SHORT COMMUNICATIONS

OESTRIOL IN AMNIOTIC FLUID: THE EFFECT OF STORAGE, FILTRATION AND GLUCOSE

J. S. BIGGS*

Clinical Research Unit, Department of Obstetrics and Gynaecology,
Maternity Hospital, Foresterhill, Aberdeen, AB9 2ZA

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Oestriol concentrations in amniotic fluid have been investigated by several workers as a possible means of assessing foeto-placental function (Berman, Kalchman, Chat-toraj & Scommegna, 1968; Michie & Livingstone, 1969). Certain basic data are required, however, in working with any biological fluid and although information is available for storage effects on urinary oestriol (Leon, Bulbrook & Greenwood, 1959) more information is needed for amniotic fluid. This paper describes an investigation of the effects of storage at different temperatures, of filtration and of the influence of glucose concentration on amniotic fluid oestriol using a gas–liquid chromatographic (GLC) method.

Amniotic fluid was obtained at surgical induction of labour in patients in the 40th or 41st weeks of normal pregnancy. For the study of storage effects, 450 ml. of amniotic fluid was obtained from one patient and immediately divided into three parts. The first was stored in a stoppered flask at room temperature (22°), the second in a stoppered flask in the refrigerator at 4°. The third was divided into 5 ml. aliquots in screw-top containers which were placed in the deep freeze at −16°. No preservative was added at any stage. Six 5 ml. aliquots were taken from the first flask to determine the initial level of oestriol. Acid hydrolysis of these samples was begun within 30 min. of amniotomy.

Filtration effects were studied using 200 ml. of amniotic fluid from another patient. One portion of the fluid was stored at 4° overnight while the other portion, also at 4°, was filtered through a Whatman No. 1 filter paper. Oestriol assays on both samples were begun 15 hr. after rupture of the membranes.

The effects of glucose concentration were studied using fluid from a normal patient. The glucose concentration of this fluid was tested by a glucose oxidase method (Middleton & Griffiths, 1957). A solution of glucose was prepared and added to further aliquots of the same amniotic fluid to provide two concentrations of glucose, one similar to that found in insulin-treated diabetic patients and one about twice this level. These aliquots were then hydrolysed and the oestriol concentration of all three portions determined.

All determinations were performed in replicate using the GLC method described by Biggs, Klopper & Wilson (1969). Tritiated oestriol was added as an internal standard and all results were corrected for losses during assay.

* Present address: Department of Obstetrics and Gynaecology, Clinical Sciences Building, Royal Brisbane Hospital, Queensland 4029, Australia.
The oestriol concentration in amniotic fluids stored at three temperatures was determined at weekly intervals for 33 days and at 2-weekly intervals for a further 28 days. Six replicate aliquots of 5 ml. of amniotic fluid tested for the initial level of oestriol gave a mean concentration of 57.3 \( \mu g./100 \) ml. with a standard deviation (s.d.) of 3.2 \( \mu g./100 \) ml. Figure 1 shows that there was no appreciable change in oestriol levels after 9 weeks' storage at 22\(^\circ\), 4\(^\circ\) or -16\(^\circ\). The possibility of spontaneous hydrolysis of oestriol conjugates under different storage conditions was not investigated.

![Graph showing oestriol concentration over weeks at different temperatures](image)

**Fig. 1.** Oestriol concentration in replicate samples of human amniotic fluid stored at 22\(^\circ\), 4\(^\circ\) and -16\(^\circ\) for periods up to 9 weeks.

In the filtration experiment six replicate determinations of oestriol in 5 ml. of unfiltered amniotic fluid gave a mean concentration of 97.3 ± 5.5 \( \mu g./100 \) ml. (s.d.). This gives a coefficient of variation of 5.7\% and a standard error of the mean of 2.5 \( \mu g./100 \) ml. Six replicate determinations in 5 ml. of filtered amniotic fluid gave a mean oestriol concentration of 101.9 ± 3.9 \( \mu g./100 \) ml. (s.d.). The coefficient of variation was 3.8\% and the standard error of the mean was 1.7 \( \mu g./100 \) ml. Filtration had no significant effect on the assay results. In the samples tested the method was made a little easier by filtration since emulsions, though rarely a problem with the method, were virtually eliminated.

Increasing the glucose concentration in amniotic fluid had no effect on oestriol determination by the GLC method employed. The glucose concentration of the amniotic fluid samples was 39 mg./100 ml. Duplicate estimations gave a mean oestriol concentration of 34.6 \( \mu g./100 \) ml. When glucose was added to the amniotic fluid to give a concentration of 106 mg./100 ml., the mean oestriol concentration was 34.2 \( \mu g./100 \) ml. Further addition of glucose to give a concentration of 262 mg./100 ml. resulted in a mean oestriol determination of 33.5 \( \mu g./100 \) ml. The method of Biggs et al. 1969, would appear to be of value in investigating the amniotic fluid oestriol in diabetic patients.

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**REFERENCES**


