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 100ml of test solution from pH6 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 ^{3}H-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 300000*g* for 9h 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 10μM-haemoglobin and proportional to [haemoglobin]$^{\frac{1}{4}}$ above 10μM. At low haemoglobin concentrations, in order to inhibit autoxidation, 1mM-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

viscosity of which can be accounted for in terms of components A and B, two transitions in viscosity were observed as KCl concentration was increased. Increasing the salt concentration to 50mM-KCl resulted in a 50% decrease in viscosity, attributed to the polyelectrolyte charge-shielding effect. The second transition, indicated by a 25% decrease in viscosity between 0.5M- and 1.5M-KCl, was examined further. Although there was no variation in the molecular weight, nor any interconversion of mucoprotein components A and B, there was a marked change in the hydrodynamic properties of the mucoprotein. The s^0 values of mucoprotein components A and B decrease from $s^0_{25,w}$ 18.7 and 4.9S to 16.1 and 3.9S respectively when the concentration of KCl was raised from 0.2M to 2.5M. Values of intrinsic viscosity, of K_s (Creeth & Knight, 1965) and of the frictional ratios show that the mucoproteins A and B contracted from expanded hydrated spheres in 0.2M-KCl to asymmetric compact equivalent hydrodynamic elipsoids in 2.5M-KCl. This transition is only partially reversible, and indicates the occurrence of definite structural changes within the mucoprotein.

The viscosity of component A was highly shear-dependent in 0.2M-KCl, this shear-dependence being almost absent in 2.5M-KCl. Further, at zero shear in 0.2M-KCl the intrinsic viscosity of component A showed an anomalously high increase in viscosity with concentration, reflecting the ability of the mucus to form a gel under physiological conditions. The absence of this anomalous increase in viscosity in 2.5M-KCl indicates that the expanded hydrated structure, as well as the highly polymerized form of the mucoprotein (component A), is necessary for gel formation. The concentration-dependence of shear suggests that gel formation does not involve strongly co-operative interactions (Rees, 1969).

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Mechanisms of Colicinogeny

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Nine colicin factors were examined in *Escherichia coli* K12 with respect to the effect on colicin synthesis of the hosts *recA* (recombination-deficiency) allele and the relative amount of each colicin that is free or cell-bound. The results can be summarized as follows: group 1 (colicin factors E1a-16, E1-K30, E2-P9 and K-235), $recA^+/recA$ ratio of bound colicin $\geqslant 16$ and bound/free colicin ratio in $recA^+$ host $\leqslant 1$; group 2 (colicin factors Ia-CT4, Ib-P9, V-K30 and V-K94), $recA^+/recA$ ratio of bound colicin $\leqslant 2$ and bound/free colicin ratio in $recA^+$ host >8; group 3 (colicin factor B-K98), $recA^+/recA$ ratio of bound colicin $\geqslant 16$ and bound/free colicin in $recA^+$ host >8.

Colicin synthesis may result from de-repression of the colicin operon in group 1, from conversion (i.e. constitutive synthesis) in group 2, and from conversion accompanied by a high rate of vegetative plasmid replication in group 3. A hypothetical group 4, with $recA^+/recA$ ratio of bound colicin $\leqslant 2$ and bound/free colicin ratio in $recA^+$ host, may be impossible.

Relationships between the Uptake of Uracil and Amino Acids by *Candida utilis*

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Candida utilis was grown at 30°C in a minimal medium with ammonia as the nitrogen source. When leucine was added to such a culture, there was no change in either the rate of protein synthesis, as measured by the incorporation of radioactive sulphate, or growth rate. However, when the medium also contained radioactive uracil, the rate of incorporation of this latter into RNA increased by about 50%. Chemical analyses showed that the addition of leucine had no effect on the protein and RNA contents of the cells. Instead, the contribution of exogenous uracil to the pyrimidine nucleotide pools increased from about 40 to 60% of the total, so that the RNA made after the amino acid was added was of greater specific radioactivity.

These findings suggested that leucine increases the rate of uptake of uracil by *C. utilis*. This was confirmed directly. The maximum velocity of uracil uptake was almost doubled in yeast grown with leucine present, whereas the Michaelis constant was unaltered. Experiments with cycloheximide showed that this apparent increase in the number of sites available for uracil uptake depended on protein synthesis. The kinetics of the appearance of the increased uptake of uracil, and also the decay of this activity when leucine was removed from cultures, support the interpretation that leucine acts through an inductive process.

These actions of leucine are restricted neither to this amino acid nor to the incorporation of uracil. Growth in the presence of leucine also stimulated the uptake of uridine, cytosine and adenine as well