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.

of .2 to .5 cc into the yolk sacs of other embryos. Embryos of various ages were used and transfers were made at various intervals following inoculation.

The morphology of the microorganism varies depending somewhat more upon the age of the embryo from which the smear is made than upon the duration of the infectious process. Cultures from early generations showed mixtures of encapsulated and non-encapsulated forms. As passages increased smears from older embryos showed predominantly unencapsulated forms while smears from young embryos showed a predominance of encapsulated ones. Experience has determined that inoculation of 5- or 6-day embryos into the yolk yields consistently in 72 hours a rich culture that is almost wholly encapsulated. The encapsulated form has been maintained in series. This form inoculated into 12-day embryos grows out largely unencapsulated. Embryos from 1-through 13-days incubation support subsequent development of infection following inoculation into the yolk sac. Our experience indicates that the yolk of every embryo inoculated (700–800) has yielded a growing culture.

This microorganism grows evidently extracellularly in the yolk of the embryo. Smears and histological sections also show that it occurs both in its encapsulated and unencapsulated form inside epithelial cells of the yolk sac membrane, also within mononuclear cells of inflammatory exudate in the yolk and its sac. Notwithstanding direct inoculation evidence that it grows on the chorioallantois or invades the embryo proper from the yolk sac is as yet lacking. Inoculation into the amniotic fluid of the intact embryo seems to support growth feebly.

Infected yolk of the 10th passage was drawn from the embryo and stored in sealed test tubes at 5° C, 25° C, 37° C and at −78° C. After 17 days stored yolk from the first three groups was diluted 50 per cent. with .85 per cent. NaCl and injected in .5 cc amounts into 6-day yolk sacs. That stored at 25° C grew out promptly in 72 hours; that stored at 5° C and at 37° C grew slowly, but all embryos showed a good growth at the end of a week. Similar tests for survival made at the end of 33 days showed that the microorganism survived only at 25° C. Yolk stored at −78° C has not yet been tested for survival of the organism.

Mice inoculated intraperitoneally showed no evidence of infection. Dogs inoculated intra- and subcutaneously have not yet shown evidence of infection. *Macacus rhesus* monkeys were inoculated intra- and sub-dermally. Organisms resembling Donovan microorganisms were demonstrated by smears from nodules that persisted for 4 days, but the nodules regressed and have shown no further activity.

The fact that the microorganism appeared to grow in the yolk of the intact developing embryo made its culture in that medium *in vitro* seem feasible. Yolk alone from uninfected 5- and 6-day embryos in test-tubes did not support growth, but with the addition of bits of embryonic chick heart it gave a fairly good culture in 6 days at 37° C. After 2 serial passages in yolk-heart medium a subculture in yolk without heart was initiated. Strains have thus been maintained through ten serial passages *in vitro* during 7 weeks in yolk with and without heart.

Experiments are in progress at the present time to determine the relationship of this microorganism to the human infection, granuloma inguinale. A series of experiments to determine something of its antigenic relation to the disease is also being carried out. More detailed consideration of its morphological, cultural, antigenic and pathogenic characteristics will be the subject of further study.

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EFFECTIVENESS OF VITAMIN A IN THE TREATMENT OF DEFECTIVE COLOR VISION

SEVERAL reports have appeared in this journal durin the past year on problems of color-blindness, tests for color sensitivity and the value of vitamin A as a remedial agent in conditions of defective color perception.[1,2,3,4] The first suggestion that vitamin A could be used with effect in cases of impaired sensitivity to color was made in a report by Dunlap and Loken[5] before the Southern Society for Philosophy and Psychology. This was followed by the statement that cases were "cleared up" with vitamin A in from three to eight weeks, using doses of 25,000 units per day.[1] Later, these writers stated that 80 per cent. of their cases were able, after vitamin A treatment, to pass chart tests which they had failed previously.[3]

The practical importance of color vision has increased greatly since the beginning of the war. With well over a million men of draft age showing some degree of color deficiency, the possibility of salvaging even a small percentage of this man-power for the armed services or for vital work in industry was certain to attract attention.

Present knowledge of function of the visual receptors, plus the fact of a demonstrable hereditary

[1] K. Dunlap and R. D. Loken, SCIENCE, 95: 2474, 554, May 29, 1942.
[2] E. MURRAY, SCIENCE, 96: 2484, 133–5, August 7, 1942.
[3] K. Dunlap and R. D. Loken, SCIENCE, 96: 2489, 251–2, September 11, 1942.
[4] E. MURRAY, SCIENCE, 96: 2498, 448, November 13, 1942.
[5] K. Dunlap and R. D. Loken, *Psychol. Bull.*, 39: 585, October, 1942.