that of the control group (0.041 cc of serum). This was true even with massive doses (up to 500 dog units of Cortin and 30 mg of Percorten). No immediate or late ill effects from these substances were noted.

A group of 5 animals were injected with 0.5 mg of desoxycorticosterone acetate 3 times daily for 10 days, and complement titrations were carried out before starting the experiment, after the fifth day, and also after the tenth day in the 4 surviving animals. One animal died as a result of cardiac puncture. No change in the serum complement titer was observed in any of these animals.

In further experiments, in which the cortical extract was added to guinea pig sera *in vitro*, no change in complement was demonstrated.

Five animals were sensitized to horse serum by the intramuscular injection of 0.1 cc of horse serum. Complement titrations carried out 14 days later showed no significant change after a period of 2-6 hours following the intraperitoneal injection of cortical extract in doses similar to those listed above.

Comment. Cope and Kapnick[13, 14] have reported that adrenal cortical insufficiency, both in the rabbit and dog, results in no change in the concentration of serum complement. In the work reported here, there is no significant change in serum complement on the injection of cortical hormone into normal guinea pigs and into normal sensitized guinea pigs. It is probable, therefore, that adrenal cortical hormone does not influence anaphylaxis or immunity through an effect upon serum complement.

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Comparison of Growth of *Trichomonas foetus* and *Trichomonas vaginalis* in Chick Embryos.*

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Nelson[1] first reported the cultivation of *Trichomonas foetus* in

[14] Kapnick, I., and Cope, O., *Endocrinology*, 1940, **27**, 543.

* This study was made possible by Dr. E. D. Plass, Head of the Department of Obstetrics and Gynecology, College of Medicine, University of Iowa, and Dr. Chas. Murray, Dean of the Division of Veterinary Medicine, Iowa State College, to whom the authors express their sincere appreciation.

[1] Nelson, P. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 258.

chick embryos after inoculations of 1 cc of a culture into the allantoic sac. The eggs were opened 3 to 4 days after inoculation. Allantoic fluid from the most heavily infected embryos was used to carry the protozoan through 14 subcultures. Later, Levine, Brandly, and Graham,[2] following the same procedure, carried *T. foetus* through 23 passages and found that 12-day embryos were better than others in an 8- to 16-day series tested. Hogue[3] inoculated *T. foetus* into the allantoic sac of developing chick embryos and allowed the embryos to hatch or die. In one of 4 chicks that hatched, the protozoa were found in the yolk sac, esophagus, intestines, gall bladder, and cloaca 2 days after hatching. The other 3 gave negative results on fecal examinations. In embryos that died after inoculation, the trichomonads were also found in the digestive tract, yolk, and gall bladder.

The present report concerns a comparison of the growth of bacteria-free cultures of *Trichomonas foetus*[4] and *Trichomonas vaginalis*[5] in chick embryos. Also, successful subculturing of *T. vaginalis* in chick embryos is recorded for the first time.

After a few preliminary trials, *T. foetus* was established in chick embryos and was carried through 14 passages in 78 days. The inoculations were made into the allantoic sac as described below for *T. vaginalis*. The organisms seemed to grow better after the first few subcultures. Seven- to 14-day-old embryos were employed and were inoculated with 0.1 cc to 0.4 cc of trichomonas-containing material. The small inoculum may explain the slight difficulty encountered in establishing the infection. The eggs were opened from 4 to 7 days following inoculation. Observations and experiences were essentially the same as those already reported by others. Examination of the different egg parts showed that the protozoa developed in the allantoic fluid or the amnionic fluid, but were not found in the egg white, the yolk sac, the actual embryo or its blood stream.

The work with *Trichomonas vaginalis* was much more difficult. In initial trials with this organism, less than 0.5 cc of a heavy culture was injected into the allantoic sac. (Table I.) Irregular results led to trials with other methods of inoculation. Attempts to grow the trichomonads in an artificial air cell on the chorio-allantoic membrane met with total failure. Inoculations into the yolk gave

[2] Levine, N. D., Brandly, C. A., and Graham, R., *Science*, 1939, **89**, 161.

[3] Hogue, M. J., *Am. J. Hyg.*, 1939, **30** (Sec. C), 65.

[4] Glaser, R. W., and Coria, H. A., *Am. J. Hyg.*, 1935, **22**, 221.

[5] Trussell, R. E., *J. Iowa M. Soc.*, 1940, **30**, 66.

TABLE I.

Showing Number of "Takes" in Embryos When Injected with Small Numbers of *T. foetus* as Compared with the Number of "Takes" When Injected with Small and Large Numbers of *T. vaginalis*.

T. foetus		*T. vaginalis*			
Inoculations of 0.1 to 0.4 cc per egg		Inoculations of 0.1 to 0.4 cc per egg		Inoculations of 0.75 to 1.0 cc per egg	
No. eggs Protozoa present	No. eggs Protozoa not found	No. eggs Protozoa present	No. eggs Protozoa not found	No. eggs Protozoa present	No. eggs Protozoa not found
184	34	9	66	306	96

Note: After *T. foetus* was once established in chick embryos, it was readily carried in series through 12 subcultures in about 8 weeks, even though small inoculums were employed. On the other, *T. vaginalis* was not subcultured in embryos when a small inoculation was made, but was easily carried in series through 24 subcultures in 15 weeks when a large inoculum was used. The initial material for the larger inoculations had been grown through several subcultures in chick embryo allantoic fluid over liver infusion agar slants before it was employed.

negative results in preliminary trials although subsequent work with larger doses was more successful.

Since the allantoic sac inoculations appeared most promising, these were investigated further and 5- to 17-day-old embryos were tested in an attempt to find the time of greatest susceptibility. This appeared to be 6 days. In addition, the *T. vaginalis* was cultured in chick embryonic fluids over liver infusion agar slants (pH 6.8-7.0) in an effort to adapt the trichomonads to such an environment. An excellent growth occurred and the organism has now been carried for 7 months in this medium. After several weeks' growth, embryo inoculations were attempted with larger doses (0.75 to 1.0 cc) of the supernatant embryonic fluid culture. Six-day embryos were inoculated in the allantoic sac. A small hole was punched into the natural air cell and another hole was drilled through the shell over the embryo,avoiding the blood vessels. The needle was inserted about 5 to 8 mm into the latter opening and the injection completed after which the hole was sealed with paraffin. It is recognized that an occasional inoculation may have been made into the amnion. This is especially true in the younger embryos. After inoculation the developing embryos were incubated at a temperature of 35°C. Beginning 2 hours after inoculation the eggs were opened at intervals up to 10 days. No apparent multiplication occurred during the first 24 hours; in fact, it was often difficult to find trichomonads in hanging drops during that time. Maximal growth was noted after 2 to 4 days. Here again, the protozoa were found only in the allantoic or amnionic fluids.

Only eggs showing maximum multiplication of the protozoa

were used for subculture into new 6-day embryos. In the subcultures, an equal number of inoculated eggs were opened on the second, third, and fourth day after injection and within an hour the most heavily infected allantoic fluids were reinoculated into fresh embryos. Following this procedure, a series of 24 subcultures has been maintained. A few embryos died following inoculation, some because of bacterial contamination, some because of mechanical injury, and others from unknown causes. The protozoan continued to grow well for several days in dead embryos.

It appears that *T. vaginalis* is not particularly pathogenic for chick embryos although Hogue[6] found that *Trichomonas foetus* produced a substance that destroyed tissue culture cells.

In summary, it was found that *Trichomonas vaginalis* and *Trichomonas foetus* can be grown in chick embryos but that the former was more difficult to maintain and did not grow as profusely as *T. foetus*. These 2 protozoa are probably nonpathogenic under such conditions. *T. vaginalis* was successfully cultured in series in developing chicken embryos for the first time. There appears to be no advantage in maintaining a stock series of such cultures since the organisms can be maintained more readily in artificial media with much less danger of bacterial contamination and with a richer growth.

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Experimental Production of Dietary Liver Injury (Necrosis, Cirrhosis) in Rats.*

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Liver injury, mainly in the form of acute diffuse necrosis combined with fat infiltration, has occurred irregularly in young rats fed a diet devoid of vitamin B (casein 18%, sucrose 68, melted butter fat 8, cod liver oil 2, salt mixture 4) and supplemented with thiamine, ribo-

[6] Hogue, M. J., *Am. J. Hyg.*, 1938, **28**, 288.

* Independently and at the same time Dr. Graham T. Webster of the Department of Medicine, Western Reserve University, has made similar observations and has reached essentially identical conclusions. Publication of his results is pending.