5318

Hydrolysate of Proteins as the Basis for a Bacteriological Culture Medium.*

ALDEN K. BOOR AND C. PHILLIP MILLER, JR.

From the Department of Medicine, University of Chicago.

The advantage of a medium, free from antigenic proteins and suitable for the growth of organisms, particularly pathogenic bacteria, is obvious.

Long and Seibert[1] introduced a synthetic, non-protein medium suitable for growth of tubercle bacilli. ZoBell and Meyer[2] have described the adaptation of the Brucella group to a protein or peptone-free environment. Miller and Castles[3] have found that a tryptic digest of egg white, from which the coagulable proteins have been removed, is an excellent basis for a medium for gonococcus.

Twenty grams of commercial, dried, powdered egg white were subjected to the action of 50 cc. of boiling, 20% hydrochloric acid for 10 hours, using a reflux condenser for the purpose. It was found that other proteins, *e. g.*, casein and gelatin, could be substituted for the egg white. Excess hydrochloric acid was removed from the hydrolysate by evaporation to a thick paste and the product diluted with water to 500 cc. This solution was treated with 63 cc. of a solution containing 10.35% sodium hydroxide, 0.67% potassium hydroxide; and 0.03% calcium hydroxide. This composition was chosen to insure the presence of the salts of these metals in a quantity to best meet bacterial requirements. The solution was filtered. About one-fifth of its volume of a carefully prepared aluminum hydroxide cream† was added to precipitate some colloidal matter, and the solution again filtered. The volume of this clear, transparent filtrate was then brought to 1,750 cc. with distilled water and 0.10 gm. Na_2HPO_4 and 0.02 gm. NaH_2PO_4 added for buffer effect. One percent dextrose was added and in the case of the solid medium, 2% agar. The final adjustment of the reaction to pH 7.4 was made by addition of the hydroxide mixture.

* This study was made possible by a research grant from the Public Health Institute of Chicago.

1 Long and Seibert, *Am. Rev. Tuber.*, 1926, **13**, 393.

2 ZoBell and Meyer, *Science*, 1930, **72**, 176.

3 Miller and Castles, PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 123.

† The aluminum hydroxide cream was prepared by adding slowly and with constant stirring a 1% solution of ammonium hydroxide to a 1% solution of ammonium alum and repeatedly washing the precipitate by decantation until the supernatant liquid no longer gave a reaction with Nessler Reagent.

That the medium was free from those proteins generally agreed to be antigenic is indicated by the following negative tests: biuret reaction (after removal of ammonium salts), sulfosalicylic acid, picric acid and trichloracetic acid. No precipitate appeared when the solution was half saturated or completely saturated with ammonium sulfate.

The following organisms have multiplied on the medium herein described; gonococcus, *Staphylococcus aureus, B. coli, B. paratyphosus A, B. paratyphosus B, B. fecalis alkaligines,* pneumococcus, *B. typhosus, B. abortus, B. melitensis, B. dysenteriae, B. anthracis, B. diphtheria,* meningococcus, and others. Some of these organisms have exhibited a luxuriant growth. Others have multiplied to a lesser extent.

5319

Effect of Various Stomach Preparations in Pernicious Anemia.

J. P. BURGESS AND J. E. MORGAN. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

This work was started in April, 1929, under the direction of Dr. Ivy on the principle that the active constituent of liver effective in pernicious anemia might be produced by the gastric mucosa and stored in the liver. Very soon after starting our work articles appeared showing that desiccated whole stomach is effective,[1, 2] that gastric mucosa is slightly effective, whereas gastric muscle is ineffective,[1] that a normal *in vivo* digest of meat is effective,[3] that pepsin is ineffective,[3] and that gastric juice is ineffective.[4, 5] Our report is simply to record a confirmation of some of these findings.

We have fed to pernicious anemia patients fresh hog's gastric mucosa (300 gm. daily) brought to a boil within 20 minutes, pepsin (75 gm. of scale pepsin daily), desiccated mucosa (prepared by the method of Sturgis, Isaacs and Sharp, 75 gm. daily or the equivalent of 450 gm. of fresh mucosa), desiccated gastric muscle (120 gm. daily or the equivalent of 450 gm.) and desiccated whole stom-

[1] Sturgis and Isaacs, *J. Am. Med. Assn.*, 1929, **93**, 747.

[2] Sharp, *J. Am. Med. Assn.*, 1929, **93**, 10.

[3] Castle, *Brit. Med. J.*, 1929, **1**, 1120.

[4] Castle and Townsend, *Am. J. Med. Sci.*, 1929, **178**, 748.

[5] Coggeshall, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1044.