Technical methods

Potentiometric method for the determination of inorganic fluoride in biological material

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A number of methods for the determination of inorganic fluoride in biological material are available. These are dependent on the formation of hydrogen fluoride which is separated by means of diffusion or distillation; it is collected in a form suitable for the production of a dye-complex which may be determined colorimetrically. (Hall, 1960, 1963; Johnson and Leonard, 1961; Chang and Thompson, 1964; Singer and Armstrong, 1959; 1965).

An electrode possessing a specific response to fluoride ion is now commercially available and consists essentially of a lanthanum fluoride single crystal membrane, doped with europium. It has the advantage that it is simple and rapid in use and avoids difficulties inherent in the diffusion and allied procedures. It also enables samples of widely varying range to be studied. The electrode is, however, responsive to ionic activity and not directly to concentration; for this reason the pH and ionic strength of the solutions under evaluation must be controlled.

This communication describes certain precautions which must be taken, and the reagents found to be needed in the light of our experiences with various types of biological material.

APPARATUS

The fluoride electrode (Orion Research Inc., Cambridge, Mass., U.S.A.) was used in conjunction with a standard calomel reference electrode and an expanded scale pH meter (Radiometer 25 SE). Readings were taken on the millivolt scale.

REAGENTS

- 1 Stock fluoride standard, 1 mg/ml, 2.21 g of sodium fluoride was dissolved in water and diluted to 1 litre
- 2 M sodium acetate, adjusted to pH 7.2
- 3 M sodium acetate, adjusted to pH 5.4
- 4 SOLUTION 'A' Sodium chloride 5.8 g, potassium chloride 0.3 g, sodium carbonate 2.1 g, dissolved in water and diluted to 1 litre.
- 5 SOLUTION 'B' Sodium chloride 3.5 g, potassium chlor-

ide 2.5 g, sodium dihydrogen phosphate (anhydrous) 4.8 g, dissolved in water and diluted to 1 litre.

The following solutions were prepared as described by Hall (1963).

6 LITHIUM HYDROXIDE (FLUORIDE FREE) About 4 g of metallic lithium, freed from its crust of oxide, was added in small pieces at a time to 500 ml of distilled water. When the metal was dissolved, the normality of the solution was checked by titration against potassium hydrogen phthalate and the concentration adjusted to 1 N.

7 MAGNESIUM SUCCINATE (FLUORIDE FREE) About 3 g of magnesium turnings was washed twice with 100 ml of distilled water in an Erlenmeyer flask and the washings discarded. One-hundred ml of distilled water and 50 ml of 5% succinic acid were then added. After about one minute, the acid solution was decanted and the magnesium again washed with 100 ml of water. The turnings were suspended in 100 ml of water and dissolved by the addition of 100 ml of 5% succinic acid. When the reaction subsided, the solution was filtered through Whatman no. 42 filter paper. The filtrate was evaporated to dryness at 100°C and the white residue ground to a fine powder in a glass mortar. Magnesium succinate solution (0.2 M) was prepared by dissolving 5.13 g of the magnesium succinate in water and diluting to 100 ml. It was stored at 2°C in a polythene container.

METHOD

GENERAL PROCEDURE All glassware was washed in dilute hydrochloric acid and rinsed several times in distilled water.

Standards were prepared in the appropriate solutions to give a similar ionic composition and pH to that of the test sample. Approximately 10 ml of the solution was placed in a small polystyrene beaker; the electrodes were just immersed in the liquid which was constantly stirred.

A typical response of the electrode to various concentrations of fluoride is shown in Figure 1. In this example dilutions were prepared in solution A.

PLASMA To 10 ml of plasma in a glass stoppered test tube 2.5 ml of 25% trichloroacetic acid was added. The tube was shaken vigorously, allowed to stand for 20 minutes and then centrifuged at 4,000 rpm. To 4 ml of the clear supernatant, 3 ml of M sodium acetate (pH 7.2) was added. The resultant pH was about 4.9.

When 0.5, 0.7, 1.0, 1.5, and 2.0 µg of fluoride were added to 10 ml aliquots of pooled dog plasma the recovery was found to be 90-105% as shown in Table I.

Human plasma, collected from 12 volunteers living in this area (where the water contains 0.1 ppm of fluoride), was assayed. A mean value of 0.023 µg/ml ± 0.005 SD was obtained. At these levels, reproducibility is within 2 mV, which represents a variation of about ± 15%.

FIG. 1. Curve of a typical response of the electrode to various concentrations of fluoride.

TABLE I

RECOVERY OF FLUORIDE ADDED TO DOG PLASMA

Fluoride Added (µg/ml)	Fluoride Found (µg/ml)	% Recovery
—	0.035	—
0.050	0.080	90
0.070	0.105	100
0.100	0.140	105
0.150	0.190	103
0.200	0.235	100

These results seem to confirm the opinion of Taves (1966) that the normal values quoted by Singer and Armstrong (1959, 1965, 1967) are too high.

URINE Twenty-four-hour collections were preserved by the addition of concentrated hydrochloric acid (5 ml/litre). Three ml aliquots were diluted with two volumes of acetate buffer pH 5.4. The mean value of 18 human samples was found to be 1.16 mg/24 hours $\pm$ 0.52 SD.

BONES AND TEETH These were crushed with a pair of pliers and 0.2 g of the resultant fragments weighed into a tared platinum crucible. To this was added 0.3 ml of N lithium hydroxide and 0.2 ml of 0.2 M magnesium succinate. The crucible, with lid, was inserted in a closed steel canister and placed in a muffle furnace. The temperature was raised from ambient to 400°C over a period of three hours and maintained at this temperature for 15 hours. The canister was removed from the furnace

whilst still hot and allowed to cool. The ash was washed into a 100 ml volumetric flask with 5 ml of 5% perchloric acid (added in small quantities at a time), followed by 10 ml of water. One ml of perchloric acid (72% w/w) was added and the flask placed in a water bath at 60°C until the ash was completely dissolved. When cool the solution was made to 100 ml with water (Hall, 1963).

An aliquot of this solution was buffered with an equal volume of M sodium acetate solution (pH 7.2) so as to give a final pH of 5.4. The electrode potential of the sample was measured and compared with a calibration curve prepared from standards in 1% v/v perchloric acid to which an equal volume of the buffer was added.

Femurs from rats, which had been treated with 2-oximino 3', 4', difluoropropiophenone, were analysed potentiometrically and also by the diffusion technique of Hall (1963). Comparative figures are shown in Table II and demonstrate close agreement.

TABLE II

COMPARISON OF POTENTIOMETRIC METHOD WITH DIFFUSION TECHNIQUE¹

Dose (mg/kg/day)	Method	
	Diffusion	Potentiometric
Control	546 $\pm$ 48 (5)	542 $\pm$ 107 (5)
100	1,340 $\pm$ 184 (5)	1,417 $\pm$ 311 (5)
400	3,490 $\pm$ 253 (4)	3,350 $\pm$ 172 (4)
1,000	4,574 $\pm$ 1,618 (5)	5,072 $\pm$ 748 (5)

¹Fluoride content of rat femurs from animals treated for six months with 2-oximino 3', 4', difluoropropiophenone. Results are expressed as µg/g (dry wt) $\pm$ SD.

Numbers of animals in each group are shown in parenthesis.

COMMENTS

As mentioned earlier, the pH and ionic strength of the standards and test samples must be similar. The pH range is governed by the fact that in acid solution, fluoride exists as undissociated hydrogen fluoride; this sets the lower limit of pH at about 4.5. At above pH 8.0, hydroxyl ions interfere. However, in preparations of biological material such as bones, teeth, and urine, calcium phosphate will precipitate at values above pH 7.0 and may result in the co-precipitation of fluoride. Practical limits are thus between pH 4.5 and 6.5.

The effect of ionic strength in the unknown samples can be compensated by making the standards in solutions matching the ionic concentration of the test samples. In preliminary experiments standards for plasma were first prepared in Krebs and Henseleit solution (1932). Standards in the simplified solution (solution A) gave comparable readings. The same principle was applied to urine, the standards being prepared in a solution approximating the inorganic content of a 24 hour urine *ie*, solution B.

SUMMARY

A potentiometric method for the estimation of fluoride in biological material is described which is less time

consuming, simpler and more sensitive than the standard chemical methods.

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Quick-drying sterile cabinet for culture plates¹

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One of the most time-consuming processes in a busy bacteriology laboratory is the drying of agar culture plates after they are poured. This is usually done in ordinary incubators or ovens and a large load may take up to two hours to dry. I therefore tried drying plates in an oven equipped with a circulating fan, and with air ducts to allow dilution with fresh air (Gallenkamp OV 150 BS size 3). This speeded up drying enormously, but caused an unacceptable level of aerial contamination of the plates. Ultraviolet bactericidal units (Hanovia 06029 model 11 with 12-in. tubes) were therefore fitted to the back of the oven (Fig. 1) and a marked reduction in contamination was thereby achieved (Table I).

Only small loads could be dried satisfactorily in this oven and some plates were found at first inspection to be not completely dry and had to be replaced in the oven for a few minutes. This of course slowed up the process and increased the risk of contamination. Also, drips of condensate from the agar surface fouled the shelves, which had to be cleaned frequently.

A new cabinet (Figs. 2-3) was therefore designed with much larger air intakes and outlets and with longer UV tubes (Hanovia 06031, model 12, 24-in.). This allowed the shelves to be spaced far enough apart to minimize shadowing of air by the load or shelves. Air is admitted to the underfloor space via 11 $1\frac{1}{2}$ -in. holes in the rear wall, and thence to the chamber by 36 $\frac{1}{2}$ -in. holes in the floor. Immediately above these holes are three 500-watt elements which heat the incoming air. They are controlled by an adjustable thermostat and by an over-temperature cut-out. Additional air is also admitted through two $1\frac{1}{2}$ -in. tubes beside the UV tubes. The air exits are six $1\frac{1}{2}$ -in. holes in the roof. A microswitch, operated by the door, is fitted to the UV transformer circuits so that both units are switched off by opening the door and on by closing it. A plate glass window is let into the door so that the UV tubes can be seen to be working. The UV transformers, fan, and heater are all separately controlled by switches on the front panel. Construction of the cabinet is in 20 SWG stainless steel. No thermal insulation is required. Heating up is very rapid (15 min.) due to the elements lying free in the chamber.

METHOD OF TESTING

Two ovens were tested, the Gallenkamp with 12-in. UV tubes and the special cabinet made by Messrs Laboratory and Thermal Equipment Ltd, which had

¹All enquiries regarding purchase of this equipment should be addressed to Messrs Laboratory and Thermal Equipment Ltd, Greenfield near Oldham, from whom further details, including price, are available.

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