base of the centrifuge.

None of the Anderson samplers showed any *Flavobacterium* colony-forming units at any time. There were viable contaminants present (no spreaders), indicating that the units were functioning. The same was true for all settling plates used since no attempt was made to use a selective medium to minimize contamination. The settling plates taken in the test cubicle (P-1, P-2, P-3, and P-4) and in the upper half of the centrifuge cabinet (P-5, P-6, and P-7) showed no
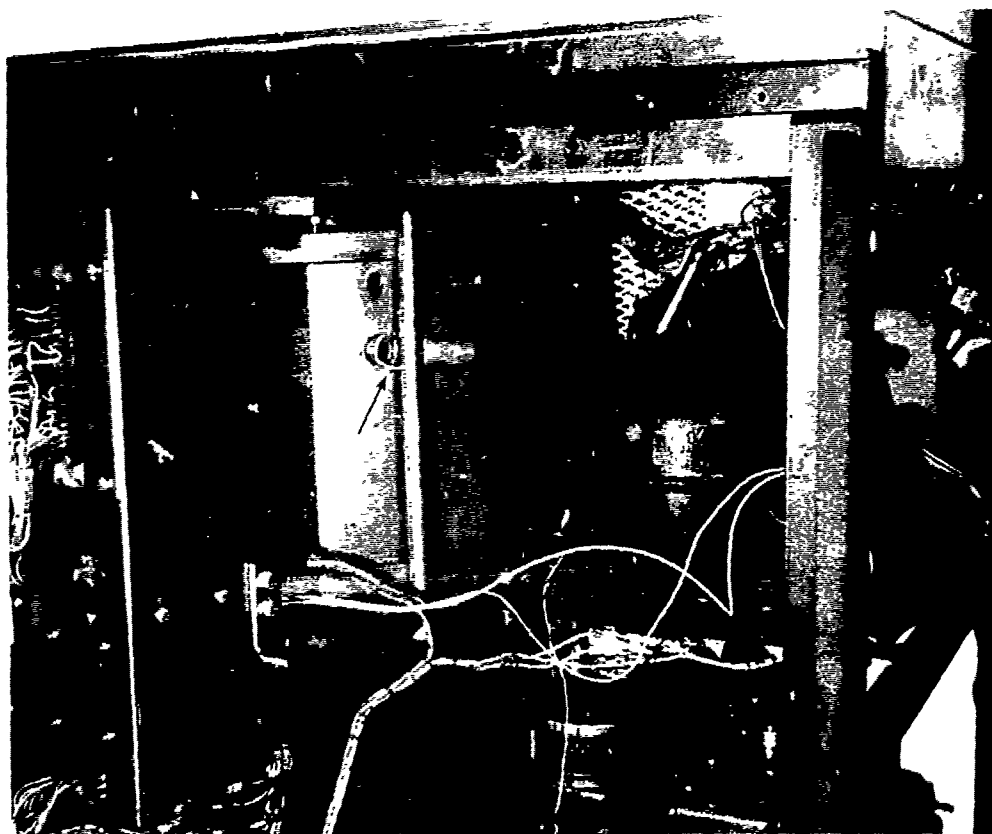

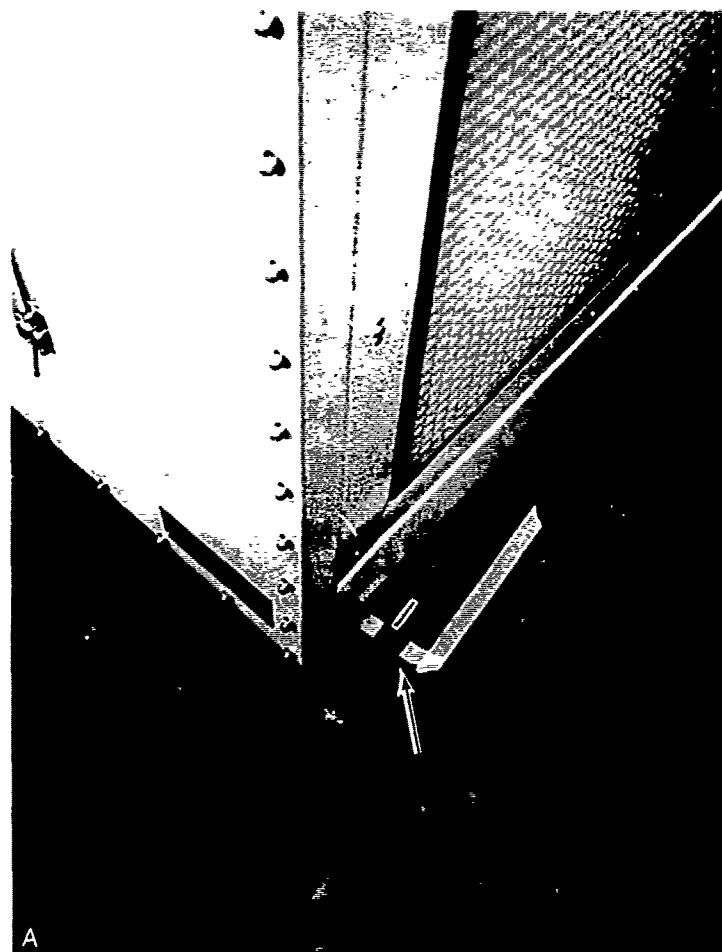FIG. 3. View of vacuum pump connection and rotor enclosure in base of centrifuge.

FIG. 4. *Views of special centrifuge enclosure showing movement after rotor rupture. (A) View of rear lower corner; (B) view looking at front.*

Flavobacterium contamination. Sampling plates P-8 and P-9, inside the closed base of the cabinet, showed colony counts of 14 and 45 colony-forming units, respectively. At the conclusion of the test, some of the original suspension was recovered and returned to the laboratory for assay. Laboratory tests indicated that the suspension available at the time ꞏture contained approximately 10^{10} v   ꞏs (colony-forming units) per ml.

DISCUSSION

It is apparent that the titanium rotor tested in this experiment was extremely durable. It must be emphasized that the rotor was weakened to an extent far beyond that which would be found in normal practice. The explosive nature of the failure is noted by the dislocation and rotation of the entire safety cabinet and the distortions of the rotor enclosure. This lends considerable emphasis to the requirement for total enclosure of the centrifuge if the agents under test are highly hazardous to the operator or the environment. Catastrophic failure of the rotor is extremely unlikely, provided manufacturer's recommendations for care and inspection of the rotor are followed. However, the combination of high-risk agent, high titer, and probability of incidence can act as a combination of power law factors, and the resultant dosage or "risk" estimated may be unacceptable. Work with hazardous agents like Marburg virus, Lassa fever virus, or the agents of Kuru or Creutzfeldt-Jakob disease at high titers are examples.

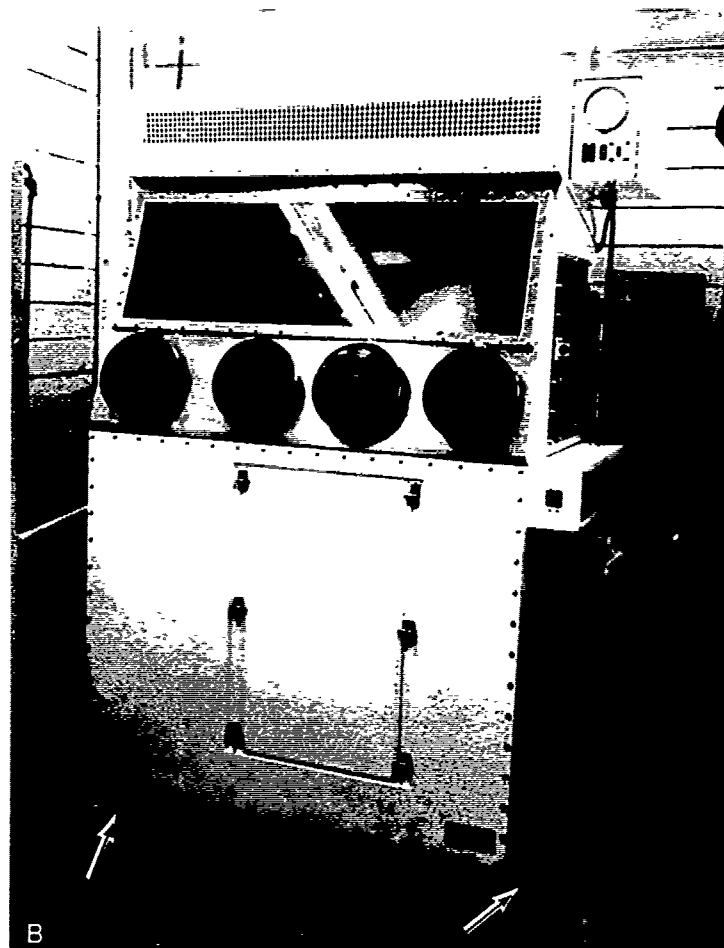The low counts on settling plates P-8 and P-9 are attributed in large part to the rapid flush-out

FIG. 4. B

rate of the ca. 300 cfm passed through this lower space. This air flow served to cool the operating mechanism but also acted to remove the cloud of small (3 to 8 μm) airborne particles which we must presume, on the basis of previous studies (3), to have been produced.

Although it would have been ideal to have had more tests and more sampling sites within the cabinet and outside the cabinet to assure the validity of the experiment, the conditions of the trial were such that we believe the data acquired are valid.

The sole purpose of this experiment was to determine the effects of catastrophic failure of the rotor. No attempt was made to overfill the tubes or to create a leaky condition either in the centrifuge closure or in the rotary seals of the drive unit. These items have been tested separately, and results are reported elsewhere (1, 2,

5). In view of the movement of the centrifuge, on rupture of this rotor, the use of a full cabinet safety enclosure is believed warranted for protection against microbial aerosols which could have been of substantial magnitude (had the vacuum chamber been distorted or ruptured), from the physical damage which could have been created (had the centrifuge cabinet been allowed to move freely away from a top-mounted enclosure), and from any aerosol emanating from the vacuum system before rupture.

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