PENNSYLVANIA ACADEMY OF SCIENCE

THE regular spring meeting of the Pennsylvania Academy of Science was held in Harrisburg on April 2 and 3. Because of the emergency, the usual evening dinner was omitted and the session curtailed. Nevertheless, about 200 persons attended. The evening of the second was opened with papers by Dr. William L. Rhein and Dr. John M. Fogg, Jr., on natural history, particularly as applied in Pennsylvania. On the third, the customary procedure was changed. Instead of the reading of many papers by the members, a few selected papers were read which in their entirety were in the nature of a symposium on research and the status of science education. President Charles E. Mohr presided. The following officers were elected: *President,* C. A. Horn; *President-elect,* Homer C. Will; *Vice-presidents,* Bradford Willard and Leroy K. Henry; *Editor,* E. M. Gress; *Secretary-Treasurer,* V. Earl Light; for the Junior Academy, Mary E. Hawthorne.

BRADFORD WILLARD

SPECIAL ARTICLES

THE CULTIVATION FROM GRANULOMA INGUINALE OF A MICROORGANISM HAVING THE CHARACTERISTICS OF DONOVAN BODIES IN THE YOLK SAC OF CHICK EMBRYOS[1]

IN spite of careful work by a number of investigators no agent acceptable as the etiologic factor of granuloma inguinale has as yet been cultivated. Recent reports by Dienst, Greenblatt and Sanderson,[2] by Greenblatt, Dienst, Pund and Torpin[3] and by Carter, Jones and Thomas[4] agree that the agent is not cultivable on a wide variety of media known to be useful for the cultivation of certain fastidious pathogenic microorganisms. Ordinary experimental animals are resistant to infectious material from natural lesions. Neither of the above groups of workers was able to cultivate the agent on the chorioallantois of chick embryos. Greenblatt and his associates were able to reproduce the infection in human beings with material containing Donovan bodies apparently free from contaminants. They concluded that the Donovan body is the etiologic agent, that it is not related to the Friedlander-aerogenes group of bacteria and has not been propagated outside the human body.

This paper reports the cultivation in the yolk sac of living chick embryos of a microorganism that has all the morphological characteristics of the Donovan organism and is as yet neither cultivable on ordinary culture media nor pathogenic for mice, dogs or monkeys.

Tissue from a human lesion especially rich in Donovan bodies and with remarkably little evidence by smear of contamination with bacteria was obtained by Dr. W. A. DeMonbreun from a patient at the Nashville General Hospital. Small bits of this tissue were smeared over the surface of cystine agar slants subsequently incubated at 37° C. After 96 hours two slants appeared free of any bacterial growth. Smears showed the presence of a few gram-negative bipolar forms seemingly closely associated with degenerating tissue. These microorganisms appeared to be viable. There was little or no evidence that they had multiplied on the slant. They were not unlike non-encapsulated Gram-negative forms characteristically associated with Donovan bodies in granuloma inguinale lesions.

Each uncontaminated cystine slant was washed with 3 cc .85 per cent. NaCl; the washings were pooled and .5 cc was inoculated into the yolk of six 8-day-old embryos. On the third day two embryos, dead without evidence of bacterial growth, were discarded. Smear from the yolk of one live embryo at this time did not show evidence of bacterial growth. On the eighth day smears from the yolk of each of the four remaining living embryos, stained with Wright's and Gram's stains, revealed the presence in abundance of both encapsulated and non-encapsulated Gram-negative microorganisms indistinguishable from Donovan bodies and from those pleomorphic Gram-negative non-encapsulated forms always present in lesions of granuloma inguinale.

Subcultures of the microorganism present on the original cystine slants to other cystine slants did not grow. The microorganism present in the yolk sac of embryos has repeatedly failed to grow on enriched blood media, potato-dextrose-agar, anaerobic broths and meat, Loeffler's slants and egg-yolk slants.

As far as we could determine these original yolk sac cultures were pure and the microorganism has been uninterruptedly and easily cultivable in the yolk sacs of living chick embryos through 25 successive passages during a period of three months.

Transfers have been made by drawing infected yolk from its sac with a needle and syringe and injection

[1] This work was aided by a grant from the John and Mary R. Markle Foundation.

[2] R. B. Dienst, R. B. Greenblatt and E. S. Sanderson, *Jour. Infect. Dis.*, 62: 112–114, 1938.

[3] R. B. Greenblatt, R. B. Dienst, E. R. Pund and Richard Torpin, *Jour. Am. Med. Asn.*, 113: 1109–1116, 1939.

[4] Baynard Carter, C. P. Jones and W. L. Thomas, *Jour. Infect Dis.*, 64: 314–316, 1939.