

The glycogen was easily dissociated from the borate-C.P.C. complex by lowering the pH to neutrality. This was followed by a dialysis against tap water. The luteic acid was separated from the C.P.C. by the following procedure<sup>2</sup>.

The complex was dissolved in a solvent consisting of methanol 20 % v/v, saturated sodium chloride 30 % v/v and water to 100 %. This solution was treated with Lloyds reagent and the disappearance of the C.P.C. was followed spectrophotometrically.

It should be pointed out the method cannot be used when large amounts of protein contaminate the polysaccharide solution, because the proteins tend to form highly viscous cakes with detergents, thus enclosing the polysaccharides.

In microbiological work extracellular polysaccharides can in many instances be isolated by the above methods if the salt concentration of the medium is not too high. When the salt content is high it is advisable to dilute the medium or to dialyse it before precipitation with C.P.C.

1. Raistrick, H. *et al.* *Phil. Trans. Roy. Soc. London, Ser. B* **220** (1931).
2. Scott, J. E. *Chemistry & Industry* **1955** 1568; *Biochem. J.* **62** (1956) 31P.
3. Barker, S. A. *et al.* *Chemistry & Industry* **1957** 330.

Received November 13, 1957.

## Isolation of Oestradiol-17 $\alpha$ from Bovine Meconium

WEIERT VELLE

*Department of Sexual Physiology and Sexual Pathology, Veterinary College of Norway, Oslo, Norway*

Investigations on oestrogens in the newborn calf have shown that significant amounts of oestrone and oestradiol-17 $\alpha$  are excreted with the urine during the first days of life<sup>1</sup>. These observations led to investigations on the oestrogen contents of meconium in this species.

In this preliminary communication results are presented which strongly indicate the presence of significant quantities of oestradiol-17 $\alpha$  in meconium from calves.

*Experimental.* Meconium was obtained from normal, healthy, newborn calves of both sexes and the analyses were undertaken immediately.

Reagents were of the same grade and manufacture as those used in a previous investigation<sup>2</sup>. The material was examined for the contents of both free and conjugated oestrogens:

a. For determination of free oestrogens specimens of meconium were suspended in distilled water and disintegrated in an "Ato-Mix" blender. The suspensions were then extracted repeatedly with ether. The combined ether extracts were processed as described in the following.

b. For determination of conjugated oestrogens the water phase from a. was freed from ether by evaporation, and concentrated HCl added to a final concentration of 10 %. Fitted with reflux condenser, the flask was kept at 100° C for 1 h. After cooling ether extraction was performed as before. The combined ether extracts were analysed according to the following procedures:

1. The chemical method for determination of urinary oestrogens, described by Brown<sup>3</sup> was applied with minor modifications<sup>2</sup>. This method is based on the chromatographic separation of the 3-methyl ethers of the oestrogens, using standardized alumina as adsorbent.

2. Chromatographic separation of the unaltered oestrogens were performed according to the method of Stimmel<sup>4</sup> as modified by Kakusjkina and Orlova<sup>5</sup>.

3. The *cis-trans* test for the 17-hydroxy group of oestradiol was performed according to Kägi and Miescher<sup>6</sup>, omitting the addition of bromine.

4. Absorption spectra of the isolated fractions were examined from 400–600 m $\mu$  using the Kober reagent described by Bauld<sup>7</sup>, and from 240–500 m $\mu$  using fuming sulphuric acid according to Axelrod<sup>8</sup>. The registered spectra were compared with those of pure compounds. All readings were made in a Beckman Model DU spectrophotometer supplied with photomultiplier.

*Results and discussion.* Using the modified Brown procedure, a strongly reacting Kober chromogen was found present in the oestradiol fractions from both a. and b., indicating that the substance is partly excreted in free and partly in conjugated form. Considerably greater quantities were observed in the "conjugated" fractions.

In the oestriol and oestrone fractions no trace of Kober chromogen could be detected.

The elution pattern of the methylated substance appearing in the oestradiol fraction closely followed the elution pattern of the 3-methyl ether of oestradiol-17 $\alpha$ .

Oestradiol fractions isolated according to the modified Stimmel procedure showed intense pink colour and green fluorescence when subjected to the test of Kägi and Miescher. An identical reaction was shown by oestradiol-17 $\alpha$ , while oestradiol-17 $\beta$  showed only a faint colour reaction.

The absorption spectra of the methylated oestradiol fractions, using the modified Kober reagent, closely followed the one of oestradiol-17 $\alpha$ -3-methyl ether with the absorption maximum at 524 m $\mu$ .

The absorption spectra of the non-methylated oestradiol fractions, using fuming sulphuric acid as reagent, closely followed the one of oestradiol-17 $\alpha$ , with distinct maxima at 300 and 430 m $\mu$ .

The Kober reaction is known to be highly specific to the oestrogenic hormones. Using the Brown method for isolation of the oestrogens, both the  $\beta$ - and  $\alpha$ -isomer of oestradiol will appear in the oestradiol fraction. No other Kober chromogen is known to appear in this fraction. The conformity between the absorption spectra of the methylated and non-methylated substance isolated in the oestradiol fractions, and the absorption spectra of oestradiol-17 $\alpha$ -3-methyl ether and oestradiol-17 $\alpha$ \* respectively, and the results of the *cis-trans* test for the 17-hydroxy group, support the validity of the concept that the Kober chromogen isolated from meconium of calves is identical to oestradiol-17 $\alpha$ .

The quantities found is of the order of magnitude of 20 mg/kg in unconjugated, and 25–35 mg/kg in conjugated form, both figures representing free oestradiol-17 $\alpha$ . There seems to be no significant difference between the sexes as regards the amounts present. This would indicate that the substance is of maternal origin.

As far as the present author is aware, the only previous paper reporting the presence of oestrogens in similar material is the one presented by Kinsella *et al.*<sup>3</sup>, who in 1956 isolated oestriol from human meconium. No other oestrogen was present in this material.

Isolation of greater quantities of oestradiol-17 $\alpha$  from bovine meconium is being carried out. The work shows that the substance is easily crystallized from ethanol-water mixtures.

\* Gift from Ciba, Basel.

A detailed report of the work in progress will be published later.

*Added in proof:* By use of infrared spectroscopy it has now been definitely established that the isolated substance is identical with oestradiol-17 $\alpha$ .

This investigation was financially supported by the Agricultural Research Council of Norway.

1. Velle, W. *To be published.*
2. Velle, W. *Acta Endocrinol.* **27** (1958) 64.
3. Brown, J. B. *Biochem. J.* **60** (1955) 185.
4. Stimmel, B. F. *J. Biol. Chem.* **162** (1946) 99.
5. Kakusjkina, E. A. and Orlova, V. G. *Biochimia (Moskva)* **21** (1956) 26.
6. Kägi, M. and Miescher, K. *Helv. Chim. Acta* **22** (1939) 683.
7. Bauld, W. S. *Biochem. J.* **63** (1956) 488.
8. Axelrod, L. R. *Recent Progr. in Hormone Research* **9** (1954) 69.
9. Kinsella, R. A., Francis, F. E., Thayer, S. A. and Doisy, E. A. *J. Biol. Chem.* **219** (1956) 265.

Received October 27, 1957.

## Carbohydrates in a Cold Water Extract of a Pine Forest Soil

E. ALVSAKER and K. MICHELSEN

*Chemical Institute, University of Bergen, Bergen, Norway*

In a preliminary communication Hawthorth *et al.*<sup>1</sup> state that levans have been isolated from polysaccharides obtained by extraction of soil with buffer solutions. We have not, however, been able to find any information about the existence of free fructose in soil. Forsyth<sup>2</sup> mentions the occurrence of small amounts of free glucose, xylose, rhamnose, and galactose in water extracts of several types of soil, but no details are given about methods of identification. Hot (85°C) water soluble soil polysaccharides have been investigated by Duff<sup>3,4</sup>. In the polysaccharide hydrolysates several aldoses could be identified by chromatographic methods.

An attempt was made to isolate levans from the uppermost layer (F-layer) of a pine forest soil from Western Norway, according to the experimental conditions described by Hawthorth, but the attempt