to the duration of treatment. In no instance was there a significant increase or decrease in the mean red blood cell diameters.

Stasney and Higgins reported only the largest diameters and mean corpuscular volume of the red blood cells in the newborn rats whose mothers received normal human and hog gastric juice but did not report the mean diameters. These "largest" diameters were measured from photographs of smears. The cells were selected at random and the largest diameter of the cell was measured, *i. e.,* if the cell was oval, the long axis was used rather than the short. This method appears to us to be too arbitrary to be used in determining the effect of anti-pernicious anemia material.

Conclusions. The administration of normal human gastric juice to pregnant rats by intraperitoneal injections of concentrated juice and by stomach tube of unconcentrated juice was without effect on the mean red blood cell diameters of the newborn rats.

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Cultivation of Relapsing Fever Spirochetes in Embryonic Chick.

S. W. BOHLS, J. V. IRONS AND THELMA DE SHAZO.
(Introduced by C. W. Duval.)
From the Division of Laboratories, Texas State Health Department.

Since its use for the cultivation of a spirillum of fowls by Levaditi[1] and the fowl-pox virus by Woodruff and Goodpasture,[2] the fertile hen egg has been employed in the cultivation of many viruses. This medium was employed by Cox[3] for the cultivation of Rickettsiae, and has also been utilized in the study of bacteria[4] and protozoa.[5] Morrow, Syverton and their collaborators[6] employed the developing hen egg in the study of *Leptospira icterohemorrhagiae,* but Bessemans and De Meirsman[7] failed with *Treponema pallida.* Employing

1 Levaditi, C., *Ann. Inst. Past.*, 1906, **20**, 924.

2 Woodruff, A. M., and Goodpasture, E. W., *Am. J. Path.*, 1931, **7**, 209.

3 Cox, H. R., *Pub. Health Rep.*, 1938, **53**, 2241.

4 Goodpasture, E. W., and Anderson, K., *Am. J. Path.*, 1937, **13**, 149.

5 Longley, B. J., Clausen, N. M., and Tatum, A. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 365.

6 Morrow, G., Syverton, J. T., Stiles, W. W., and Berry, G. P., *Science,* 1938, **88**, 384.

7 Bessemans, A., and De Meirsman, E., *C. R. Soc. biol.*, 1938, **127**, 847.

murine-passaged strains, both Oag[8] and Chabaud[9] were successful with *Borrelia duttoni*.

Owing to the difficulty of cultivating spirochetes of relapsing fever in laboratory media, we have attempted to grow these organisms in the developing hen egg. In this communication we are reporting successful cultivation of Texas relapsing fever spirochetes in this medium with blood from each of four patients. Specimens were received in the routine mail and after Giemsa preparations of the clots were examined, each specimen was divided in two portions. The first portions of the 4 inocula were introduced intraperitoneally in rats without delay, while an additional day of delay was incurred by sterility testing of the second portions prior to the inoculation of fertile eggs. Meanwhile, previously incubated white leghorn eggs were candled and scrubbed clean with warm soap and water.

For the first inoculum 10-day-old eggs were selected and the chorio-allantoic membranes were exposed by means of a modified dental drill, for the window method of study.[2] On each of 8 chorio-allantoic membranes was dropped 0.2 cc of the specimen and the membranes were observed through the window daily for possible changes or lesions. Although chorio-allantoic lesions were not observed, beginning with the third day after inoculation, an egg was sacrificed daily for the removal of portions of chorio-allantoic membrane, yolk, amniotic fluid and embryonic blood. Portions of these tissues and fluids were examined in appropriate darkfield or Giemsa-stained preparations for possible content of spirochetes. Spirochetes were first found in blood-tinged amniotic fluid on the fifth day after inoculation. Spirochetes were not demonstrated in non-vascular portions of the chorio-allantois, amniotic fluid devoid of blood cells, or yolk.

For each of 3 subsequent primary inoculations, 9-day-old eggs were selected and the yolk-sac method was utilized.[3] Several fertile eggs, as immediately determined by candling, were placed in wine glasses or beakers with the air-sac end upwards. After the shell had been thoroughly scrubbed with alcohol a hole was tapped with the end of a heavy pointed probe and 0.2 cc of material was injected at an approximate depth of 2 cm, after which the hole was sealed with paraffin. Beginning with the third day and daily thereafter, the chorio-allantois of an egg was exposed for the collection of amniotic fluid and blood. By the 3rd to 7th day spirochetes had appeared in bloody amniotic fluid with each of the 3 inocula. In no instance were spirochetes found in any preparation which was devoid of blood. In

[8] Oag, R. K., *J. Path. and Bact.*, 1939, **49**, 339.

[9] Chabaud, A., *Bull. Soc. Path. Exot.*, 1939, **32**, 483.

many instances the spirochetes were found in enormous numbers, often occurring as matted masses. In some preparations they were nearly as numerous as blood cells, attesting to the suitability of the fertile egg as a culture medium.

Two strains are being passaged weekly in 8-10-day-old eggs. Apparently the passages may be continued indefinitely. Five-day-old eggs have been less satisfactory than 8-10-day-old eggs. Twelve-day-old eggs also seem to be satisfactory. Since most of these eggs have been sacrificed by the sixteenth day of development, the ultimate effect of infection on embryonic life has not been determined, but spirochetes have not been found in the blood of a few embryos which survived until the hatching stage. Rats developed typical infections as a result of inoculation from the original specimens, but tests for possible alterations in virulence as a result of egg passage have not been done. Cultivation in the embryonic blood of chicks may be useful as an aid in the diagnosis of relapsing fever or for the maintenance or study of the spirochetes.

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Is Chromodacryorrhea a Diapedesis of the Red Corpuscles?

SHIRO TASHIRO, ELIZABETH BADGER AND WALDO YOUNKER.

From the Department of Biochemistry, College of Medicine, The University of Cincinnati, Cincinnati.

When dacryorrhetin, a substance prepared from muscle, is injected into rats, the animals shed bloody tears. This peculiar phenomenon of weeping bloody tears by rats was designated as chromodacryorrhea.[1, 2] These bloody tears come from Harder's glands without apparent injuries to the blood vessels.[3] These facts together with the amazing speed with which this phenomenon occurs and the fact that other cholinergic substances produce it raises an interesting question, namely: Is chromodacryorrhea a diapedesis of blood?

According to Freud,[4] who discovered a similar phenomenon with

[1] Tashiro, Shiro, and Stix, Helen, *Biol. Bull.*, 1935, **64**, 327.

[2] Tashiro, Shiro, *Proc. Am. Soc. Biochemists*, 1937, **8**, xcviii; Kongressbericht. II les XVI Internat. Physiologenkongress 1938, 46.

[3] Stix, Helen, unpublished data.

[4] Freud, J., *Acta Brevia Neerl.*, 1933, **3**, 159.