male) over a period of time 6 to 17 weeks in duration. The daily doses varied from 25 to 800 rat units. At the end of 4 months histological examination of the dog receiving the largest dosage revealed essentially no pathology in the heart, arteries, liver, spleen, adrenals and thymus. The tubules of the kidneys contained some albumin; the glomeruli were normal. Hyperplasia of the thyroid was marked. Maxillary prognathism in these dogs was demonstrable. The anterior lobe of the hypophysis was much smaller than in the normal dog. The cytoplasm of the chromophobes of this lobe was diminished in amount. The posterior lobe seemed relatively much larger than in the normal dog. The nipples of all dogs enlarged. The external genitalia and uterus of the female dogs *increased* in size. Hyperplasia of the glands of the uterine endometrium was pronounced but no hemorrhagic discharge was observed. The ovaries of the experimental dogs were less than half the size of the control dogs, containing relatively more stroma and no mature follicles.

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## An Egg White Digest Medium for the Gonococcus.*

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The culture medium described below has been found to yield a more plentiful growth of gonococcus than any we have employed. Additional advantages are transparency, low content of protein, and ease of preparation.

Dissolve 40 gm. of powdered egg white† in a liter of 0.45% NaCl solution and adjust the reaction to pH = 7.0-7.4. Rub into a paste and add to the solution 4 gm. of a special high-test trypsin;‡ plug the mouth of the flask with cotton and incubate on a water bath at 48° C.—with occasional agitation—for 10-12 hours. This temperature prevents spoilage by inhibiting the growth of most micro-

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

† We have used both Merck's and Mallinckrodt's preparations of egg white and found them equally satisfactory. They are purchased under the label: "Albumin egg, impalpable, powdered."

‡ This trypsin, which we found superior to the others we have tried, was kindly furnished us by Dr. David Klein of the Wilson Laboratories, Chicago.

organisms. By the end of 10-12 hours, a sample of the digest should give an amino acid titration of 130-180 (*i. e.*, cc. of N/10 NaOH per 100 cc. of digest) by Sörensen's method. This determination is not essential, except as proof that digestion has proceeded satisfactorily, for we have found that a digest with a Sörensen titer of 80 supported growth as effectively as one with a titer of 180.

Now add sufficient HCl to bring the reaction down to pH = 5.0, the isoelectric point of most of the proteins, and autoclave for 15 minutes. Add sufficient distilled water to bring the volume up to its original quantity. Then separate the precipitated proteins by filtration through paper or by centrifugation, and then by filtration through a Berkefeld candle. This sterile filtrate can be stored in the ice-box until needed.

To this clear, straw-colored filtrate add 6 cc. of 0.04% phenol red solution per 100 cc. and readjust the reaction to pH = 7.0-7.2 with NaOH. The indicator in this concentration exerts no bacteriostatic action on the gonococcus, and its addition directly to the digest simplifies the adjustment of pH. It may be necessary to withdraw samples of the fluid 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.

To make a liter of medium put together:

250.0 cc. digest
750.0 " distilled water
0.2 gm. KCl
3.0 " NaCl
1.25 " $NaHCO_3$
10. " dextrose
15-20. " agar depending on the consistency desired.

Heat and stir the mixture until all of the ingredients are dissolved. If necessary, adjust the reaction to about pH = 7.2-7.4. A simple and sufficiently accurate method of making readings is to drop about a cubic centimeter of the hot solution on to a piece of clean white paper and note the color of the cooled agar. Then add: 8 cc. of a M/15 solution of $Na_2HPO_4$ and 2 cc. of a M/15 solution of $NaH_2PO_4$.

The medium is now ready to be tubed, autoclaved, and slanted. Freshly slanted medium yields a better growth than that which has dried out even a little. The final reaction should be pH = 7.0-7.4.

The digest prepared according to the directions given contains no

heat-coagulable proteins and gives a negative phosphotungstic acid test. Half saturation with ammonium sulphate yields a precipitate which gives a positive biuret test. On full saturation of the filtrate a precipitate is obtained which also gives a biuret test. It therefore contains both proteoses and peptones.

If the presence of undigested proteins in the medium is a matter of indifference to any one using it—that is, if their possible adherence to organisms removed from the culture does not interfere with the use to which the organisms are put—the acidification and autoclaving of the digest can be omitted. The medium in that case is rendered cloudy on sterilization by the precipitation of the heat-coagulable proteins.

Another step which can be omitted is the filtration of the digest through a Berkefeld candle. Its purpose is to render the digest sterile—in order that it may be stored with safety—and perfectly clear, for occasionally a very fine precipitate forms on autoclaving which passes a paper filter and makes the medium cloudy.

The only fault we have found with this medium is that cultures of gonococcus do not remain viable longer than about 12 hours. They develop very rapidly within that time but die shortly afterward. This condition obtains for both freshly isolated and stock strains.

## 5190

### Transplantation of Ureteral Segments to the Abdominal Wall.

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In previous communications[1, 2] an osteogenic effect of the epithelium of the urinary bladder, ureter and renal pelvis was demonstrated when the epithelium was brought into association with certain connective tissues such as the muscles and fasciae of the abdominal wall, extremities, etc., in the dog.

These observations on the ureter have been repeated and extended. In 4 dogs a 3 cm. segment of the ureter was excised and split along its long axis. This rectangular piece was then sutured on the right internal oblique muscle with silk and the wound closed. The experiments were terminated at 35, 42, 62, and 79 days and in all cases an epithelial lined cyst was found partly surrounded by bone.

[1] Huggins, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 349.

[2] Huggins, *Arch. of Surg.*, in press.