



FIG. 2.

Cat in which 4 ganglia, the last 2 lumbar and the first 2 sacral, were removed on the left side (lower tracing) 20 days before the experiment. A, B and C correspond to the injection of 0.3, 0.8 and 2.0 γ acetylcholine, respectively. The chronically denervated side shows a considerably larger response to acetylcholine than does the normal side.

taken to remove completely the sympathetic supply to the limb in question in order to observe sensitization.

13311

A Protein-Free Medium for Primary Isolation of the Gonococcus and Meningococcus.

J. HOWARD MUELLER AND JANE HINTON.

From the Department of Bacteriology and Immunology, Harvard Medical School, and School of Public Health, and the Boston Dispensary, Boston, Mass.*

It is desired to report the development of a transparent, reproducible medium, containing no heat-labile material such as serum, which is sufficiently stable to withstand autoclave sterilization and storage, and which will support satisfactory primary growth of either meningococci or gonococci. The latter were used as the test

* Aided by a grant from the Commonwealth Fund.

organisms during the experimental work, since a constant source of primary cultures of the meningococcus is ordinarily not available. The similarity between the two organisms, and the reputedly greater difficulty expected in the cultivation of the gonococcus, indicated that a medium suitable for this organism should prove equally satisfactory for the meningococcus.

As a basis for comparison, the Difco "Proteose No. 3 Chocolate Agar" has been used. This medium has been adopted as a standard in certain laboratories for the diagnosis of gonorrhoea¹ and one of us (J.H.) was thoroughly familiar with its use.

The plan as formulated in beginning the experiments was essentially that followed with considerable success with other organisms, namely, to select the most suitable empirical medium available and attempt to break it down to its essential component parts. The experience of the senior author in 1918 with the Gordon and Hine² pea meal extract agar for meningococcus carrier detection had shown that this medium, when carefully prepared, was eminently satisfactory. It proved, upon trial, to be equally so for primary cultivation of the gonococcus, and it was therefore chosen as the starting point in the investigation. It consists essentially of a tryptic digest of meat, agar, and a 5% NaCl extract of pea flour, the latter presumably supplying some essential protein material.

The fractionation of the pea extract proved surprisingly simple, since it could be accomplished by mechanical means. The active portion proved to be, not protein, but starch. This might perhaps have been anticipated on the basis of Vedder's early work.³ Substitution of ordinary laundry starch completely replaced the growth promoting effect of the pea extract. The effect is evidently due to the starch itself, rather than to some adherent impurity of small molecular size. This has been shown in two ways. First, it was found that a variety of starches,—corn, potato, wheat, rice, arrowroot, etc. were weight for weight identical in their effect. Second, relatively brief hydrolysis of the starch with ptyalin completely destroys the growth-promoting effect. This occurs even before the disappearance of the blue starch-iodine test, and indicates that so-called "soluble starches" are likely to prove unsatisfactory or irregular in their action. The explanation for the effect of the starch is

¹ Cox, D. F., McDermott, M., and Hinton, W. A., *The Gonococcus and Gonococcal Infection*, Science Press, 1939, p. 79-82.

² Gordon, M. H., and Hine, T. G. M., *Brit. Med. J.*, 1916, **2**, 678.

³ Vedder, E. B., *J. Infect. Dis.*, 1915, **16**, 385.

not yet clear. Possibly, as McLeod⁴ has suggested in connection with the action of ascitic fluid or serum, it acts as a "protective colloid" against the inhibitory effects of amino acids.

The tryptic digest of meat was next examined. This can be replaced by a rapidly prepared meat infusion together with a complete acid hydrolysate of casein prepared as described elsewhere by Mueller and Johnson⁵ and Mueller and Miller.⁶ This hydrolysate is now obtainable from the Difco Laboratories, Inc., Detroit, Michigan, under their trade name of "Casamino Acids, Technical." Further examination of the essential components of the meat infusion is now under way. Since it is quite possible that this will prove to be a laborious matter, this report is being made with the hope that the medium in its present form may prove useful to others.

It has been tested in parallel with the Difco chocolate agar in a considerable series of primary cultivations of the gonococcus at the Boston Dispensary. The results have been highly satisfactory. Colonies are frequently larger on the starch medium than on the chocolate. They are easily recognizable, particularly with the aid of the oxidase reagent. There is no appreciably greater tendency for overgrowth of the plates by other organisms, and no significant discrepancies between positives and negatives have been noted. We have recently been fortunate in being able to test the medium on a series of primary isolations of the meningococcus from both spinal fluid and naso-pharyngeal swabs of cases and carriers in Halifax, Nova Scotia. This was made possible through the courtesies of Dr. A. R. Morton, City Health Commissioner and Dr. D. J. MacKenzie, Director of the Provincial Public Health Laboratory. Consistently good results were obtained in connection with this material, and a considerable number of cultures of a Type I meningococcus were readily secured. A correct appraisal of the value of this medium can result only from a more rigorous series of tests in the hands of other workers as well as our own. The details of the preparation follow.

For 1,000 cc Starch Agar. Add 17 g dry shredded agar to 500 cc tap water in a 2 liter flask. Autoclave 15 minutes at 15 pounds to dissolve. While still hot, add the following solution which may be prepared while the agar is being autoclaved:

⁴ McLeod, J. W., Wheatley, M. B., and Phelon, H. V., *Brit. J. Exp. Path.*, 1927, **8**, 25.

⁵ Mueller, J. H., and Johnson, E. R., *J. Immunol.*, 1941, **40**, 33.

⁶ Mueller, J. H., and Miller, P. A., *J. Immunol.*, 1941, **40**, 21.

Meat infusion*	300 cc
Casein hydrolysate†	17.5 g
Starch paste‡	100 cc
Water	100 "
Adjust pH to 7.4-7.6.	

Mix and distribute at once either into test tubes (about 20 cc each for pours, 5 cc for slants) or flasks of 120-200 cc. Autoclave *not more than 10 minutes at 10 pounds*. Over-autoclaving spoils the medium. The flasks can be used to pour plates at once. The tubes may be melted in boiling water and used as needed. The medium seems to give best results when not too moist, either with gonococcus or meningococcus. For either organism a candle jar must be used, and the cultures incubated at about 36° for 24 to 48 hours in the case of gonococcus, or 16 to 24 hours for meningococcus.

13312

Mechanism of Action of Estrogens on Insulin Content of the Rat's Pancreas.*

HEINZ L. FRAENKEL-CONRAT, VIRGIL V. HERRING,
MIRIAM E. SIMPSON AND HERBERT M. EVANS.

From the Institute of Experimental Biology, University of California, Berkeley, Calif.

The action of estrogens on the pancreas of rats has been demonstrated in a twofold manner. Griffiths, Marks and Young^{1, 2} have shown the insulin content of rat pancreas to be increased after implantation of tablets of various estrogens for periods of 2-4 weeks;

* Meat Infusion—1 pound of meat (chopped lean beef or beef heart), 500 cc water. Suspend meat in water, bring to active boiling, strain through cheese-cloth and filter through paper. For routine use, this infusion may be preserved in the cold room in stoppered bottles containing a few cc of chloroform.

† Casein Hydrolysate—Quantity specified for Difco preparation, Lot S-64123 of "Casamino Acids, Technical."

‡ Starch Paste—Suspend 1.5 g ordinary starch (corn starch or laundry starch, not "soluble starch") in 10 cc cold water. Pour slowly into 90 cc boiling water, while stirring.

* Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation, New York City, and Parke, Davis and Company, Detroit, Michigan. We wish to acknowledge assistance from the Works Projects Administration, Project No. OP-65-1-08, Unit A-5.

¹ Griffiths, M., and Young, F. G., *Nature*, 1940, **146**, 266.

² Marks, H. P., and Young, F. G., *Lancet*, December 7, 1940.