Device for eluting proteins from starch gel by freezing and thawing

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If starch gel is frozen and thawed, its structure is so changed that most of the water and protein can be extracted by centrifugation (Smithies, 1955) or by squeezing. This is conveniently done in the device illustrated here. Portions of the completed gel are cut out, placed in the barrel of the syringe, and then frozen by keeping at −20° for two hours. After allowing to thaw on the bench for two hours, fluid is expressed, and the syringe and gel matrix are then washed as often as desired by drawing up water or a suitable buffer solution and re-expressing after an interval of 10 minutes to 24 hours. The recovery of haemoglobin was virtually complete after two rinses, but serum albumin proved more difficult, only some 80 to 90% being extracted after three rinses.

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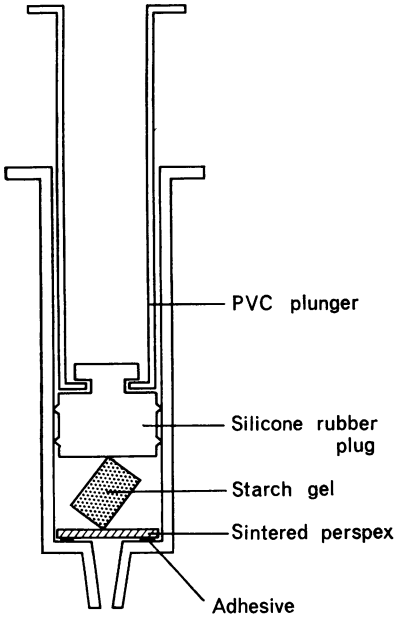

FIG. 1. *The device is based upon a 10 ml disposable syringe made of polyvinyl chloride, but having a close-fitting plug of artificial rubber attached to the end of the plunger. The minimum quantity of perspex cement (4% perspex in chloroform) is placed round the perimeter of the end of the barrel (inside), using a Pasteur pipette. A disc of sintered perspex (Fisons Scientific Instruments Ltd, Loughborough) is cut to fit inside the barrel, and is then forced down with the plunger until it lies in contact with the adhesive. The plunger is then withdrawn, and the syringe laid aside for 24 hours to harden. Alternative adhesives might be tried with advantage.*

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The advantages of this device are that it is convenient, at least for small numbers, and that it is easy to rinse the gel matrix. Only about 75% of the water contained in the gel is recovered at the first expression or centrifugation, and rinsing is essential if recoveries are to be quantitative. The chief disadvantages are shared with any procedure for extraction by freezing and thawing—the sample is contaminated with polysaccharides, and even some protein, derived from the gel itself.

Bocci (1963) described the use of metal-glass syringes fitted with filter paper discs to extract protein by the freezing and thawing process, from starch gel expanded with Pevikon C870. Filters of this kind have proved too weak to stand up to the pressures used in expressing gels made of starch alone. Also, if the gel matrix is to be rinsed by aspirating buffer, the filter must be firmly attached to the end of the syringe barrel. A demountable system of screw clamps can be used instead of simply glueing the porous disc in place, but it is more expensive.

Letter to the Editor

A MODIFIED SCREENING TEST FOR GLYCOSAMINOGLYCAN EXCRETION

The cetylpyridinium chloride turbidity screening test for glycosaminoglycan excretion discussed by Manley, Severn, and Hawksworth (1968) has been used in this laboratory. In this method (cetylpyridinium chloride turbidity) the precipitation time of four minutes is critical since, after this time interval, turbidity may continue to rise or may fall due to aggregation of particles. As a result of this, false positive results may be found in normal subjects. Precipitation of glycosaminoglycan by cetylpyridinium is also affected by ionic strength and *p*H, false positive results also being found in urine of high *p*H or high ionic strength. Therefore, it seemed desirable to try and overcome the critical time dependence and adjust the urine *p*H with a buffer in such a way that differences in final ionic strength and *p*H between samples would be negligible. Preliminary experiments showed that a citrate buffer at *p*H 4·8 was

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suitable and the modified method (cetylpyridinium chloride citrate turbidity) is as follows:

For the blank 1 ml of filtered urine is added to 1 ml of a citrate buffer (*p*H 4·8) containing 9·68 g of citric acid per litre and 15·88 g of trisodium citrate per litre (Dawson and Elliott, 1959). For the test 1 ml of urine is added to 1 ml of the same buffer containing 1 g of cetylpyridinium chloride per litre. Mix well and allow to stand at room temperature for at least 30 minutes before mixing again and reading the optical density at 680 mμ. Standard solutions containing 5 mg and 10 mg of chondroitin sulphate (Calbiochem Ltd) per 100 ml are treated in the same manner.

Optical density is linearly related to concentration up to 10 mg/100 ml and reagents have a shelf life in excess of one year. Results are reported as cetylpyridinium chloride units/100 ml or cetylpyridinium chloride units per gram of creatinine where one unit is equivalent to the optical density of a 1 mg/100 ml solution of chondroitin sulphate under the conditions of the test. Results are not reported as milligrammes of glycosaminoglycan since mucoproteins are coprecipitated.

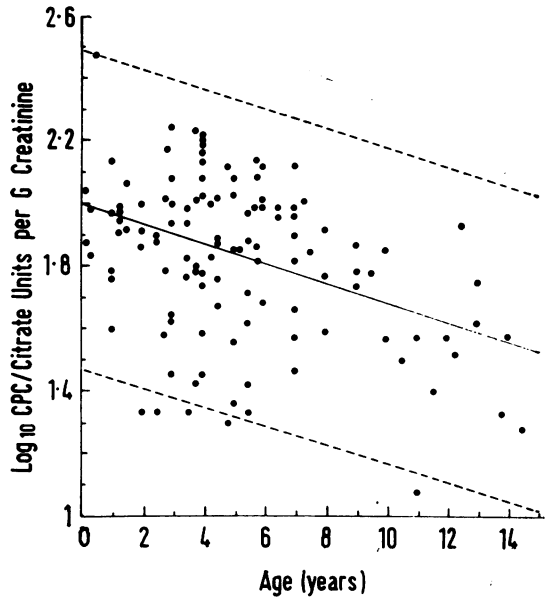
The optical density increases with time reaching a maximum at 30 minutes but thereafter time has little effect provided the sample is well mixed before reading. Thus the method described here largely overcomes the critical time dependence of the method of Manley and Hawksworth (1966) as citrate appears to stabilize the precipitate. Furthermore, the inclusion of a standard, not possible using aqueous cetylpyridinium chloride since glycosaminoglucan will not precipitate in the absence of salts, also corrects for time and temperature effects. Samples should be allowed to reach room temperature before testing as cetylpyridinium chloride will precipitate at a low temperature. Early examination is also desirable since prolonged storage may produce false results.

Cetylpyridinium chloride precipitable uronic acid (a useful guide to glycosaminoglycan concentration although it will not reflect the amount of kerato sulphate or mucoprotein coprecipitated) was isolated as described by Di Ferrante (1967), assayed by the method of Bitter and Muir (1962), and compared with the cetylpyridinium chloride turbidity test described here. There was good correlation between cetylpyridinium chloride precipitable uronic acid and both screening tests (Table I) but correlation was better for the modified method.

In the screening of small children it is not always possible to obtain accurate 24-hour urine collections.

TABLE I

Comparison	*No. of Samples*		*P*
Cetylpyridinium chloride precipitable uronic acid with cetylpyridinium chloride turbidity (Manley and Hawksworth, 1966)	30	0·7690	<0·001
Cetylpyridinium chloride precipitable uronic acid with cetylpyridinium chloride/citrate turbidity (this work)	30	0·9229	<0·001



In this laboratory it is usual to screen random samples in the first instance and relate cetylpyridinium chloride units to creatinine excretion in an effort to overcome fluctuations which occur in cetylpyridinium chloride precipitable material throughout the day. Mid-morning specimens are satisfactory and cetylpyridinium chloride units/g creatinine show the same changing pattern with age described for cetylpyridinium chloride turbidity/g creatinine (Manley *et al*, 1968). The correlation coefficient of $\log_{10}$ cetylpyridinium chloride units/g creatinine with age in normal children was —0·3870 n = 118 p < 0·001. The normal range is shown in the Figure, the dashed lines enclosing two standard deviations from the regression line ($y = 1{\cdot}9764 - 0{\cdot}03x$).

The modified method described here minimizes the possibility of false positive results encountered in the method of Manley and Hawksworth and has proved a useful screening test for abnormal glycosaminoglycan metabolism.

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