Summary and Conclusions. Immature eggs of the sea urchin *S. franciscanus,* like fertilized eggs, stain more rapidly in solutions of Methylene Blue, Gentian Violet, and Methyl Violet than do resting unfertilized eggs. Like the fertilized eggs they are also more permeable to water. The immature and fertilized eggs are developing while the unfertilized egg is in a state of developmental inhibition. Increase in water permeability, as well as staining rate, at fertilization (when the egg is released from inhibition) is thus the converse (in direction) of the change when the immature egg enters the resting stage.

8763 P

Concentration of Gonadotropic Substance from Pregnancy Urine.*

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Katzman and Doisy[1, 2] reported that the gonadotropic substance found in the urine of pregnant women (P.U.) can be concentrated conveniently by adsorption on benzoic acid or by precipitation with phosphotungstic acid. Hellbaum *et al.*[3] have shown that the same substance is precipitated quantitatively from the urine by tannic acid. However, the purification of the tannic acid precipitate is rather difficult.

We have found that cresol extracts P.U. efficiently and its use offers definite advantages over the methods mentioned in simplicity, ease of manipulation and recovery of reagents. It removes the active material from the urine as completely as does tannic or benzoic acid and the product is a powder which is a convenient starting material for further purification.

The urine used in these experiments was from a stock supply which had been collected and kept in the coldroom for several days. The cresol was the U.S.P. grade of J. T. Baker Co.

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1 Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

2 Katzman, P. A., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1188.

3 Hellbaum, A. A., Fevold, H. L., and Hisaw, F. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1566.

The routine extraction of pregnancy urine is carried out as follows: The urine, in a separatory funnel, is shaken with 1/10 volume of cresol. After allowing to separate, the cresol is drawn off and the urine is extracted a second time with 1/10 volume cresol. The second portion of cresol is then used for the first extraction of a fresh batch of urine. The second urine sample is extracted a second time with a fresh batch of cresol which is also used for the first extraction of a third sample of urine, etc. The cresol, containing the active material is diluted with 2 volumes of acetone which precipitates the active substance. The precipitate is collected by centrifugation or by allowing it to settle to the bottom of the flask and the acetone-cresol solution decanted. The precipitate is washed with acetone and ether and stored as a dry powder. Twenty liters of urine, extracted in this manner yielded 12.6 gm. of dry powder which contained 1 rat unit per milligram. Another batch of urine (24 liters) yielded 19.4 gm. of powder which assayed 1 rat unit per 1.5 mg.

A series of experiments were carried out on the same batch of urine to determine the efficiency of extraction, as compared with other methods. One liter of urine was extracted three times with 100 cc. portions of cresol. The adsorbate was prepared as described and taken up in water for assay. Another liter of urine was treated once with 100 cc. cresol and prepared for assay as before. A third liter was precipitated with tannic acid and the crude tannate assayed. A fourth liter was extracted according to the benzoic acid method of Katzman and Doisy. The physiological activities of the 4 preparations were determined by injecting 22-day-old female rats twice daily for 3 days and weighing the ovaries the morning of the fifth day. The rat unit employed was the minimum amount which elicited a 50% increase in the weight of the ovaries. (Table I.)

TABLE I.
Assay of P.U. Preparations.

Method	R.U. per liter
Cresol (3x)	600-750
Cresol (1)	600-750
Tannic Acid	600-750
Benzoic Acid	500-600

Preliminary recovery experiments demonstrated that cresol extracts P.U. quite quantitatively from relatively dilute solutions. Two liters of urine were extracted 5 times with 1/10 volume of cresol. One liter of the urine was then boiled for 2 hours. The second liter was not boiled. One hundred milligrams of P.U. powder,

equivalent to 50 R.U. were added to the extracted boiled urine and a similar amount to the extracted unboiled urine. Both samples were extracted twice with 1/10 volume of cresol in the usual manner. From the extracted boiled urine, 450 mg. of powder were obtained which contained 1 R.U. per 7 to 8 mg., indicating a recovery of 50 to 65 R.U. The extracted unboiled urine yielded a powder weighing 230 mg. which contained 1 R.U. per 5 to 6 mg., a recovery of 40 to 50 R.U. Both of these results would indicate a quantitative recovery as they are well within the limits of error for the assay method.

One hundred cubic cm. of pregnancy urine, containing 75 R.U. were diluted with 900 cc. of urine which had been extracted and boiled as described and the whole was extracted twice with 100 cc. cresol. The dried adsorbate weighed 330 mg. and contained 1 R.U. in 5 to 6 mg., representing a recovery of 55 to 66 R.U.

Cresol extraction has also been used in attempts to concentrate the follicle stimulating hormone from the urine of castrated women and women past the menopause (F.S.U.) but without success. While cresol is apparently very efficient in extracting P.U. it does not take up F.S.U. This demonstrates a striking difference in the properties of the 2 substances, at least as they exist in the 2 types of urine.

8764 P

Action of Crustacean Eye-Stalk Extract on Melanophores of Hypophysectomized Fishes, Amphibians, and Reptiles.*

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The discovery of the crustacean eye-stalk hormone by Perkins[1] and by Koller[2] was soon followed by announcements of its effects upon the chromatophores of vertebrates. Koller and Meyer,[3] Meyer,[4] Perkins and Kropp,[5] and Kropp and Perkins[6] reported

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4 Meyer, E., *Zool. Jahrb., Abt. Allg. Zool. u. Physiol.*, 1931, **49**, 231.

5 Perkins, E. B., and Kropp, B., *Bio. Bull.*, 1932, **63**, 108.

6 Kropp, B., and Perkins, E. B., *Bio. Bull.*, 1933, **64**, 226.