insulated terminal "N" is in contact with the metal handle of the movable plate; this makes an electrical contact and the signal magnet records the time taken for the blood to be driven through the capillary tube under a known pressure. This time when compared with that required for water under the same pressure gives the relative viscosity of the blood.

It is evident that any number of determinations may be taken without decreasing the amount of blood in the animal. The electrical recording of the time is of advantage in reducing the error due to the reaction time of the experimenter. Because of the short time the blood for determinations remains in the bulb, a bath for temperature control is not thought necessary.

In order to test the accuracy of the instrument, a series of experiments was carried out in which the relative viscosity of 7 per cent. gum arabic was determined by means of this instrument and the same procedure carried out with the Oswald viscosity pipette, water being taken as unity; in thirty determinations with each instrument it was found that the relative viscosity of the gum solution when determined with this instrument was 3.76 while with the Oswald type it was 3.78. These results appear to be well within the range of experimental error. The determinations were made at room temperature and the pressure on the fluid maintained at 70 mm. Hg.

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RUSSELL A. WAUD

SPECIAL ARTICLES

BALANTIDIA FROM PIGS AND GUINEA-PIGS: THEIR VIABILITY, CYST PRODUCTION AND CULTIVATION

THE following data concerning *Balantidium* occurring in the pig and guinea-pig are deemed of sufficient importance to warrant a report at this time. An abundance of material from the pig has been obtained from two packing plants within several squares of the laboratory, and Dr. W. R. Stokes, of the Baltimore City Health Department, has kindly furnished guinea-pigs for autopsy that died as a result of experimental work. Thus far of the twenty examined, 55 per cent. were infected with *Balantidium*. A colony of rhesus monkeys which also harbor *Balantidium* is maintained by Dr. Carl Hartman, of the Carnegie Institution. This article is a progress report on a problem of host-parasite relations which was suggested to me by Dr. R. W. Hegner.

VIABILITY

According to McDonald[1] (1922) the trophozoites of *Balantidium* of the pig become spherical when the intestinal contents are cooled to room temperature. McDonald also states that they live at room temperature not longer than eight hours. Accordingly, a thermos bottle was used to carry the material to the laboratory from the packing plant. No appreciable rounding was noted when the organisms were examined at room temperature. Therefore, the content of a bottle obtained January 3, 1927, was allowed to cool. Active, apparently normal, trophozoites were found in a sample taken from this bottle the next morning and on every subsequent morning until January 14. The relative numbers did not appear to diminish for about seven days, but then fell off very rapidly. The temperature of the contents of the bottle was taken after fourteen hours, and found to be 20° C. On several other occasions the organisms lived at room temperature for four days and on one occasion for seven days. The viability of trophozoites is also indicated by the fact that water from the trucks in which the pigs were transported from the cars was found to contain them; they remained perfectly normal in appearance at room temperature for twenty-four hours. Feces passed at least two hours previously by ten different pigs were collected from the pens at the packing plant. Trophozoites were found in seven of the ten samples. The pigs had been long in transit from Ohio and the feces were well formed so that they had to be torn apart in water before the trophozoites were freed. The latter appeared perfectly normal and swam about actively.

INFECTIVITY OF TROPHOZOITES

It seems to be the general opinion that ingestion of cysts must occur to set up an infection. (Fantham, Stephens and Theobald,[2] 1916); but Hegner[3] (1926) injected trophozoites from the pig into the stomach of the guinea-pig, and, when the animal was killed one hour later, active, apparently normal trophozoites were found in the stomach, small intestine and cecum. This experiment has been repeated with success.

[1] McDonald, J. D. 1922. "On *Balantidium coli* (Malmsten) and *Balantidium suis* (sp. nov.) with an Account of their Neuromotor Apparatus. Univ. Calif. Pub. Zool., 20: 243–300.

[2] Fantham, H. B., Stephens, M. D., and Theobald, M. A. 1916. "The Animal Parasites of Man," 900 pp. New York.

[3] Hegner, R. W. 1927. "Host-Parasite Relations between Man and His Intestinal Protozoa." The Century Co., New York. (In press.)

Trophozoites of the pig *Balantidium,* grown in culture, were injected into the stomach of a guinea-pig four weeks old. It died during the night, but after eighteen hours trophozoites, gorged with starch grains, were found in the esophagus and cecum. That these were not inhabitants of the intestine before the experiment began is indicated by the fact that the latter are always translucent and move slowly, whereas those from cultures are blackened from ingested starch and move actively. Later another guinea-pig from the same litter was given trophozoites by stomach tube direct from the pig. This animal also died during the night and trophozoites were found the next morning in the ileum, jejunum and cecum. They were of two kinds in the cecum; (1) those that parasitize the guinea-pig and (2) the starch-filled forms of the pig. When it is considered that trophozoites may live in feces for ten days at room temperature it is probable that they may serve as well as cysts in transmitting infection.

CYST PRODUCTION IN THE PIG

McDonald (1922) states that trophozoites were in various stages of encystment in the lower colon and rectum of the pig and all encysted in the formed feces. In Baltimore during February and March no cysts were found until about thirty pigs, all of which were infected with *Balantidium,* had been examined. They were not numerous; about three hours were required to pick out twenty specimens. Because of their large size, cysts of *Balantidium* can be distinguished with a binocular microscope. Material can be diluted in a Syracuse watch glass and examined much more rapidly than when slides are used. Cysts can be picked out with a micropipette and studied under the compound microscope. In this work, as well as by McDonald (1922), the cysts were found to be very resistant to fixing and staining. The nucleus and other structures are not revealed when they are treated with iodine eosine or even when Mallory's haematoxylin is run under the cover glass after fixation with Schaudinn's fluid. When bodies resembling cysts were finally found the material was treated in bulk with hot Schaudinn's and stained with iron haematoxylin (method of Kofoid and Swezy). They were then picked out and positively identified. Walker[4] (1913) and others state that fecal diagnosis for *Balantidium* in man and monkeys is unsatisfactory, because for long periods no cysts are passed. Walker (1913) states, as does McDonald (1922), that cysts are frequent in feces of pigs. Walker and others express the opinion that for this reason infection in man is usually contracted from pigs. Data obtained in Baltimore, however, indicate that cysts may be as scanty in pigs as in man, monkeys and guinea-pigs.

CULTIVATION

Much experimental work has been done recently and is being continued on cultivating parasitic protozoa. The first media tried by myself were made according to directions given by Dobell and Laidlaw[5] (1926) for *Endamoeba histolytica.* On one occasion trophozoites lived seventy-two hours and multiplied. The medium consisted of an inspissated human serum slant plus a fluid of Ringer's solution without dextrose, nine parts, and human serum, one part. Sterile rice starch was added. Transplants from this culture failed. Various egg media were tried with negative results. Walker (1913) concluded, as a result of experiments, that a 0.5 per cent. NaCl solution is best suited for *Balantidium.* Barret and Yarbrough[6] (1921) cultivated *Balantidium coli* successfully in a medium 0.5 per cent. NaCl, fifteen parts, plus human serum, one part. Their cultures were maintained thirty-two days. In order to ascertain whether Walker's data could be applied in the cultivation of *Balantidium* from the pig the concentration of the medium in which the organism lives was determined. Feces from the cecum were filtered through filter paper and the freezing point method employed, with a Beckmann thermometer. The reading of filtrate from fresh feces was minus 0.70° C.; of filtrate from feces kept forty-eight hours in the laboratory minus 0.79° C.; and of feces kept 168 hours it was minus 0.72° C. The freezing point of blood serum is about minus 0.6° C., and is isotonic with an 0.85 per cent. NaCl solution. Walker found this hypertonic for *Balantidium,* and preliminary trials carried out here seemed to confirm this conclusion. Hence it was thought that the hypertonicity of 0.85 per cent. NaCl is due to an excess of inorganic ions. Accordingly, 10 cc of each of the above fecal filtrates were evaporated to dryness; and the organic matter driven off by heating. The residue was taken up in 10 cc of distilled water and the freezing point found to be minus 0.17° C. This is isotonic with a NaCl solution of about 0.25 per cent. On the basis of the above data an attempt was made to prepare synthetic media,

[4] Walker, E. L. 1913. "Quantitative Determination of the Balantidicidal Activity of Certain Drugs and Chemicals as a Basis for Treatment of Infections with *Balantidium coli.*" *Philippine Journ. Sci.* (B), 8: 1–15.

[5] Dobell, C., and Laidlaw, P. P. 1926. "On the Cultivation of *Endamoeba histolytica* and Some Other Entozoic Amoebae." *Parasit.,* 18: 283–318.

[6] Barret, H. P., and Yarbrough, N. 1921. "A Method for Cultivation of *Balantidium coli,*" *Amer. Journ. Trop. Med.,* 1: 161.

using 0.25 per cent. NaCl solution and adding urea in one instance and aminoids, Biuret free, in another, to make the solution about isotonic with blood serum. Blood serum and starch were also added to some of the tubes. The results of culture experiments were invariably negative in the urea tubes. Growth was occasionally noted in the aminoids, but transplants were unsuccessful.

Successful results were finally secured by using the method of Barret and Yarbrough (1921) with a modified Ringer's solution instead of 0.5 per cent. NaCl. The latter contained: NaCl 6.5 gms., KCl 0.14 gms., $CaCl_2$ 0.12 gms., $NaHCO_3$ 0.20 gms., Na_2HPO_4 0.01 gms., per liter of distilled water. The ingredients were weighed from C. P. crystals on an analytical balance but no attempt was made to desiccate them before weighing. The freezing points of various solutions thus far used have ranged from minus 0.52° C. to minus 0.58° C. Eighteen cc is pipetted into clean test tubes and autoclaved. Two cc of human or horse serum is then added, plus a sprinkle of sterile rice starch. Pig serum has thus far proved unsatisfactory. A solution made from Loeffler's dehydrated blood serum is used frequently instead of the human or horse serum. This product is manufactured by the Digestive Ferments Company, of Detroit, Mich. The contents are listed as beef blood 3 parts and dextrose broth 1 part. Solution is obtained by adding 8 gms. of the powder to 100 cc of distilled water at 40° C. It is advantageous to measure the water in a 250 cc flask containing a few glass beads and sterilize. Shaking the flask with the beads aids in securing solution, but even so there is much suspended matter. A culture of *Balantidium* from the pig was maintained for fifty-four days and was still thriving on April 4. The tubes are kept in an incubator at 36° C. and transplants are made according to Barret and Yarbrough's technique. Twenty transplants have been made. They are made usually at three-day intervals, but on one occasion (7th transplant), a seven-day interval occurred. Trophozoites in abundance have been found on a number of occasions at the end of seven days; but they usually decrease rapidly after three days.

Twelve attempts have been made to secure pure lines by isolating single individuals. The findings agree with those of Barret and Yarbrough that this can not be done by the drop culture method. The organisms appear to be anaerobic. Success was obtained by picking out single individuals with a micropipette, placing into a drop of culture fluid on a cover slip and dropping the latter into a test tube containing the medium. On one occasion multiplication occurred but the transplant failed.

After three unsuccessful attempts a culture was started with *Balantidium* from the guinea-pig in the same media as are used for the culture from the pig. This strain has now been successfully transplanted six times. The organisms ingest starch as does the pig *Balantidium* and become very active. It would be impossible to distinguish the trophozoites from those from the pig if the labels were left off the culture tubes. Occasional cysts have been found in tubes in which trophozoites from the guinea-pig have disappeared. Despite repeated research no cysts have been found in cultures from the pig.

Resistance of Trophozoites to Hydrogen Sulphide

The odor of H_2S is always pronounced in the feces of pigs. On five occasions this gas has been bubbled through culture tubes for fifteen to thirty seconds. No deleterious effects have been noted on the trophozoites. The first time that the experiment was tried the culture from the pig lived seven days. It was hoped that this gas might be used to check the growth of harmful bacteria. Subsequent results were so variable that no conclusions are justified. H_2S was found toxic to the trophozoites when bubbled through feces of the pig; when treated and untreated samples were kept in the incubator the organisms disappeared in forty-eight hours from the treated tubes but lived seventy-two hours in the untreated material.

Summary

It has been shown that trophozoites of balantidia from the pig are normal in appearance and reactions when the medium is cooled to room temperature. They may live at room temperature for ten days. Trophozoites that infect the pig may pass through the stomach of the guinea-pig and reach the cecum, where they are normal after eighteen hours. Trophozoites are frequently passed in feces by the pig but cyst production is irregular and determined by unknown factors as in monkeys, guinea-pigs and man. The fluid of pig feces from the cecum is slightly hypertonic to blood serum. The method of Barret and Yarbrough for the cultivation of *Balantidium coli* has been found practicable for *Balantidium* from the pig and guinea-pig. The addition of rice starch improves the medium and Ringer's solution without dextrose was found more suitable than 0.5 per cent. NaCl solution. Loeffler's beef blood serum may be substituted for human serum or horse serum. H_2S appears non-toxic to *Balantidium* from the pig when passed into culture tubes.

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