

satisfactory but will stand up to approximately  $10^5$  gravity if sufficient care is taken in the construction.

The rotational speeds of the above centrifuges were determined by the stroboscopic method. The maximum rotational speed of course depended upon the diameter of the rotor. The  $1\frac{1}{2}$  in. all-metal rotor described in Figs. 1 and 2 would rotate up to 3,000 R.P.S. and give centrifugal forces in excess of  $4 \times 10^5$  gravity. However, the glass disk and seals in Fig. 2b are not as yet practical above  $2 \times 10^5$  gravity. With a small 1 cm all-metal rotor over  $10^6$  gravity has been obtained. With the larger  $3\frac{1}{2}$  in. rotor shown in Fig. 3a we obtained approximately  $10^5$  gravity but were apparently limited here by the capacity of our air compressor (we use a garage "Dayton" V-30, but most regular laboratory air compressors should have sufficient capacity). The air pressure of course regu-

lates the speed. We seldom operate above 150 pounds per square inch, 90 pounds per square inch being a convenient working pressure. The temperature of the centrifuge is usually just below room temperature.

In conclusion, we believe that the air-driven type of ultracentrifuge described above offers certain important advantages as follows: First, it is simple and easy to construct; second, the design may be widely varied to suit the need of the experimenter, and still remain extremely stable;<sup>6</sup> third, the cost is very little if air pressure is already available; and fourth, the centrifugal forces developed are as high as any material (as yet tried) of the rotor will safely stand.

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## SPECIAL ARTICLES

### EXPERIMENTAL MENINGOCOCCAL INFECTION IN MICE

INVESTIGATION of the biology of meningococcus has been retarded by the lack of a satisfactory means of producing experimental infection in laboratory animals. Although guinea-pigs and mice succumb to intraperitoneal inoculation, the dose required of the most virulent strains is very large. Even when a fatal issue has resulted, it is not always possible to recover living organisms from the heart's blood or from the peritoneal cavity itself. Considering the essential toxicity of meningococcus, it seems reasonable to regard such heavy inoculations as overwhelming intoxications rather than true infections. It has been found, in fact, that the minimum lethal dose of meningococci suspended in saline is so close to that of heat-killed organisms that the difference falls within the limits of experimental error. Of particular interest in this connection is the work of Branham and Lillie<sup>1</sup> on experimental meningitis produced in guinea-pigs by intracisternal injection. They report that boiled or filtered suspensions of meningococci produced essentially the same clinical as well as histopathological pictures as did living organisms.

In the course of experiments begun some time ago in the hope of establishing gonococcal infection in some laboratory animal, various menstrua for the organisms were tried out. Since the gonococcus in its natural host gains a foothold in a surface covered by mucous secretion, it was assumed that mucin, the characteristic constituent of mucus, might provide a desirable environment for the initiation of growth under experimental conditions. Of the several prepa-

rations tried, the most practicable was found to be mucin prepared commercially from hog's stomach.<sup>2</sup> While this was more satisfactory than other vehicles for the inoculation of gonococci, its value in promoting experimental infection with meningococci was even more striking. It has been possible, in fact, to produce with regularity a fatal infection in mice by the intraperitoneal injection of relatively few organisms. That the mucin is itself non-toxic is demonstrated by the fact that mice seem to suffer no more ill effect from the intraperitoneal injection of 2 cc of mucin than from an equal quantity of salt solution. A report<sup>3</sup> has recently appeared on the enhancement of virulence of pneumococci and hemolytic streptococci for mice by means of mucin, but since this species is naturally susceptible to these organisms, the effect is less pronounced than in the case of meningococci.

The suspension fluid is prepared as follows: hog's gastric mucin is washed for several days in many changes of 70 per cent. alcohol to remove unnecessary ingredients and also to kill the spores of contaminating bacteria. It is then dried between blotting-paper and dissolved in sufficient saline to make a 6 per cent. solution and buffered at pH 7.4 as is ordinary culture media. Solution is facilitated by adding the saline slowly to the mucin as it is being ground vigorously in a mortar. The solution is then tubed and sterilized in an Arnold sterilizer at 100° C. for one hour every

<sup>6</sup> See Harvey, *Jour. Franklin Inst.*, 214: 1, 1932; or Beams, *Phys. Rev.*, A 39: 858, 1932.

<sup>2</sup> We have used the preparation marketed by The Wilson Laboratories, Chicago.

<sup>3</sup> W. J. Nungester, A. A. Wolf and L. F. Jourdonais, *Proc. Soc. Exp. Biol. and Med.*, 1932, 20, 120.

<sup>1</sup> S. E. Branham and R. D. Lillie, *J. Bact.*, 1933, 25, 90.

other day for 3 days, and incubated on the intervening days. The precipitate which forms is discarded and only the clear supernatant used for injection.

Mice infected intraperitoneally with appropriate doses of meningococci suspended in 2 cc of mucin usually succumb within 6 to 24 hours. Cultures of the heart's blood have always been positive if made soon after death. The peritoneal exudate contains large numbers of organisms and relatively few leucocytes. Maximum virulence has been attained by using as the inoculum peritoneal exudate, diluted with mucin. Of nine recently isolated strains, one has been found by this method to have a minimum lethal dose of less than 100 organisms, and another of less than 200 organisms. In the case of the more virulent of these two strains, parallel titrations of peritoneal exudate made with saline and mucin showed the minimum lethal dose with the former diluent to be approximately a million times as great as with the latter. Thus far the loss of virulence resulting from cultivation on artificial media has been regained by two or three passages through mice.

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#### THE FEEDING REACTION OF SEVERED PROBOSCIDES OF *DILEPTUS ANSER*

*DILEPTUS* is a holotrichous ciliate protozoan which possesses at its anterior end a long undulating proboscis, at the posterior end of which there is a mouth. On the oral side of the proboscis trichocysts are situated. The cilia of this region are longer and stronger than those of the rest of the animal.

A finely drawn glass rod was used as a cutting instrument. The animals were isolated from stock cultures and placed into depression slides. Under a binocular dissecting microscope, having a magnification of approximately 40 diameters the glass rod was used to sever the proboscides of these animals from the main body portions. The feeding reactions were observed under a compound microscope.

The proboscides of some of the animals were cut off just anterior to the mouth and the proboscides of others were cut off just posterior to the mouth. Thus two categories of proboscides were obtained: (1) Proboscides possessing a mouth and (2) Proboscides not possessing a mouth. These were separately placed into isolation dishes containing numerous *Colpodas* in a small amount of hay medium. In both cases the well-known feeding reaction that Visscher<sup>1</sup> has described took place.

<sup>1</sup> J. Paul Visscher, "Feeding Reactions in the Ciliate, *Dileptus gigas*, with Special Reference to the Functions of Trichocysts," *Biol. Bull.*, Vol. 45, 1923.

#### A. REACTION OF PROBOSCIDES POSSESSING A MOUTH

These swam around quite actively; much more so than the main body portions from which they were severed. If, by chance, one came in contact with a *Colpoda* it immediately extruded trichocysts. Most frequently either of the following reactions took place:

(1) The cell membrane of the *Colpoda* was broken down at the point of contact with the trichocysts and thus an amorphous *Colpoda* was produced.

(2) The *Colpoda* retained its shape but became immobile. The *Colpoda* was then passed by action of the cilia of the proboscis toward the mouth, which became distended to receive its food. A food vacuole of the type described by Visscher was formed. The ingested food remained within the posterior portion of the proboscis. A plasma membrane probably had formed at the posterior end where the proboscis had been severed from the main body portion. Such a proboscis continued its activities and with each added *Colpoda* its posterior portion became more and more swollen until the proboscis lost all semblance to the shape that it had when it was first severed. I have observed as many as six food vacuoles in one of these proboscides.

#### B. REACTION OF PROBOSCIDES NOT POSSESSING A MOUTH

These also swam around quite actively and undulated in the manner similar to that when they were on the whole animals. The same reaction to *Colpodas* took place with these proboscides. The affected *Colpoda* was passed down to the posterior end where the mouth was formerly located. Then the proboscis rotated on its posterior end around the *Colpoda* for a time before swimming away. This characteristic feeding reaction as shown by a specialized small part of the total organism is an interesting physiological phenomenon. The proboscis met up with other *Colpodas* in a short time and repeated the reaction. The *Colpoda* was always passed to the posterior end (where the mouth was formerly located) and most often the proboscis with its posterior end closely applied to the *Colpoda* would gyrate around it.

These experiments were done many times and the same results were recorded in each instance.

The posterior portions usually regenerated new proboscides within two hours. The proboscides that were severed in the evening were seen to have regenerated into complete animals which were about one third as large as a normal-sized *Dileptus* the following morning.

As a result of these experiments it may be seen