unanesthetized animal after its recovery from the operation and thereby to induce changes in the tonus of the gall bladder as revealed by X-rays of its roentgen-opaque contents. Preliminary attempts to ascertain the nerves involved in these reflex pathways have demonstrated that direct stimulation of the plexus that accompanies the left gastric artery (2 animals), or of the hepatic plexus (1 animal), induces relaxation of the contracted gall bladder.

This preliminary report is based on 14 experimental animals. In the 8 animals in which the conditions prerequisite to inhibition were established (*i. e.*, emptying of the gall bladder following ingestion of egg-yolk), faradic stimulation sufficient to cause slight pain, repeatedly and consistently stopped the flow of cystic bile.

4899

A Method for Obtaining a Pure Culture of Balantidium coli.

ERNEST CARROLL FAUST.

From the Parasitology Laboratory, Department of Tropical Medicine, Tulane University of Louisiana.

Balantidium coli, the ciliate of the large bowel of the pig, and not infrequently reported from man, the monkey and the guinea pig, has been grown *in vitro* by Walker,[1] Barrett and Yarbrough[2] and Rees.[3] The latter investigator was able to isolate single individuals and start a pure line of the organism obtained from the guinea pig. In all of these attempts, however, the medium was contaminated by various fecal bacteria and no efforts appear to have been made to develop pure cultures, although the need for such a procedure is evident.

Opportunity to attempt the sterilizing of balantidia against fecal bacteria was presented to the writer during the summer of 1929, when all of 12 individuals of *Macacus rhesus,* which had been under observation by Dr. C. C. Bass for a non-protozoan infection, were found to be passing cysts and trophozoites of *Balantidium coli* in their stools. Rectal specimens from those individuals which gave the richest yields of trophozoites were washed and concentrated in a

[1] Walker, E. L., *Phil. J. Sci.,* (B), 1913, viii, 1.

[2] Barrett, H. P., and Yarbrough, N., *Am. J. Trop. Med.,* 1921, i, 161.

[3] Rees, C. W., *Science,* 1927, lxvi, 89.

modified Ringer's fluid and the active organisms freed of fecal debris and gross bacterial contamination.

Preliminary tests were then made with several bactericidal agents in various dilutions to determine if any could be found which were lethal to the bacteria without injuring the active balantidia. The most promising of these were acriviolet and neutral acriflavine. The washed trophozoites were placed in the following dilutions of each of these drugs and the organisms examined after 5 minutes, 10 minutes, 30 minutes and 60 minutes respectively: 1/1,000; 1/2,000; 1/4,000; 1/8,000; 1/16,000; 1/32,000; 1/64,000; and 1/128,000. In both series the dilutions of 1/8,000 or less, even for as short a period as 10 minutes were toxic to the trophozoites. Those in acriviolet at a 1/16,000 dilution or greater survived for 10 minutes but were killed before 30 minutes. Those in acriflavine survived and were active in a 1/16,000 dilution or greater for 30 to 60 minutes. In acriviolet the dead organisms were violet stained; in acriflavine they were orange tinted; in both media the active organisms were tinted a delicate lemon hue. The living organisms were removed under sterile precautions to various culture media where they immediately began to feed. Fractions from the material submitted to the various dilutions of the two dyes for 30 minutes were placed on glucose-agar slants and incubated at 37°C. They were all negative for bacteria and yeasts, except the 1/64,000 dilutions, in which a few colonies developed and the 1/128,000 dilutions in which abundant growth took place.

Since the 30-minute contact with the dye was most convenient for manipulation and since acriflavine proved to be less toxic for the balantidia for this amount of exposure, the experiment was repeated several times, with dilutions of 1/1,000; 1/5,000; 1/10,000; 1/15,000, etc., up to 1/70,000. It was found that healthy trophozoites could stand exposure in all dilutions from 1/70,000 to 1/20,000 for 60 minutes; in dilutions to 1/15,000 for 30 minutes; in dilutions of 1/10,000 for 10 minutes, and in dilutions of 1/5,000 for 5 minutes or less. Glucose-agar inoculations from all of these were negative up to 10 days, when the culture tubes were discarded.

These experiments indicate that acriflavine is capable of sterilizing trophozoites of *Balantidium coli* against bacteria and yeasts in dilutions well within the viable limits of the ciliate. One probable reason for this success was the thorough washing of the balantidia in an isotonic medium preceding exposure to the dye, thus freeing them from gross fecal contamination. The faint lemon tinting of the balantidia and even the staining of the nuclei apparently produced no unfavorable effect on the organisms, since they proceeded

to feed, at times ravenously, as soon as they were removed to a sterile nutrient medium. This same method may possibly be useful for the sterilization of the trophozoite stage of other intestinal protozoa.

4900

A Standardized Method for Pollen Air Analysis.*

W. T. PENFOUND AND B. G. EFRON. (Introduced by Isadore Cohn.)

From the Botany Department of Tulane University and the Allergy Clinic of Medical Department of Touro Infirmary and Hay Fever Clinic of Senses Hospital.

Pollen air analysis has come to play an important rôle in the diagnosis and treatment of allergic diseases caused by weeds. The usual method of determining the amount of wind-borne pollen is as follows: A portion of a glass slide is marked off near the center and thinly coated with vaseline, glycerine, or corn oil. It is then placed in a horizontal position or at an angle in some exposed situation for 24 hours. The slide is taken to the laboratory in a closed container, and the pollens on the ruled portion are identified and counted with the aid of a mechanical stage and a micrometer ocular.

Comparisons of slides placed in various sections and situations in New Orleans throughout 1929 revealed a great difference in the numbers and percentage compositions of pollen of various species. Very high counts were often obtained in the heart of the business district, and at the same time low counts prevailed in the residential and suburban districts. Slides placed on different sides of the same building at the same time, showed marked discrepancies in both numbers and species of pollen. In addition, relatively low counts were obtained during periods of marked hay fever, and *vice versa.* These results were, no doubt, directly related to the degree of exposure.

This diversity in the percentage of pollen of a given species in a given situation raised the question as to whether one might not get a great difference on slides placed in one location simultaneously, and exposed in positions varying from the horizontal to the vertical. Accordingly, slides were exposed at various inclinations with the horizontal and counts were made. The vertical slides showed more pollen on windy days, provided they were exposed perpendicular to

* This work was aided by a grant from the Harry Dennery Research Fund.