rapid, as one would expect from the favoring local conditions. Whether such conditions will cause the virus papillomas of cottontails to become cancerous remains to be seen. Frequently the alteration of a virus papilloma to a squamous cell cancer halts for a time at one stage or another, and in consequence cystic tumors or malignant papillomas develop, growths unusual after tarring.

In one cottontail small virus papillomas grew for a while, at 2 of 3 inoculation sites on the trunk. Fourteen weeks after their disappearance tarring of the ears was begun. One growth reappeared during the 5½ months of tarring, and a new papilloma developed at the site that had previously been negative. The new tumor progressed after tarring was stopped, but the growth which had recurred disappeared once more. In untarred rabbits a reappearance of retrogressed papillomas due to virus has never been observed. The fact is attested, however, that intercurrent stimulation may influence decisively the course of established growths.[7]

To summarize, the results of the comparison accord with the conception that the tar tumors may be due to a virus or viruses ensconsed in the epidermis and dependent for pathogenic activity upon tissue derangements such as are produced by tar.

8371 C

A Convenient Method for the Preparation of Concentrates of Follicle Stimulating Hormone from Urine.*

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In the course of investigations on the excretion of gonad stimulating hormones in the urine of mental patients[1, 2] the following method was developed for the preparation of concentrates of the follicle stimulating hormone (F.S.H.).

[7] Kidd, John G., Rous, Peyton, and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 193.

* This investigation was aided in part by a grant from the Board of Directors of the Neuro-Psychiatric Institute of the Hartford Retreat, Hartford, Conn. We are indebted to Dr. C. Charles Burlingame, Psychiatrist-in-Chief of the Institute, for his continued interest in this problem.

[1] Harris, M. M., Brand, E., and Hinsie, L. E., *Am. J. Psychiat.*, 1935, **91**, 1239.

[2] Harris, M. M., Brand, E., Hinsie, L. E., and Block, R. J., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1576.

The method is based on the observation that the stable aluminum hydroxide C (γ), prepared according to Willstaetter and Kraut[3] is a satisfactory absorbent for F.S.H. from urine. The effectiveness of this adsorption depends markedly on the pH, a definite maximum occurring at pH 4.5. Evans, Gustus and Simpson[4] have reported that solutions of the gonadotropic hormone from acetone-dried pregnant mare's serum are more satisfactorily adsorbed at pH 3.5 by aluminum hydroxides of Types A and B, prepared according to an earlier method of Willstaetter and Kraut,[5] while Type C was considerably less efficient.

The urine is collected in glass jars containing 5 cc. of a saturated alcoholic solution of thymol per liter capacity. Insoluble material is removed by filtration and the filtrate is adjusted to exactly pH 4.5 with 5 N HCl using bromcresol green as external indicator. A suspension containing 20 gm. per liter of aluminum hydroxide C (γ) is slowly poured into the urine until 1 gm. of aluminum hydroxide per liter of urine has been added. For 24-hour specimens of 500-1000 cc. a full 1.0 gm. of aluminum hydroxide is added; for samples of less than 500 cc. only 0.5 gm. is used. The resulting mixture is occasionally stirred by hand (mechanical stirring results in poor yields) during 20 minutes so as to maintain a homogeneous suspension; the solids are allowed to settle during 45 minutes. The supernatant fluid is removed by siphoning and centrifugation, readjusted to pH 4.5 and again treated with aluminum hydroxide by the same procedure. The combined precipitate is centrifuged at high speed in 100 or 250 cc. vessels, washed with 10 volumes of acetone, and transferred with acetone to 35 or 50 cc. centrifuge tubes in which the acetone washing is repeated until dehydration is complete.† The product is dried *in vacuo* over calcium chloride, pulverized and stored in the refrigerator.

For the elution of the F.S.H. the dry powder, weighing 3-6 gm. for an average 24-hour specimen, is suspended in 7 cc. of 0.01 N NaOH and the pH adjusted to 10-10.5 by the careful addition of

[3] Willstaetter, R., and Kraut, H., *Ber. Deut. Chem. Ges.*, 1923, **56**, 1117; 1924, **57**, 1082. For nomenclature *cf.* Kraut, H., in Oppenheimer, C., *die Fermente*, Leipzig, 1929, **3**, 480.

[4] Evans, H. M., Gustus, E. L., and Simpson, M. E., *J. Exp. Med.*, 1933, **58**, 569.

[5] Willstaetter, R., and Kraut, H., *Ber. Deut. chem. Ges.*, 1923, **56**, 150.

† The dehydration with acetone permits the storage of the activated aluminum hydroxide and also removes any theelin which might have been carried down in the precipitation. Dehydration, however, is not essential and elution of the wet precipitate yields satisfactory results.

5 N NaOH.‡ The mixture is thoroughly stirred and centrifuged. If at this point the pH of the supernatant liquid is found to be below 10, it is raised to this level by the addition of more 5 N NaOH, stirred and again centrifuged. After decanting the supernatant fluid, the solid is treated with 0.01 N NaOH to give a volume of 7-8 cc. and the pH again brought to 10-10.5. The process is repeated until the supernatant liquid is practically colorless; the combined extracts, amounting to 20-27 cc., are neutralized§ to pH 7.4 with 5 N HCl and employed for biological assay.|| If a more concentrated solution is required, the hormone may be precipitated by pouring the extract into 10 volumes of cold acetone, dried *in vacuo* and redissolved. Any insoluble material may be removed by centrifuging, for it is inactive.

The above processes can be satisfactorily carried out on larger volumes of urine. After elution the neutralized extract may, if desired, be decolorized with charcoal (1-2 gm. of Carbex E per 100 cc. of extract) at room temperature without apparent loss of activity. By acetone precipitation of such extracts a slightly colored, hygroscopic powder is obtained in yields of 8-9 gm. per 100 gm. of aluminum hydroxide. This material contains about 50% of ash and 2.7-3.5% of nitrogen (uncorrected). Qualitative tests on the precipitates indicate little or no organic sulfur and no reducing substances either before or after acid hydrolysis. A positive Sakaguchi reaction has repeatedly been obtained. In the standard rat test 60 mg. of such preparations produce ovaries weighing 50-60 mg. A large amount of such a homogeneous preparation is being used at present in an attempt to establish a unit of the follicle stimulating hormone suitable for clinical purposes.

Concentrates prepared by the aluminum hydroxide method are nontoxic when injected even in large amounts into immature female rats, in contrast to samples prepared by precipitation with ethyl alcohol.

Comparison of concentrates prepared by the above process and that involving benzoic acid[6] indicates that aluminum hydroxide is superior for the concentration of the follicle stimulating hormone

‡ Elution of the dry powder with ammonia, $Ba(OH)_2$, $Ca(OH)_2$ or pyridine has proved to be unsatisfactory.

§ Sometimes a precipitate is formed at this point, which is apparently inactive and should be removed by centrifugation.

|| The assay on immature female rats is carried out by the usual technique (*cf.* 2).

[6] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **106**, 125.

while the benzoic acid procedure gives better results with the luteinizing hormone,** when these methods are applied to urine. Our experiments indicate that the aluminum hydroxide method leads to a recovery of at least 60% of the F.S.H. originally present in the urine.

The above described method has been satisfactorily used for daily determinations of the amount of F.S.H. excreted in the urine of more than 150 patients suffering from various mental and nervous diseases. Various physiological and clinical aspects of the findings will be discussed in a detailed report which is being prepared for publication in the *Psychiatric Quarterly.*

The investigations are being continued.

8372 C

Influence of Ultra Violet Irradiation on Clam Heart Subjected to Potassium Excess.

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This investigation was undertaken in an attempt to determine the effect of ultra violet on tissue subjected to potassium excess. For this purpose the heart of the clam, *Venus mercenaria,* was used.

Records were obtained of the ventricular beat of the heart *in situ.* A small hook, made of glass capillary tubing, was inserted into the apex of the ventricle and, by means of a thread attached to the hook and a light balanced lever (a drinking straw), records were obtained on a moving kymograph. The clam, on the half shell, was placed in a finger bowl which was filled with sea water. To this the desired amount of potassium chloride was added.

The ultra violet source was a Cooper-Hewitt Uviarc (6-inch tube with reflector and running at 110 volts D. C.) placed at 35 cm. from the preparation. A thermopile, with a blackened couple, at the heart, served as a temperature index. The sensitivity of the galvanometer enabled temperature changes of 0.01°C. to be observed. During all of the experiments the temperature changes were negligible. Room temperature varied from 22 to 27°C. During the course of an experiment the temperature both of the room and of the heart

** Antuitrin-S was used as the source of L.H. We are indebted to Dr. Oliver Kamm of Parke, Davis and Co., for considerable quantities of this material.