is made. This latter reading will normally show an increase and this value must be subtracted from the increase in optical density at 340 $m\mu$. The background absorbance is undoubtedly due to the high concentration of protein in the semi-micro cell.

The lactose content of the sample is directly proportional to the optical density difference between the readings at 340 $m\mu$ minus any increase at 400 $m\mu$.

Each determination should be performed in duplicate, as well as the blanks; in a lactose tolerance test the fasting specimen is ideally treated as the blank determination. Standards must be prepared by adding small amounts of aqueous lactose solutions to plasma. Purely aqueous standards do not have the same ultimate pH, no doubt due to the removal of hydrogen ions by protein, in the protein-precipitation step.

CALCULATION

A standard solution of 20 mg. per 100 ml. is made by diluting a 200 mg. per 100 ml. standard with pooled plasma. If S340 is the increase in optical density of standard at 340 $m\mu$ and S400 is the increase in optical density of standard at 400 $m\mu$ and T340 is the increase in optical density of test at 340 $m\mu$ and T400 is the increase in optical density of test at 400 $m\mu$ the lactose concentration in the original sample in mg. per 100 ml.

$$\frac{T340 - T400}{S340 - S400} \times 20$$

A calibration curve has been shown to be linear up to 35 μ g. of lactose in a 0.5 ml. cell (i.e. with an aliquot of 120 microlitres, linearity is maintained up to 120 mg. per 100 ml. in the original plasma). The optical density per 5 μ g. of lactose is approximately 0.1 using a cell of capacity 0.5 ml. and optical path-length of 1 cm. Such a particular cell size is important for economy of reagents which are expensive.

URINE SAMPLES

Urine samples should be treated in the same manner as plasma; samples giving results greater than 100 mg. per 100 ml. should be diluted 1:2. Values greater than this are well beyond the linearity of the method.

COMMENT

A method is presented for the measurement of plasma and urine lactose. Significant amounts of lactose were detected only in plasma following lactose loads. These were used to provide a more accurate differentiation of lactose intolerance, alactasia, and lactosuria.

Several of the infants with lactose intolerance and lactosuria had plasma lactose levels from 14 to 22 mg. per 100 ml., with a mean value of 19 mg. per 100 ml. These levels were detected in the range 60–90 minutes after ingestion of the lactose. Normal levels did not rise above 5 mg per 100 ml.

The author wishes to thank Dr. A. Jordan and Dr. J. Liddell for their advice and helpful suggestions.

REFERENCE

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A simple device for microdialysis

A. A. CODD, *From the Public Health Laboratory, General Hospital, Newcastle upon Tyne*

One of the problems of analysing macromolecular substances by immunodiffusion and immunoelectrophoretic techniques is that of concentrating these substances from the dilute fractions obtained off columns of ion-exchange and gel-filtration materials, or eluates of substances adsorbed to cells. The difficulty is increased when only small volumes of the starting material are available or required for analysis. Loss of material is considerable when small volumes are concentrated in cellophane bags by dialysis against polyethylene glycol. Much of it remains trapped in the folds around the occluding knots. Some of the difficulty can be avoided by the use of the simple device described.

The wide end of a conical plastic needle cover is cut off squarely (A). A 3 mm. ring is cut from this end (AB) and slid over the narrow end which is then cut off below the ring (C). The sharp outer edge of the narrow end of the tube is made smooth. A small square of dialysis membrane is applied to the narrow end of the tube and secured by means of the ring as illustrated. The sample to be concentrated is pipetted into the tube which is transferred to a 1 x 5 cm. tube containing a small quantity of wet polyethylene glycol. Within a few hours a sample volume of 0.1 ml. is concentrated tenfold. The concentrate may be removed with a fine pipette and applied directly to the well or origin of the system being used in the analysis. Alternatively, for some procedures it is convenient to leave the sample in the tube until all the water has dialysed into the polyethylene glycol. The high molecular weight material deposits as a uniform circular film on the membrane. After washing the outside of the device, to remove the polyethylene glycol, a small disc, with the deposit on it, is cut from the membrane with a sharp cork-borer. The disc may be applied, deposit side down, to an agar surface in an immunological system. In this way a large number of samples may be examined easily, and, as very little material is lost, a reasonable quantitative assessment can be made of a particular immunologically precipitating constituent. This

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Letter to the Editor

A CAUSE OF ERROR IN THE DETERMINATION OF THE HAEMATOCRIT USING THE SMA-4

Sir,

One of the advances in automation of haematology has been the introduction of the AutoAnalyzer SMA-4. From a single sample of blood this instrument determines four parameters, viz., the haematocrit, the haemoglobin, the red cell and the white cell counts. In conjunction with a data logger, the MCH, MCHC, and MCV may be calculated. In our experience the results of the haematocrit determinations have not always been reliable.

In the SMA-4 the electrical conductivity of a sample is taken as an index of its haematocrit. The conductivity is a function of the concentration of ions present. Therefore, an excess of ions supplied by the anticoagulant EDTA (dipotassium salt) could cause inaccuracy. While comparing the results of packed cell volumes done on the SMA-4 and by centrifugation in the microhaematocrit method, some discrepancies were apparent. These occurred when the anticoagulated bottles were not properly filled.

It has already been demonstrated by Pennock and Jones¹ that excess concentration of EDTA reduces the

packed cell volumes when determined by the microhaematocrit method. In order to investigate the effect of excess EDTA on the conductivity method for packed cell volumes, a series of experiments was set up. Blood from several normal individuals was added to eight anticoagulated tubes to give a final concentration ranging from 2 mg to 9 mg of EDTA per millilitre of blood. The results are set out in the table below:

Concentration of EDTA (mg/ml blood)	2	3	4	5	6	7	8	9
Mean % error of SMA-4 method	0	1	4	8	11	13	18	21
Mean % error of microhaematocrit method	0	0	0	1	1	1	2	2

In the microhaematocrit method the concentration of EDTA in this range does not alter the haematocrit reading significantly. This finding is in accordance with that of Pennock and Jones¹. When samples of blood with high concentration of EDTA are used, the haematocrit determination, carried out by a conductance method, is significantly altered.

To produce a final concentration of 2 mg/ml of blood as recommended by Dacie and Lewis², we add 10 mg of EDTA to each of our haematology sample bottles.

If less than 4 ml of blood is added to the sample bottle the resultant concentration of anticoagulant is such as to produce a significant error in the haematocrit reading. Therefore, if the SMA-4 is to be used for haematocrit determinations, it is important that the blood should be mixed with the anticoagulant in the correct proportions.

In the determination of the other parameters on the instrument, the concentration of anticoagulant is less important.

T. J. R. LAPPIN AND A. LAMONT
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REFERENCES

- ¹Pennock and Jones, *J. clin. Path.*, 1966, 19.
- ²Dacie, J. V., and Lewis, S. M. (1963). *Practical Haematology*, 3rd ed. p.4. Churchill, London.

CORRECTION

We print below the corrected Table III of the paper by J. McGeachie and W. McCormick 'Importance of potency in typing by colicine production' (*J. clin. Path.*, 20, 887-891).

TABLE III

PATTERN OF COLICINES AT THE RANGES OF TITRES NORMALLY PRODUCED

Colicine	Reciprocal of Titre	Indicators															
		2	56	17	2M	38	56 56	56 98	R1	R6	M19	2 7	2 64	2 15	R5	Row	Phi
A	4	--	--	--	--	--	--	--	--	--	--	--	--	--	+	+	
	256	+	+	1	--	--	+	+	+	+	+	+	+	+	+	+	
B	16	+	+	+	--	+	+	+	+	+	--	+	+	+	+	+	
	128	+	+	+	--	+	+	+	+	+	--	+	+	+	+	+	
D	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+	
	256	+	+	+	--	+	+	+	+	+	--	+	+	+	+	+	
K	1,000	+	--	+	--	+	--	--	+	--	--	1	+	+	+	+	
	8,000	+	--	+	--	+	--	--	+	--	--	1	+	+	+	+	
S4	Neat	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+	
	256	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+	
V	2	--	+	+	--	--	--	--	--	+	--	1	1	1	--	+	
	1,024	+	+	+	+	+	--	--	+	+	--	1	+	+	+	+	

+ = inhibition of indicator
— = no inhibition of indicator

¹inhibition at this level with Colindale subculture
*no inhibition at this level with Colindale subculture