Letters to the Editor

Alternative Solutions for the Operation of the Coulter Model S

The cost of operating the Coulter model S as reported by Barnard, Carter, Crossland-Taylor, and Stewart (1969) is approximately 5 (2½p) cents (Australian) per test for reagents purchased from the manufacturer. In countries where importation of these solutions is necessary the operating cost is approximately 9 cents (4½p) per test. This compares unfavourably with the cost of operating systems such as the semi-automated system described by Davis and Kelly (1969).

The Coulter model S requires two solutions for operation: a buffered sodium chloride solution and a lysing and haemoglobin conversion solution. The formulae for these solutions have not been published to date. The high cost of the commercial products has prompted formulation of alternative solutions. Excellent results have been obtained with the substitute buffered salt solution made up as follows:

Sodium chloride.. ..	8.3 g
Disodium hydrogen phosphate	2.0 g
Potassium dihydrogen phosphate	0.45 g
Potassium chloride	0.16 g
Sodium azide	1.0 g
Glucose	0.25 g
Water	to 1 litre

To facilitate preparation of large volumes of this solution, a dry mix is prepared. Solutions are made up in 25 litre plastic containers and are membrane filtered (millipore filter, pore size 0.45μ). Using the substitute buffered salt solution no variation in results was obtained when compared with the commercial product.

To be compatible with the Coulter S system, the lysing/haemoglobin conversion solution should have the following properties: it should convert haemoglobin to cyanmethaemoglobin within 25 seconds and it should selectively and rapidly lyse the erythrocytes and leave the leucocytes in stable suspension. The formula of a solution fulfilling these requirements is made up as follows:

Sodium chloride.. ..	8.3 g
Disodium hydrogen phosphate	2.0 g
Potassium dihydrogen phosphate	0.45 g
Potassium chloride	0.16 g
Potassium cyanide	0.5 g
Coco-alkyltrimethylammonium chloride (Arquad C-50%, Armour Hess Chemicals)	150.0 ml
Water	to 1 litre

The solution is prepared in bulk and membrane filtered. Because of the powerful lytic action of this reagent, it is necessary to retain a filter unit solely for this purpose. Relative instability of potassium cyanide in dilute solution gives the preparation a limited shelf life and it is best prepared fresh every three months. Blood diluted in the substitute lysing reagent gives an absorption curve similar to that obtained with Drabkin's solution. Conversion is virtually complete within the time limit set by the machine.

Although the leucocytes undergo considerable shrinkage in the substitute lysing solution, the total cell count does not vary appreciably over a period of at least thirty minutes.

Using the substitute solution the cost per test has been reduced from approximately 9 cents (4½p) to 0.5 cents (½p) per test.

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References

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Sites of Maximal Intestinal Absorptive Capacity for Amino Acids and Peptides: Evidence for an Independent Peptide Uptake System or Systems

In this letter we wish to make a brief report of an investigation of the sites of maximal absorptive capacity for amino acids and peptides, since the results throw additional light on the problem of intestinal peptide uptake and its role in protein absorption (Matthews, 1971).

Absorption of L-methionine (200 m mol/l) and the equivalent L-methionyl-L-

methionine (100 m mol/l) was measured by disappearance from the lumen of the loops of small intestine in anaesthetized rats over a period of 10 min (Matthews, Lis, Cheng, and Crampton, 1969). Six loops were made in each animal at regular intervals down the small intestine from a site close to the pylorus (loop 1) to one close to the ileocaecal valve (loop 6).

The results in the Table show that the sites of maximal absorptive capacity for the amino acid and the peptide are completely different. That for the amino acid is in the distal small intestine, whereas that for the peptide is in the proximal small intestine. In the jejunum (loop 2) absorption from the peptide is three times as great as from the equivalent amino acid, whereas in the distal ileum both peptide and amino acid are absorbed to approximately the same extent. A similar pattern has been found using a tryptic hydrolysate of casein (consisting mainly of small peptides) and a mixture of amino acids of equivalent amino acid content (Crampton, Lis, and Matthews, 1971).

Loop No.	Absorption of L-methionine (200 m mol/l) (μ mol methionine/cm/10 min)	Absorption of L-methionyl-L-methionine (100 m mol/l) (μ mol methionine/cm/10 min)
1	1.36 ± 0.40 ¹	2.98 ± 0.18
2	1.47 ± 0.11	4.37 ± 0.28
3	1.71 ± 0.25	4.32 ± 0.41
4	2.01 ± 0.19	3.85 ± 0.33
5	2.41 ± 0.30	3.52 ± 0.43
6	1.86 ± 0.10	2.19 ± 0.09

¹Mean ± SE n = 6

Table Absorption of L-methionine and L-methionyl-L-methionine from different sites in the small intestine of the rat

The difference in the sites of maximal absorptive capacity for peptides and amino acids provides additional support for the hypothesis, suggested by observations on Hartnup disease (Asatoor, Cheng, Edwards, Lant, Matthews, Milne, Navas, and Richards, 1970), that peptides and amino acids are absorbed by independent mechanisms. Furthermore, if mucosal uptake of peptides (rather than amino acids) plays the major part in protein absorption, as seems possible, the results may help to explain the previously puzzling observation that whereas protein is largely in the proximal small intestine