Buffer Capacity of Nutrient Media in Relation to that of Rumen Fluid

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In order to grow rumen bacteria under relatively defined conditions, it is often desirable to delete rumen fluid from the nutrient medium. This results in media with particularly low buffer capacity in comparison with that of rumen fluid itself, and it is suggested that, in view of the known influence of pH on growth of rumen bacteria (see, e.g., Hobson & Stewart, 1970), buffer capacity should be considered in the design of media for growth studies.

If buffer capacity is defined as mmol of HCl required to lower the pH of 100 ml of test solution from pH 6 to 4, approximate values obtained in a test were: (1) rumen fluid from (i) sheep on 100%-dried-grass diet, 9.1; (ii) sheep on 60%-concentrate + 40%-dried-grass diet, 10.2; (iii) deer on commercial ration, 8.0; (2) growth media (i) M2 of Mann (see Hobson, 1969) + 20% (v/v) of rumen fluid, 6.5; (ii) M2 without rumen fluid, 3.2. Other commonly used media showed a similar relationship.

When the buffer capacity of medium M2 (ii) was increased to a value comparable with that of rumen fluid by supplementation with either phosphate or bicarbonate, improved bacterial growth occurred with stimulation of both growth rate and yield of cells. Thus *Streptococcus bovis* grown on medium M2 (ii) with 1% (w/v) of glucose gave an increased dry-weight yield of 34% in phosphate-buffered media and of 38% in bicarbonate-buffered media. Other rumen bacteria (*Lactobacillus* sp. 17 and *Bacteroides ruminicola*) so far tested showed broadly similar responses, with gains in dry-weight yield of about 36% (*Lactobacillus*) and 32% (*Bacteroides*) in buffered media.

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Binding of Oestrogens to Human Erythrocytes and Haemoglobin

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Lefevre & Marshall (1959) showed that phloretin and various oestrogen analogues were concentrated by human erythrocytes. By using ^3H -labelled oestradiol and diethylstilboestrol, we have observed a similar concentration effect, and have separately measured the binding of oestrogen to the cytoplasmic

macromolecules by an ultracentrifuge technique. Diluted erythrocyte cytoplasm containing oestrogen was centrifuged at 300000g for 9 h at 20°C, and the oestrogen concentration in the supernatant was determined; controls showed this corresponded to the oestrogen concentration at equilibrium with that bound to cytoplasmic macromolecules. The amount of oestrogen bound to the cytoplasmic macromolecules varied as the square root of their concentration.

Since the major protein of erythrocyte cytoplasm is haemoglobin, comparative studies were continued with purified bovine oxyhaemoglobin. The amount of bound oestrogen was directly proportional to haemoglobin concentration below 10 μM -haemoglobin and proportional to [haemoglobin]^{1/2} above 10 μM . At low haemoglobin concentrations, in order to inhibit autoxidation, 1 mM-EDTA was added to the system (Kellett & Schachman, 1971).

A possible interpretation is that oestrogens bind to haemoglobin dimers but not to tetramers. With this model, the number of molecules of oestrogen bound to a dimer unit, together with their association constant, K_m , and the tetramer-dimer oxyhaemoglobin equilibrium constant, K_d , could be evaluated.

The amount of oestrogen bound to diluted cytoplasm was extrapolated to the true concentration of erythrocyte cytoplasm. With both diethylstilboestrol and oestradiol this value was found to be 30% of the total amount bound to the erythrocytes. Thus at least 70% of the oestrogen attached to the erythrocytes must be bound to the membrane. This is five times the value obtained by Lefevre & Marshall (1959) for phloretin, and suggests that significant quantities of oestrogens may be associated with erythrocyte membranes *in vivo*.

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Tertiary Structure in Pig Gastric Mucus

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The principle macromolecular component of water-soluble mucus from the cardiac region of pig stomach is a mucoprotein consisting of two components, A and B, which are chemically, biosynthetically and immunologically very similar (Snary & Allen, 1971). Components A and B have molecular weights of 1.9×10^6 and 1.1×10^5 respectively (Snary *et al.*, 1970).

With the unfractionated water-soluble mucus, the