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.

11689 P

Preservation of Cultures of *N. gonorrhoeae.**

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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.

in Huntoon's[5] semi-solid stab cultures, practically all gonococci would remain viable for a month or more, but it required 3 to 15 transfers before some of the strains would grow in this medium. Repeated transfers are known to alter cultural characteristics and so a method of preservation which could be used immediately after isolation was sought.

Lumière and Chevrotier[1] reported that they had maintained gonococci viable for several months if the cultures were kept either *in vacuo* or sealed with paraffin oil or vaseline. Morton and Pulaski[6] had considerable success with mineral oil seals in the maintenance of a large collection of stock cultures of many bacterial species. They reported but one strain of *N. gonorrhoeae.* It had been grown on blood agar and was still alive at the end of 13 months. This method has the advantage that it not only preserves the cultures but keeps them readily available at all times for multiple transfer.

In order to determine the efficacy of this method of preservation, 30 strains of gonococci of varying age and sensitivity, isolated from several sources were studied over a period of more than 2 years. The age of the strains varied from 1 week to 8 years. Two showed slight growth on nutrient agar, and 17 grew fairly well on blood agar. Five of the strains were from male patients, 23 from female (18 from the cervix, 3 from the urethra, 2 from Bartholin's gland) 1 was isolated from a baby's eye and 1 from fluid aspirated from a thumb joint.

The results obtained were compared with cultures preserved in stoppered tubes and in Huntoon's stab cultures. In Huntoon's stabs the strains could be kept alive without transfer from 4 to 33 weeks.

Half the strains were lyophilized according to the method of Flosdorf and Mudd.[7] The lyophilized strains were prepared from 18 hour growths on McLeod's chocolate agar and suspended in sterile sheep serum. Only 4 of the 15 strains were alive at the end of 7 months. It is possible that better results might have been obtained by this method had the organisms been suspended in some material other than sheep serum.

The method of mineral oil seals was that described by Morton and Pulaski. Short slants of actively growing, young (usually not older than 18 hours) cultures were completely covered with sterile mineral oil. Heavy mineral oil (Parke, Davis and Co.) sterilized in a hot air oven at 150-170° for one hour was used. The tubes were

5 Torrey, J. C., and Buckell, G. T., *J. Inf. Dis.*, 1922, **31**, 125.

6 Morton, H. E., and Pulaski, E. J., *J. Bact.*, 1938, **35**, 163.

7 Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1935, **29**, 389.

stored in an incubator at 35.5° C. Several media were used; (1) McLeod's chocolate agar,[8] (2) McLeod's base with heated serum rather than whole blood, (3) Huntoon's semi-solid agar, (4) Difco's proteose peptone No. 3—hemoglobin agar and (5) Difco's dextrose starch agar. All the media gave practically the same results, except that those which remained viable longest were in Huntoon's agar. That, however, may have been because they were hardier and had been altered sufficiently before growth could be sustained in that medium. Monthly transfers were made from the original mineral oil covered slants for from 14-18 months. After 12 months all strains were transferred and then not transferred again for another 12 months. All 30 strains have survived 2 transfers made at 12-month intervals.

Better results were obtained when the tubes were stoppered with both cotton plugs and rubber stoppers than if cotton plugs alone were used.

Since this study was begun over 400 other strains have been isolated and placed under oil the second generation after isolation. Many of them have been transferred after 3- and 6-month intervals and found to be alive.

Conclusions. Strains of *N. gonorrhoeae* preserved by sterile mineral oil seals have been shown to be viable after transfer at 6 and 12 month intervals.

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Cell Opacity Method for Determination of Cell Volume on a Single Drop of Rat Blood.*

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The rat is admirably adapted to the study of certain problems in the physiology and pathology of blood. It has been used extensively in experimental nutritional anemia. For such studies it is desirable to know not only the hemoglobin content and red cell count of the blood but also the volume of the packed red cells. Sufficient blood for the first two determinations is easily obtainable by incision of the tail vein, but not for the third determination, with methods at present available. The red cell volume can be

[8] McLeod, J. W., Coates, J. C., Happold, F. C., Priestley, D. P., and Wheatley, B., *J. Path. and Bact.*, 1934, **39**, 221.

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