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[3] Stix, Helen, unpublished data.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.

acetylcholine, and pilocarpine, the tears are tinged red with *blood* and he concludes that "substances with parasympathetic action cause a diapedesis of blood into the conjunctiva in rats". For this conclusion, as Selye[5] first pointed out, Freud does not present any evidence. In justice to Freud, however, we may state that ordinarily, for the bloody fluid coming out of the mammalian tissue, no one is expected to offer scientific proof for blood. In fact none of those who had seen our demonstration of the bloody tear flow in rats following dacryorrhetin injection, or the actual color photographs on the kodachrome film, even raised the question.

Nevertheless, we thought it would be advisable not to take anything for granted, and to see first if bloody tears contain blood; and if so, to see whether there is a transudation of erythrocytes through unruptured blood vessels.

Experimental. Collection of tears. Chromodacryorrhea is produced by intraperitoneal injections of dacryorrhetin. In later experiments, acetylcholine and carbamylcholine are also used. For microscopic examinations, the microscopic slides are directly applied over the tears, and for other experiments, the tears are pipetted off into physiological salt solutions. In either event, only the red tears alone were used for examination.

Routine blood tests. When examined under a low power microscope, one finds a large number of spherical bodies, tinged red quite similar to the red cells. The number of these bodies amounted to 500,000 per cmm in one sample from a rat, the blood of which contained 6 million erythrocytes per cmm. The saline suspension of the tears is turbid red as though the cells were not hemolysed. Such a solution shows 2 characteristic bands of α and β of oxyhemoglobin when examined casually with a hand spectroscope, and it gives a benzidine test.

In spite of the fact that the casual examinations were suggestive of the presence of red blood corpuscles in the tears, we were surprised to find that "the cells" could not be stained by ordinary blood staining methods. More careful microscopic examinations under high power revealed that these round bodies are not evenly tinged red, and the red pigment is not confined in them alone. In fact, very often the red pigment is scattered, being cramped in amorphous granular shapes outside of these spherical bodies. Besides all the bodies are not exactly the same size and here and there one finds "giant" bodies which have the appearance of hollow globules. The most outstanding difference from erythrocytes is that they lack the appearance of having

[5] Selye, Hans, *Canadian Med. Assn. J.*, 1937, **36**, 200.

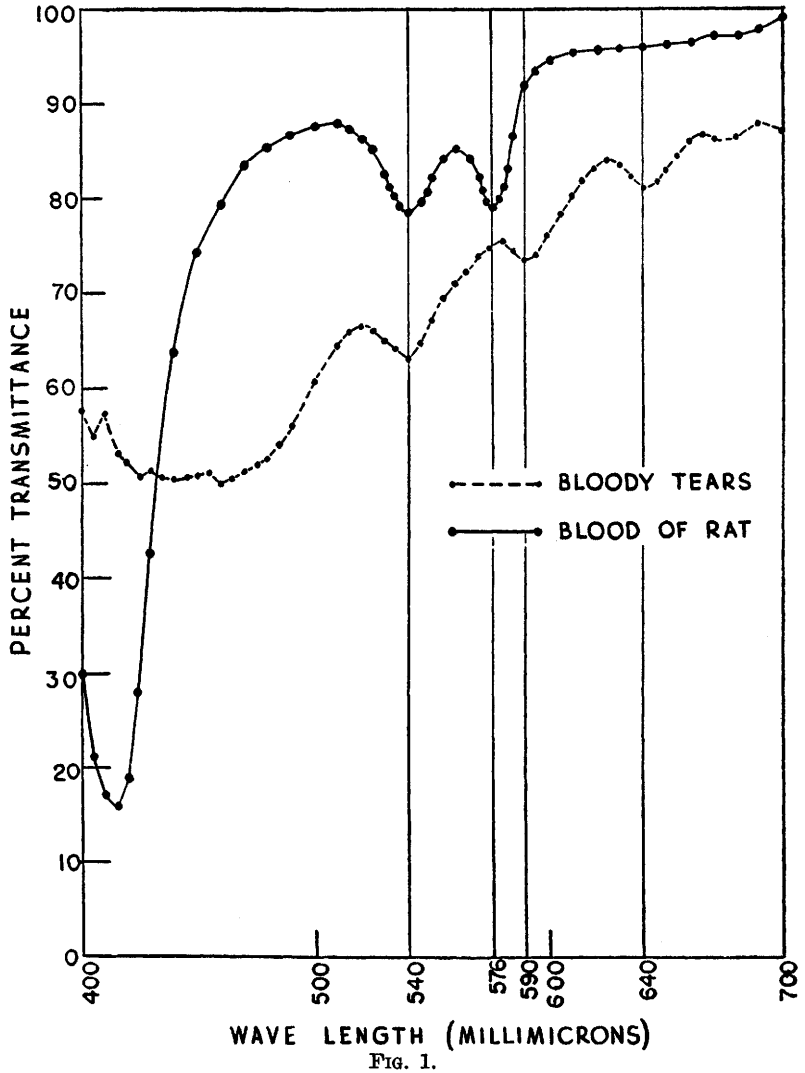

FIG. 1.

a definite cell wall. When the saline or aqueous solution (in suspension) is centrifuged, these globules are separated on the top as a colorless layer, and the red pigment is thrown down at the bottom.

The nature of the red pigment. The pigment is not very soluble in distilled water, although the solution gives a pink color. When this solution is compared with hemolysed blood with a hand spectroscope, by placing 2 spectra exactly side by side, the 2 absorbing bands are not exactly at the same position. Although the bands at β position

are identical, the α of oxyhemoglobin is slightly to the right as compared with that of the bloody tear pigment.

The spectrophotometric analysis. The further analysis of the absorption spectra of the red pigment was done with a spectrophotometer by comparing it with that of rat blood (1:500 dilution). The result is given in the figure, which shows that both have the β band at 540 millimicrons, but the tear pigment has the α band at 590, instead of the customary α band of hemoglobin at 576 millimicrons. The red pigment of the tear also has another band at 640 millimicrons, but has no band at 415 millimicrons as does the blood.

These characteristic bands can be detected with saline suspension of the tears, or supernatant fluid of the centrifuged sample, or resuspension of the pigment in water after the centrifuging.

Ultraviolet fluorescence. A further difference between the red pigment of bloody tears and hemoglobin is the fact that a solution of the red pigment of the tears gives a red fluorescence when excited by ultraviolet ray filtered through a Corex filter (90% efficiency at line 366 millimicrons).

Could chromodacryorrhetic pigment be due to the action of clear tears on hemoglobin?

We added a small amount of hemolysed blood to the clear tears of rat and examined the mixture. The results show that the absorption spectra of the mixture were identical with those of blood; and the mixture gave no ultraviolet fluorescence. Thus, the red pigment of the chromodacryorrhetic tear is not identical with the mixture of blood and clear tear, and is not a reaction product between clear tears and hemoglobin.

Conclusion. These facts definitely prove in our minds that the bloody tears contain no erythrocytes, and the red pigment in chromodacryorrhesis is not hemoglobin. The minute pink corpuscular bodies are in reality colorless fatty globules, tinged red by the pigment that surrounds them. In other words, chromodacryorrhesis is not a diapedesis of erythrocytes, and the tears do not even contain hemoglobin. It is really a most surprising fact for those who are familiar with the phenomenon of chromodacryorrhea that such obviously bloody fluid coming out of mammalian tissue does not contain any blood.

On the other hand, these findings now seem to help explain many puzzling facts. One of us (with Stix)[1] found that dacryorrhetin causes bloody tear production only in rats (with occasional exceptions with mice), milky tears in guinea pigs, rabbits and mice, and clear tears in cats and dogs. Just why rats alone should shed bloody

tears has been one of the perplexing problems. On the basis of the above findings, we can safely attribute the cause of chromodacryorrhea to a peculiarity of rats in respect to Harder's glands.

Derrien and Turchini[6] describe a substance, a porphyrine, in Harder's glands of rats and in lesser amount in those of mice. One wonders if this porphyrine is identical with the bloody pigments of the tears, or gives rise to it.

Preliminary experiments (by B) suggest that these two are probably identical as judged by ultraviolet fluorescence and the solubility characteristics of pigments as well as of their salts. Just exactly what the chemical nature of this pigment in the tears is and what the function of dacryorrhesis is, is another problem.

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Preservation of Cultures of *N. gonorrhoeae.**

LUCILE R. HAC. (Introduced by H. Close Hesseltine.)

From the Department of Obstetrics and Gynecology, The University of Chicago, and the Chicago Lying-in Hospital.

Strains of gonococci after isolation and establishment on culture media, exhibit considerable difference in viability. Ordinarily it is necessary to make transfers of recently isolated strains at least twice a week and of older strains once a week. The maintenance therefore, of even a small stock collection of *N. gonorrhoeae* strains involves a considerable amount of time, labor, and expense.

Lumière and Chevrotier,[1] Szilvasi,[2] Cohn,[3] Parish,[4] and others have reported the preservation of an occasional strain or two of gonococci for from several weeks to 10 or 12 months. In most cases the strains were reported to have been grown on ordinary or blood agar, which meant necessarily that they were hardy strains. No one seems to have made a systematic controlled study to determine whether it was possible to preserve all strains of gonococci and sensitive ones in particular.

It had been our experience that once growth had been established

6 Derrien, Eugene, and Turchini, Jean, *Comp. Rend. Biol.*, 1924, **91**, 637.

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1 Lumière, A., and Chevrotier, J., *Compt. rend. Acad. Sci. Paris*, 1914, **158**, 1820.

2 Szilvasi, J., *Dermat. Wehnschr.*, 1932, **94**, 245.

3 Cohn, A., *Z. f. Hyg. u. Infekt.*, 1928, **108**, 395.

4 Parish, H. J., *J. Path. and Bact.*, 1932, **85**, 143.