Through the courtesy of Prof. T. N. A. Jeffcoate, we have been able to obtain urine from two women immediately after parturition, who had received, in 24 hours, five 4-hourly doses of 20 mgm. of stilbœstrol. From this urine we were able to isolate the benzylamine salt of stilbœstrol monoglucuronide in a pure crystalline state. This salt had melting point and mixed melting point 223° C. and showed $[\alpha]_D^{20} = -55°$ ($c = 0{\cdot}2$ in 50 per cent aqueous acetone) and was identical with a sample (m.p. 223° C. and $[\alpha]_D^{20} = -55{\cdot}3°$ (in 50 per cent aqueous acetone)) which we had previously[3] prepared from pure stilbœstrol monoglucuronide isolated from rabbit urine. The yield of benzylamine salt corresponded to 35 per cent of the stilbœstrol administered. Small amounts of free stilbœstrol were also detected in the urine.

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Preparation of Œstrogens from Urine by Application of High Temperatures

Œstrogenic substances are usually extracted from the urine of pregnant women, pregnant mares or stallions with organic solvents not miscible in water. There are certain disadvantages inherent in this method: large extraction vessels are required; in view of the great bulk of the initial liquid containing the œstrogenic substances in low concentration and the relatively small total interface between the urine and the solvent, a relatively large volume of the latter has to be used; this in turn involves the use of a large distillation apparatus and a high fuel consumption for the evaporation of the solvent from the extract. Moreover, a high proportion of ballast substances which later require separation accompanies the œstrogenic substances in the extract.

We found the following process helpful for the concentration and preparation of œstrogens: evaporate the initial liquid until a sticky, gum-like residue is left; and heat the residue to a temperature not exceeding 245° C. for five minutes so as to carbonize the bulk of the ballast substances, while substantially preserving the œstrogenic substances.

Extracts of the carbonized mass with organic solvents contain the œstrogenic substances in rather high concentration, so that the evaporation is a step of only minor importance. Owing to the virtually complete destruction of the ballast substances by the heating, the dry residue remaining after the evaporation of the solvent already possesses a high degree of purity. It can, of course, be further purified by the known methods.

None of the usual œstrogenic substances, such as œstrone, œstradiol, œstriol, hippulin, equilin, equilinine, and the like, are destroyed by the procedure of heating.

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A Rapid Method for the Estimation of Pregnanediol

The presence in urine of pregnanediol can be detected easily and rapidly by the following procedure, elaborated in the course of recent investigations and based upon protection of pregnanediol by zinc dust during acid hydrolysis, thereby also preventing discoloration affecting the final colour reaction.

To 100 ml. of urine are added 1·5 gm. of zinc dust and the mixture is brought to the boiling point. Concentrated hydrochloric acid (10 ml.) is added and the mixture is boiled for five minutes. The flask is immersed in cold water, when the zinc dust settles rapidly; the supernatant fluid is then decanted on to a sand column. The zinc retained in the flask is washed successively with 25 ml. $N/1$ hydrochloric acid, 25 ml. $N/10$ hydrochloric acid and three lots of water, each 25 ml.

The washing fluids are also decanted on to the sand column after the zinc has settled. The sand column is dried by sucking hot air through it. The zinc dust retained in the flask is shaken up with about 20 ml. hot alcohol (95 per cent), and the hot extract is decanted on to the sand column. As an appreciable proportion of pregnanediol is retained by the zinc dust, the hot extraction has to be repeated three times. The alcohol is passed rapidly through the sand and the filtrate is evaporated to dryness. The residue is redissolved in 5 ml. alcohol (95 per cent), to which 20 ml. of $N/10$ aqueous caustic soda are added. After standing for one hour in the cold a precipitate forms, and this contains the pregnanediol fraction; it is collected on a fritted glass filter, washed with water and dried. About 10 ml. of hot alcohol are then slowly passed through the filter.

The filtrate may be used for the Gutterman colour reaction. For this purpose it is evaporated to dryness and the residue is dissolved in 5 ml. of concentrated sulphuric acid. In the presence of not less than 0·5 mgm. of pregnanediol per 100 ml. of urine a deep yellow or orange colour develops rapidly. In the absence of pregnanediol, the solution is pale yellow or colourless.

The method may be adapted to quantitative estimations by the use of larger quantities of urine and gravimetric methods. In a series of cases, 24- or 48-hour specimens of urine were divided into portions and pregnanediol was determined quantitatively both by Astwood and Jones' method and by the method described above, utilizing gravimetric and colorimetric procedures. Similarly, weighed samples of sodium pregnanediol glucuronidate were subjected to hydrolysis, pregnanediol being recovered by the two methods. It was found that the sand–zinc method gives yields of practically the same order as the Astwood–Jones method. The sand–zinc method is rapid and easy to carry out. It has been found useful in replacing the established but laborious methods in the diagnosis of pregnancy, impending miscarriage and other clinical conditions for which the detection of pregnanediol is required.

It is hoped to publish the work on which this method is based, along with some account of the cases to which it has been applied, in fuller detail elsewhere.

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