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.

12039 P

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.

flavin and pyridoxine.[1] In some of these livers there was diffuse periportal fibrosis. Rats fed the same basal diet plus yeast, or concentrated yeast extract, were free from any pathological changes in the liver. Later, the occurrence of cirrhosis of the liver on a nutritional basis and its prevention similarly by the addition of yeast to the diet were reported in rabbits[2] and in guinea pigs.[3]

The unpredictable and irregular incidence of liver injury in rats has been indirectly regarded as a basis for the assumption that the proper experimental conditions for the regular production of liver injury have not been provided by the experimental technic used.

In view of the well known lipotropic activity of casein,[4] the high proportion (18%) of casein in the basal diet was considered to be possibly one important factor which might counteract other conditions that would be conducive to liver injury. Consequently, rats weighing between 100 and 250 g were put on a diet that had the following composition: casein 10%, sucrose 64, lard 20, cod liver oil 2 and salt mixture 4. This modified basal diet was supplemented with thiamine, riboflavin, pyridoxine and pantothenic acid. In this group of rats the incidence of liver injury rose from an irregular occurrence, as it was in the rats on the original diet (with casein 18% and butter instead of lard), to a regular complication. Necrosis with or without cirrhosis and cirrhosis without necrosis were observed in rats that died between the 100th and 150th experimental days or were killed on the 150th day.

Addition of from 10 to 20 mg of choline daily reduced the incidence and the severity of the liver injury but not to a great extent. On the other hand, addition of from 10 to 50 mg of cystine daily proved to be the most potent factor in the accentuation of cirrhosis of the liver.

In this connection it should be noted that necrosis of the liver following high doses of cystine has been observed in the past.[5] In short term toxicological experiments even cirrhotic changes have been observed recently in rats which received excessive amounts of cystine (5 to 10% of the diet).[6]

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1 György, P., and Goldblatt, H., *J. Exp. Med.*, 1939, **70**, 185.

2 Rich, A. R., and Hamilton, J. D., *Bull. Johns Hopkins Hosp.*, 1940, **66**, 185.

3 Spellberg, M. A., and Keeton, R. W., *Am. J. Med. Sc.*, 1940, **200**, 688.

4 *Cf.* Best, C. H., and Ridout, J. H., in Luck, J. M., Ed., *Annual Review of Biochemistry*, Stanford University, 1939, **8**, 349.

5 Curtis, A. C., and Newburgh, L. H., *Arch. Int. Med.*, 1927, **39**, 828; Sullivan, M. X., Hess, W. C., and Sebrell, W. H., *Pub. Health Rep.*, 1932, **47**, 75; Lillie, R. D., *Pub. Health Rep.*, 1932, **47**, 83.

6 Earle, D. P., and Victor, J., *J. Exp. Med.*, 1941, **73**, 161.

Daily addition of from 10 to 20 mg of choline or of 1 g of yeast or, better, of both, neutralized more or less completely the effect of cystine on the liver. The greater benefit achieved by choline in the presence of cystine over that seen in rats fed choline without additional cystine may be explained by the conception that choline acts through the intermediary of cystine.

The pathogenesis of dietary liver injury (necrosis, cirrhosis) presented here is closely connected with the lipotropic effect[4] of casein and with fat infiltration of the liver which has been considered a prerequisite of cirrhosis.[7]

The results presented here are based on experiments made on a total of 264 rats. The work is being continued.

12040

Effect of Parenteral Administration of Vitamin B_1 and Vitamin B_6 on a Coccidium Infection.*

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In a paper now in press[1] it has been shown that the addition of both vitamins B_1 and B_6 to a special ration generally brought about a very striking reduction in the number of oöcysts produced in rats infected with standardized doses of the coccidium *Eimeria nieschulzi*. Since vitamin B_1 supplement alone only somewhat reduced the number of oöcysts eliminated and vitamin B_6 supplement increased the counts, the striking reduction brought about by combination of the two was entirely unexpected. In order to determine whether the synergistic action of the vitamins was dependent upon their admission to the site of the infection through the intestinal route, a series of tests has been carried out in which the two vitamins were administered intraperitoneally in normal saline solution instead of being mixed with the ration. Otherwise the procedure and technics were in general the same as previously described.[1]

The animals in lots 1 and 2 averaged about 70 g when started on the rations; those in lot 2, 120 g; lot 3, 90 g. The test and the reference

[7] Connor, C. L., *Am. J. Path.*, 1938, **14**, 347; *J. A. M. A.*, 1939, **112**, 387; Chaikoff, I. L., and Connor, C. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 638.

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[1] Becker, E. R., and Dilworth, R. I., *J. Inf. Dis.*, 1941, in press.