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Note

Determination of glycerol in biological samples

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The use of glycerol in neurosurgery was first reported in 1961 [1] and since then it has been widely recommended as a safe and effective means of acutely reducing intracranial pressure [2—4]. The great therapeutical success of glycerol induced us to begin a study of its mechanism of action as a cerebral dehydrating agent. For this purpose we worked up a method for qualitative and quantitative analysis of glycerol in biological tissues and fluids.

In this note we describe the gas chromatographic determination of glycerol in biological samples.

MATERIALS AND METHODS

Biological samples, serum, cerebrospinal fluid and cerebral tissue were from patients hospitalized in the Neurosurgical Clinic of the University of Rome. Cerebral tissue was from surgical removal of cerebral tumors with a surrounding part of sound tissue, in patients either treated with glycerol (1 g/kg oral glycerol every 5 h) or not so treated.

For extraction and gas chromatographic analysis of glycerol we modified the method reported by Slansky and Moshy [5].

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We added to the sample (serum or cerebrospinal fluid) methanol in a 1:10 ratio (v/v). For cerebral tissue, samples were homogenized in a Potter homogenizer with methanol and then further diluted 1:10 (w/v). Methanol has a twofold function: to deproteinize the sample and to extract glycerol. Samples were well shaken and left standing at room temperature overnight. Then they were centrifuged and an aliquot of supernatant, with a suitable quantity of erythritol diluted in methanol added as internal standard, was evaporated to dryness by a nitrogen stream at room temperature.

One milliliter of Tri-Sil/N,O-bis(trimethylsilyl)-acetamide (BSA), Formula D, was added to each sample; the test tube was tightly closed with a screw cap fitted with a Teflon liner, and well shaken. After 1 h at room temperature, 2 μl of reaction mixture were injected into the gas chromatograph.

Analyses were carried out on a Perkin-Elmer 990 gas chromatograph fitted with a flame ionization detector. The glass column (1.8 m × 2 mm I.D.) was packed with 3.5% SE-30 on Gas-Chrom Q AW DMCS, 80—100 mesh. Column temperature was 80°C for 3 min, then programmed to reach 150°C at a rate of 3°C/min. Injector and detector temperature was 220°C. Flow-rates were 30 ml/min for nitrogen carrier gas, 20 ml/min for hydrogen and 300 ml/min for air.

Glycerol anhydrous extra pure and erythritol were obtained from Merck (Darmstadt, G.F.R.); Tri-Sil/BSA, Formula D, in vials, was obtained from Pierce Chemical Co., Rockford, IL, U.S.A.

RESULTS AND DISCUSSION

Gas chromatograms of glycerol, with erythritol as internal standard, in standard solution and biological material are reported in Figs. 1 and 2, respectively. Data for glycerol concentration in the various biological samples are reported in Table I. The recovery of glycerol was tested by analysing a sample of cerebrospinal fluid containing 0.041 mg/ml glycerol after the addition 0.050 mg of glycerol: recovery was total. Also intra-assay variability was tested: it was lower than 7%. Evaporation of the solvent by a stream of

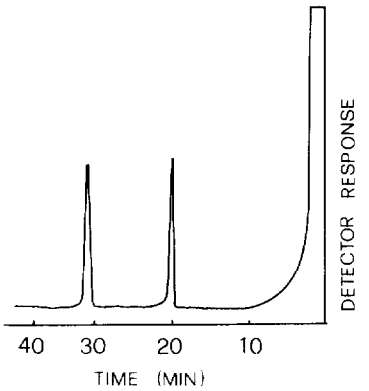

Fig. 1. Gas chromatogram of glycerol with erythritol as internal standard in standard solution: retention times are 20 and 31 min, respectively. For conditions of analysis see text.

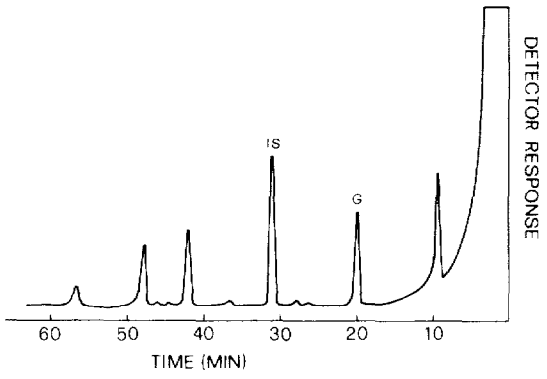

Fig. 2. Gas chromatogram of a sample of a tumor (paraventricular glioma) of a patient treated with glycerol (operation was performed about 12 h after last dose of glycerol). For conditions of analysis see text. A 2-μl volume of reaction mixture was injected, corresponding to 0.2 mg of tissue. G = glycerol, IS = internal standard; other peaks were not investigated.

TABLE I

MEAN VALUES OF GLYCEROL CONCENTRATIONS IN SOME BIOLOGICAL SAMPLES FROM PATIENTS NOT TREATED OR TREATED WITH GLYCEROL

Biological sample	Untreated patients	Treated patients
Serum (mg/ml)	0.05*	0.26 (0.05—0.65)**
Cerebrospinal fluid (mg/ml)	0.02*	0.13 (0.04—0.42)
Tumoral cerebral tissue (mg/g)	0.20 (0.05—0.44)	0.44 (0.11—0.89)
Sound peritumoral cerebral tissue (mg/g)	0.21 (0.12—0.35)	0.23 (0.11—0.44)

*Single value.
**Range in parentheses.

nitrogen and without heating or vacuum is a guarantee against any loss of sample. The use of erythritol as internal standard assures accuracy of analysis. The calibration graphs were linear in the experimented range (0.01—10 mg/ml of reaction mixture). The sensitivity of the method was 5 ng in standard solutions. In biological samples the lower value we obtained was 0.02 mg/ml of cerebrospinal fluid; but the method allows a lower limit of detection because the glycerol and erythritol internal standard peaks are isolated from the other unknown peaks of biological material.

Results with this method are very reproducible. The technique is simple and suitable for our research, which requires screening of a large number of samples.

Our first results have not allowed us to draw any conclusions about a possible mechanism of action of glycerol as a means of acutely reducing intracranial pressure, but they are very encouraging for the continuation of this research.

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