jection, the female region was yet more distinctly visible as a salmon bar in the black, male feather.

Permanent preparations may be obtained by mounting the feathers in euparal after drying.

Apart from the interest attached to the determination of any new indicator for the female hormone, the method described seems to offer special advantages since it requires the minimum of equipment and errors in reading can scarcely occur.

It is to be hoped as well that the individual variation of the test animals may prove less than in the rat.

Further work is being done on the same line of investigation.

4949

## A Synthetic Substitute for Ascitic Fluid in a Medium for Cultivation of Gonococcus.*

C. PHILLIP MILLER, JR., AND A. BAIRD HASTINGS.

*From the Department of Medicine of the University of Chicago.*

Ever since its first cultivation by Bumm in 1885, the gonococcus has maintained a reputation for fastidiousness as regards its growth requirements. In consequence, a great number of media have been devised for it, the most successful and reliable of which are those containing human ascitic or hydrocele fluid. It is generally believed that the efficacy of these transudates depends, in large part at least, upon constituents other than their inorganic salts. To determine whether the rôle played by transudates in supporting the growth of gonococci may be attributed to substances other than the biologically important inorganic ions, a study of the function of the individual ions has been undertaken. Although this study is still in progress and will be published in detail later, we wish now to report that it is possible to cultivate the gonococcus on an agar medium containing only beef infusion and dextrose, providing it is made up in an aqueous solution of inorganic salts in kind and concentrations corresponding to those found in an ultrafiltrate of mammalian blood plasma. The solution, which for the sake of brevity we have called van Dyke-Hastings solution, is similar to that used

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

by van Dyke and Hastings[1] in their experiments on the response of guinea pig uterus to pituitary extract and is approximately isohydric, isosmotic, isionic with protein-free transudates. Its composition is given in Table I:

TABLE I.
*Final Concentration of Inorganic Cations and Anions.*

| Cation | mM / Liter | Anion | mM / Liter |
|---|---|---|---|
| $Na^+$ | 145.4 | $Cl^-$ | 123.6 |
| $K^+$ | 6.0 | $HCO_3^-$ | 30.0 |
| $Ca^{++}$ | 1.5 | $HPO_4^=$ | 0.8 |
| $Mg^{++}$ | 0.5 | $H_2PO_4^-$ | 0.2 |

pH = 7.4 $CO_2$ tension = 46 mm. Hg.

Such a solution is simply prepared by mixing appropriate quantities of isotonic solutions of the individual salts. The composition of the stock solutions to be mixed is given in the second column of figures and the amounts used in preparing the van Dyke-Hastings solution are given in the last column of Table II.

TABLE II.

| Salt | Composition of Stock Solutions | | Amount of Each Stock Solution Required per Liter Final Solution |
|---|---|---|---|
| | mM / Liter | Grams / Liter | |
| NaCl anhydrous | 154 | 9.0 | 738.0 |
| KCl " | 154 | 11.47 | 39.4 |
| $NaHCO_3$ " | 154 | 12.93 | 195.0 |
| $Na_2HPO_4$ " | 107 | 15.19 | 7.6 |
| $NaH_2PO_4$ " | 154 | 18.48 | 1.3 |
| $CaCl_2 \cdot 6H_2O$ | 107 | 23.45 | 14.0 |
| $MgCl_2 \cdot 6H_2O$ | 107 | 21.77 | 4.7 |

It is essential that the calcium and magnesium chlorides be added after the solution containing bicarbonate has been acidified below pH 7.4 with $CO_2$; otherwise these salts will be precipitated. Ideally the medium should contain enough $CO_2$ to be in equilibrium with a tension of 46 mm. (*i. e.*, about 6%) of the gas at 37°C. But as the procedure described below was found to yield a satisfactory medium, it proved to be unnecessary to work at this exact tension throughout its preparation.

The medium is made as follows: Fresh beef heart, freed from fat and fibrous tissue, is ground in a meat grinder and added to twice its weight of van Dyke-Hastings solution (*without* Ca and Mg).

[1] van Dyke, H. B., and Hastings, A. Baird, *Am. J. Physiol.*, 1928, lxxxiii, 563.

The mixture is cooked at 70°C. for 3 hours and then boiled for 15 minutes. It is strained hot through cotton and made up to its original volume with distilled water. One and one-half per cent of powdered agar and 1% of dextrose are added. After they have dissolved, 15 cc. of a 0.02% aqueous solution of phenol red per liter are added to facilitate pH control in the subsequent operations.

The solution at this point is usually alkaline, about pH 7.6. If, as occasionally happens, it is much less alkaline, it can be adjusted to about that reaction with NaOH. It is then put into an Ehrlenmeyer flask fitted with a two-hole rubber stopper through which pass an aeration tube and an outlet tube, each plugged with cotton and the neck wrapped with paper to protect it against subsequent contamination. It is then autoclaved at 15 pounds for 15 minutes. After cooling to about 70°C. $CO_2$ is bubbled through the medium by means of the aeration tube until the color of the phenol red indicates a reaction of about pH 7.0. At first it may be necessary to withdraw samples of the medium in order to determine its reaction in a comparator block but with a very little experience one is able to estimate from the color of the large bulk of fluid its pH accurately enough for the purpose at hand. At this point the calcium and magnesium solutions, previously sterilized by autoclaving, are added in appropriate amounts. The medium is then tubed as quickly as possible and slanted. After cooling the reaction is usually pH 7.2-7.4, which is quite satisfactory for the growth of the gonococcus. The medium will become alkaline on standing unless kept in an atmosphere of 6% $CO_2$. During cultivation this is prevented by the fermentation of the dextrose by the growing organisms.

This medium has been found to support the growth of gonococci, both in primary and subculture, quite as luxuriantly as any of the more complex media, or as any of those containing human ascitic fluid.