Chemical Identification of Œstrone in Human Male Urine

Laqueur, Dingemanse, Hart and de Jongh[1] first reported in 1927 that œstrogenically active substances could be obtained from the urine of healthy men. The necessity arose shortly afterwards of identifying these active substances chemically. The small amounts in which these active substances occur in adult male urine has so far made this impossible. Dorfman, Gallagher and Koch[2] tried to solve the problem in 1935 by means of comparative biological studies, but such investigations merely produced indications as to the nature of the œstrogenically active substance.

On the average, adult male urine was found to contain 70 international units per litre. If one assumes a biological activity equal to that of œstrone, this represents a substance content of 7 gamma per litre. This figure makes it perfectly clear that the chemical identification of the œstrogenically active substance in male urine can only be brought to a satisfactory conclusion when large amounts of urine can be worked up. Through the intervention of the N. V. Organon-Oss, for which we take this opportunity of expressing our thanks, it has been possible for us to work up 17,000 litres of male urine. The various stages in the process are briefly described in the scheme below. Tests of the œstrogenic activity of the various fractions on castrated mice served as a guide for judging the success of the separation.

In this way we succeeded in obtaining 6 mgm. of a single crystalline substance. This proved to be identical with œstrone.

	Crystals from male urine	Œstrone.
Melting point ..	256–260°	257–261°
Mixed melting point ..	No depression	
Optical rotation $[\alpha]_D$ ..	+153°	+160°
Physiological activity ..	$0 \cdot 1\gamma = 1$ I.U.	$0 \cdot 1\gamma = 1$ I.U.

In the melting point determination practically the same crystalline form was observed as with œstrone. The identity with œstrone of one œstrogenically active substance isolated from male urine is definitely established by the above results.

Œstrone, however, is only partly responsible for the œstrogenic activity of male urine. The extract obtained according to the scheme below can be separated into ketonic and non-ketonic fractions. Only a third to a half of the œstrogenic activity passes into the ketonic fraction. We are at present occupied with the chemical identification of the non-ketonic œstrogenic fraction.

Process Scheme

Urine: extraction of the hydrolysed urine by the usual method and separation of the comb growth promoting substances.

Benzene solution: evaporated to dryness and the residue partitioned between 70 per cent alcohol and light petroleum.

Alcoholic solution: extracted with benzene after dilution with water.

Benzene solution: washed with soda solution, then with sulphuric acid and extracted with 5 per cent sodium hydroxide solution.

Alkali solution: neutralization of the alkali until practically neutral and extraction with benzene.

Benzene solution: evaporated, the residue dissolved in ether, and extracted with 0·1 *N*-sodium hydroxide solution.

Ethereal solution: treated with Girard's reagent.

Ketonic fraction: submitted to several repeated fractional sublimations in a high vacuum at less than 0·0001 mm. mercury.

125–130° Fraction: recrystallized from methyl alcohol.

Œstrone.

E. Dingemanse.
E. Laqueur.
O. Mühlbock.

Pharmaco-therapeutic Institute,
University of Amsterdam.
April 7.

[1] *Klin. Wochenschr.*, 6, 1859 (1927).
[2] *Endocrinology*, 19, 33 (1935).

Points from Foregoing Letters

On the basis of the theory of several 'time-scales', and from the fact that the equations of motion for a particle are unaltered in form when the direction of the trajectory of a particle is reversed, Prof. E. A. Milne and C. J. Whitrow conclude that all the other particles in the universe are relatively stationary, and consequently the universe is non-expanding.

Prof. V. V. Narlikar proposes a new boundary condition. It is in the tensor form and it is satisfied in the one-body problem as solved by Schwarzschild. He deduces from the condition a general result about the motion of a fluid at its surface and shows that this result implies the content of the geodesic postulate.

A discussion of isostasy leads Sir Joseph Larmor to the view that large accumulations of ice at the poles are not possible without an appreciable slowing down of the earth's daily rotation.

Prof. R. Emden points out that the energy content of the air in a room is independent of temperature and concludes that the reason why we heat our rooms in winter is not to add energy but 'entropy'.

A statistical investigation of the number of secondary cosmic rays produced in an iron-screened and unscreened Wilson cloud chamber, divided into five sections by four parallel lead plates 3 cm. thick, is described by Prof. B. Trumpy. From the results obtained he concludes that the soft components of the cosmic rays amount to about 27 per cent at the earth's surface, and that the hard components cannot be electrons.

Graphs showing the intensity of cosmic radiation at various heights near the pole of the earth's magnetic axis as compared with latitude 49° are supplied by Dr. H. Carmichael and E. G. Dymond. The results obtained both with ionization chamber and coincidence counters show that the radiation intensity is about the same, and indicate that cosmic rays of less than 3,000 M.e.v. do not exist in space, or are prevented from reaching the earth by some agency such as a solar magnetic field.

J. G. Daunt and Dr. K. Mendelssohn describe experiments indicating that the surface of a solid in contact with helium II is covered with a helium film through which the liquid is transferred to the lowest available level, the rate of transfer increasing the lower the temperature. From the amount of electrical energy which has to be supplied as current, before any apparent heating is observed in the upper part of a tube immersed in liquid helium II, A. K. Kikoin and B. G. Lasarew estimate that the thickness of the film is about 10^{-5} cm.