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Cultivation of *Trichomonas foetus* in the Chick Embryo.*

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At the suggestion of Doctor A. L. Tatum, attempts were made to cultivate a bacteria-free strain of *Trichomonas foetus* upon the chorio-allantoic membrane of the chick embryo according to the window technic devised for the cultivation of viruses by Goodpasture.[1] These attempts were unsuccessful. However, by modifying the original technic, it was found that the living chick embryo could serve as a favorable medium for the cultivation of *Trichomonas foetus*.

The strain of *Trichomonas foetus* used in the experiment was the strain H of Glaser and Coria,[2] kindly sent to this laboratory by R. W. Glaser in November, 1935. Since that time it has been kept on Locke-egg-blood medium. Within 3 months after its arrival the strain was injected serially into the peritoneal cavities of 2 guinea pigs. It was recovered from the uterus of the first pig and freed from associated bacteria by the migration technic of Glaser and Coria. Like Rees,[3] the writer found that *Trichomonas foetus* migrates down more readily than up. The strain was recovered from the peritoneal cavity of the second pig in pure culture. This particular strain is now known in this laboratory as HGP2.

The inoculations of the chick embryos were performed in this manner: The chorio-allantoic membrane of an 11- or 12-day-old chick embryo was exposed. Sterile air was forced into the allantoic cavity through an artificially produced opening to make the membrane adhere to the edges of the shell opening. For the first inoculation, about one cc of a 4-day-old culture in L.E.B. medium was introduced into the allantoic cavity with a glass pipette. For subsequent inoculations, about one cc of allantoic fluid, withdrawn by means of a glass pipette from the allantoic cavity of an embryo inoculated 3 or 4 days previously, was used. Only those allantoic fluids were selected for injection which were found by direct microscopic examination to be teeming with living trichomonads, and by

* This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

1 Goodpasture, E. W., and Buddingh, G. J., *Am. J. Hyg.*, 1935, **21**, 319.

2 Glaser, R. W., and Coria, N. A., *Am. J. Hyg.*, 1935, **22**, 221.

3 Rees, C. W., *Am. J. Hyg.*, 1937, **26**, 283.

examination of smears stained by the Gram method to be free from bacteria.

The primary purpose of the experiment was to maintain the trichomonads in the chick embryo. Because of inadequate facilities, no infected embryos were kept for more than 3 or 4 days after being inoculated. During this 3- or 4-day interval, the total mortality of all embryos was 23%. No experiments have been performed to determine the effect of the trichomonads on the chick embryo. However, two observations indicate that, in the experiment described, no increase in pathogenicity of strain HGP2 occurred. First, there was no progressive increase in mortality of the embryos. A high mortality which occurred suddenly in the thirteenth and fourteenth sets of embryos was probably due to difficulties encountered with the incubator during that time. Secondly, embryos which were inoculated with material from dead embryos showed no significantly higher mortality than those inoculated on the same day with material from live embryos. The genealogy of some of the trichomonads could be traced through 14 embryos alive at the time of inoculation and at the time of removal of allantoic fluid.

Conclusion. *Trichomonas foetus* has been grown successfully in chick embryos through 14 generations (and probably could be continued indefinitely) when the parasites are inoculated beneath the chorio-allantoic membrane as described above.

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Further Studies on the Regeneration of the Aqueous in Man.

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If the aqueous of a human eye is aspirated through a fine hypodermic needle, introduced into the anterior chamber through the periphery of the cornea, several reactive processes are set up within the eye.* The formation of new intraocular fluid and characteristic reactive fluctuations of the intraocular pressure are probably the most important of these processes. We reported[1] the results of ex-

*This proceedure is hereafter referred to as anterior chamber puncture (ACP).

[1] Kronfeld, P. C., and Lin, C. K., *Transactions Amer. Acad. Ophth. and Otolar.*, 1937.