of protein and fat) than rats that do not receive thiamine. Unfortunately I have no direct data concerning any possible protein sparing action of thiamine in addition to that of the dextrose given. The loss of weight of both groups of animals was very similar, being rapid at first and slowing as time continued. At the time of death of the dextrose-fed rats the weight of those receiving thiamine was similar. With the further survival of the thiamine-treated rats loss of weight continued so that they weighed less than the dextrose rats at the time of death. The amount of dextrose consumed each day was similar in both groups of animals.

Summary. Young rats weighing 65 to 90 g were maintained for 14 to 21 days on a vitamin B-free diet (casein 10, sucrose 80, butter 4, salts, agar and vitamins A and D). At this time these animals survived fasting but 2 or 3 days. Substitution of a diet of dextrose, supplemented by dextrose solution by stomach tube, permitted survival of from 7 to 16 days.

Vitamin-depleted rats receiving dextrose and thiamine survived 2 to 4 times as long as those receiving only dextrose. Nicotinamide appeared to have some effect, but riboflavin, pyridoxine hydrochloride, pantothenic acid and choline had no appreciable effect in prolonging the life of the dextrose-fed rats. All of the foregoing vitamins given together had no further beneficial effect than when thiamine alone was given.

When dextrose was substituted immediately following an adequate vitamin-containing diet, rats survived 19 to 29 days. The injection of thiamine hydrochloride or the vitamin mixture did not appreciably alter the survival time of these animals.

13688 P

A Difference in Metabolic Requirements of Meningococcus and Gonococcus.*

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Gordon and McLeod[1] found that the gonococcus does not grow on a medium based on tryptic digest of casein and gave this point con-

* Aided by grants from the A. B. Kuppenheimer Fund and the U. S. Public Health Service.

1 Gordon, J., and McLeod, J. W., *J. Path. and Bact.*, 1926, **29**, 13.

siderable study. They came to the conclusion that this lack of growth is due to inhibition by large amount of amino acid, and, especially, the high tryptophane content of the digested casein. McLeod[2] and, recently, Mueller and coworkers[3] have reported finding that cystine in high concentrations produces constant, marked, inhibitory effects to growth of this Neisseria. McLeod went further to suggest that cystine *may* favor growth of certain strains of gonococcus, especially when the medium contains considerable blood. Higginbotham[4] has described the use of cystine in chocolate agar medium for growth of gonococcus in specimens shipped to the laboratory for examination. Kobayashi and Nishikawa[5] have said that the growth of gonococcus, meningococcus, streptococcus and pneumococcus on a medium containing beef extract, serum, agar, etc., is favored by 0.5% to 0.005% cystine.

We require rather large quantities of gonococcus and meningococcus cells to carry on our comparative study of the chemical nature of these bacteria. Our stock strains grow very well on a medium[6, 7] containing tryptic digest or acid hydrolysate of egg white, buffer, dextrose and agar.

In recent months, however, casein was substituted for egg white. It was found that none of the gonococcus strains available will grow on this medium although the meningococcus types I, II and atypical variations thereof grow very well. Pure casein contains much less cystine than egg white, although the methionine contents of these 2 proteins are not far different; furthermore, it has been stated[8] that cystine is destroyed when liberated from casein by tryptic digestion as it is usually carried on in the alkaline condition. Analysis by Sullivan's[9] procedure showed none of this amino acid in our product of tryptic digestion. An addition of about 0.05% cystine, however, improves the casein digest medium, with respect to gonococcus growth, to a par with the egg white digest medium. The growth of meningococcus is not improved perceptibly by this altera-

2 McLeod, J. W., *Brit. J. Exp. Path.*, 1927, **8**, 25.

3 Mueller, J. Howard, Hinton, Jace., and Miller, Pauline A., *J. Bact.*, 1942, **43**, 100.

4 Higginbotham, Margaret, personal communication to the author.

5 Kobayashi, B., and Nishikawa, E., *Japan Med. World*, 1921, **1**, 11.

6 Miller, C. Phillip, and Castles, Ruth, PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 123.

7 Boor, Alden K., and Miller, C. Phillip, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 370.

8 Jones, D. Breese, and Gersdorff, Charles E. F., *J. Biol. Chem.*, 1939, **129**, 207.

9 Sullivan, M. X., Supplement No. 78 to U. S. Public Health Service Reports, pages 1-13, 1929.

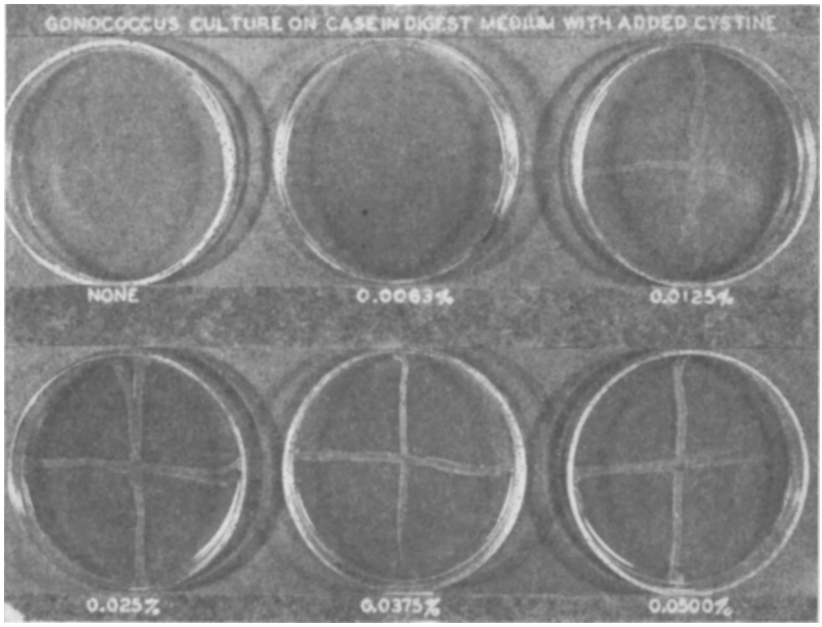

FIG. 1.
Gonococcus suspension applied with swab equally to each of the six plates. Sixteen-hour culture.

tion. The addition of cystine to the medium prepared from tryptic digest of egg white does not induce a larger yield of gonococci, nor meningococci.

A very slight growth of our stock strains of gonococcus can be detected on that medium containing as little as 0.00125% cystine, but the best growth develops with 0.0250% to 0.075% of this added amino acid. With increases in cystine above 0.10% a decrease in growth ensues, but with cystine present even to the point of saturation, some growth develops and those colonies which do multiply, do so very rapidly and after a few days are as heavy as the cultures on the media with the lesser, optimal, amounts of cystine.

These gonococcus cultures allowed to stand on the medium still contained viable organisms after 10 days, although the bacteria were not alive after 14 days. A meningococcus strain is still alive and growing after being on this semisolid medium in the incubator for more than a month.

The use of cystine by the gonococcus is evidently something more than as a "building stone" in cellular protein formation. An analysis[9] of the gonococcus cells grown on this medium showed only a trace of cystine, not more than was found in meningococcus cellular sub-

stance. It is an interesting observation in comparative cellular requirements that methionine does not replace cystine in the stimulation of gonococcus growth. This cystine requirement of the gonococcus was found to be only a type requirement; certain other substances containing sulfur, and potentially -SH formers, satisfactorily replaced this amino acid. Among these were cysteine hydrochloride, glutathione, thioglycolic acid, flowers of sulfur and barium sulfide.

Summary. A rapid and heavy growth of meningococcus developed on an agar medium containing tryptic digest of casein, dextrose and buffer at pH 7.4-7.6. However, this medium failed to support growth of the gonococcus. Addition to the medium of a small amount of cystine, however, stimulated growth of this organism. The growth of gonococcus on this casein digest medium, with cystine, was as heavy as that of meningococcus. The growth of meningococcus did not seem to be improved by this addition of cystine to the medium.

13689

Susceptibility to Convulsions in Relation to Age. II. Influence of Bile in Rats.

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In a previous communication a hypothesis was developed that the high susceptibility to convulsions of infants and young children may be due to the action of irritants entering the brain in the absence of a hypothetical hematoencephalic barrier.[1] Thus, it was observed that acid fuchsin, a water soluble neurophilic, sulfonated dye, will produce convulsions in rats of from 17 to 20 days of age and not thereafter. This phenomenon may be associated with the ability of acid fuchsin to penetrate the brain of the young animals and not of adults.

In the attempt to obtain further information concerning this peculiarity of the young brain, the influence of bile was studied. It has been known for some time that the brains of young children dying with jaundice may be icteric, while those of severely jaundiced adults are not.[2] Also, it has been observed that jaundiced infants

[1] Frohlich, A., and Mirsky, I. A., *Arch. Neur. and Psych.*, 1942, **47**, 30.

[2] Orth, J., *Virch. Arch. f. Pathol. Anatomie*, 1875, **63**, 447.