(Fanburg & Gergely, 1965). However, Amytal, an inhibitor of mitochondrial function at the level of the flavoprotein at site 1, has also been reported to decrease Ca^{2+} uptake by sarcoplasmic reticulum (Muscatello, Andersson-Cedegren & Azzone, 1962; Briggs, Gertz & Hess, 1966). Similarly phenothiazines, antagonists of FAD-dependent enzymes, have been shown to abolish Ca^{2+} transport in sarcotubular vesicles (Azzone, Azzi, Rossi & Milic, 1966).

The sarcotubular calcium pump is stimulated by K^+ up to about 200 mM concentration (Duggan, 1968), and to observe any inhibition by barbiturates or phenothiazines K^+ must be included in the medium. In the absence of K^+ both barbiturates and phenothiazines markedly increase the Ca^{2+} uptake rate. Dichlorophenol-indophenol, a flavo-enzyme-oxidizing dye, also diminishes Ca^{2+} uptake in muscle microsomal vesicles, which inhibition can be overcome either by NADH or NADH plus FMN (Azzone *et al.* 1966). Whereas NADH and NADH plus FMN have only slight effects in the absence of dichlorophenol-indophenol, the combination of NADH plus FMN considerably lowers the high rate of Ca^{2+} uptake in 100 mM- K^+ . There thus appears to be a relationship between the ATP+ K^+ -dependent calcium pump and electron flow in the sarcoplasmic reticulum, which may be the rate of formation and utilization of a high-energy intermediate analogous to that postulated to exist in mitochondrial membranes undergoing oxidative phosphorylation.

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The Permeability of the Rumen to Simple Nitrogenous Compounds

By PHYLLIS MOONEY and D. J. O'DONOVAN.
 (Department of Physiology, University College, Galway, Irish Republic)

Most biological membranes are capable of transporting ammonia and urea. Ammonia can occur in the gaseous (NH_3) and ionized (NH_4^+) forms and the proportion of these two species will

be determined by the pH of the fluid. In the kidney it has been well documented that NH_3 diffuses across the luminal membrane of the tubular cell and that the rate of diffusion depends on the pH of the luminal fluid (Pitts, 1948). Urea is a much larger molecule than ammonia and occurs in the undissociated form. The pH of rumen fluid is often as high as 6.5–7.0. Owing to microbial degradation of dietary protein (McDonald, 1954) the ammonia concentration in the rumen increases rapidly after meals. There is a significant recycling of urea from blood to rumen (Houpt, 1959). Therefore the rate of transport of urea and ammonia from rumen to blood plays an important role in the nitrogen status of ruminating animals. We compared the relative rates of transport of urea and ammonia across the isolated rumen of mature sheep by means of the Ussing apparatus. A 5 ml sample of the solution under investigation and buffered saline were placed in the two chambers, which were separated by 7 cm² of the rumen wall. A 0.1 ml portion was taken from each chamber at intervals to determine the rates of transport and total recoveries of ammonia or urea. Ammonia was determined by a modification of the enzymic method described for blood ammonia (Reichelt, Kvamme & Tveit, 1964). Urea was determined in a similar manner after treatment with urease.

Ammonia transport in both directions across the rumen wall was very rapid. When the pH of the solutions in the two chambers was maintained at 7.0 ammonia reached equilibrium when the concentrations were equal on both sides of the membrane. When the pH was lowered on one side of the membrane equilibrium was reached when the ammonia concentration was greater on the side of low pH. This phenomenon occurred irrespective of the direction of the ammonia gradient initially. These findings support the hypothesis that ammonia transport across the rumen wall is passive and that the membrane is more permeable to NH_3 than to NH_4^+ .

In contrast the transport of urea was very slow in both directions across the rumen wall. When ATP was added to the mucosal side the transport of urea from the mucosal to the serosal surface was substantially increased. Addition of ATP supported the transport of urea against its concentration gradient. The introduction of deoxycholate to the mucosal membrane completely inhibited the effect of ATP on urea transport. Deoxycholate also abolished the rate of transport of urea in the absence of ATP, indicating that residual ATP may be responsible for this low rate of transport.

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Kinetic Mechanism of Cytidine Amino-hydrolase

By J. D. FINDLATER, M. SMYTH, N. V. MCFERRAN and B. A. ORSI. (*Department of Biochemistry, Trinity College, Dublin 2, Irish Republic*)

Previous reports from this laboratory have indicated cytidine aminohydrolase (EC 3.5.4.5) as a possible regulatory enzyme in nucleic acid metabolism (McFerran, Smyth & Orsi, 1969). Results presented in the present communication suggest cytidine aminohydrolase possesses many of the characteristics associated with other regulatory enzymes.

Gel-filtration studies on cytidine aminohydrolase with Sephadex G-200 over a range of pH values between 5.5 and 8.6 and a range of ionic strengths between 0.1 and 1.0 have shown no decrease in molecular weight at high pH and high ionic strength. However, under these conditions a marked increase in enzymic activity is observed. Temperature also plays a role in this activation: at 10°C 1.0M-NaCl has no effect on the enzyme's activity, but at 37°C a 3.6-fold activation over an NaCl-free control was observed.

A series of univalent-cation chlorides was tested for their effect on this activation. At a constant ionic strength of 1.0 the order of activation by metal ion was according to the sequence: $\text{Na}^+ > \text{Rb}^+ > \text{Cs}^+ > \text{K}^+ > \text{Li}^+ > \text{HN}_4^+$. This sequence could correlate with their hydrated ionic radii as determined by Padova (1964) by using electrostrictive techniques; however, gross variations in values for hydrated ionic radii may be found in the literature. At high pH KCl and NH_4Cl both exhibit a saturation effect in their activation with a cytidine concentration equal to its K_m ; however, with NaCl there is a linear relationship between the NaCl concentration and the activity in the range tested (<1.5M).

Product-inhibition studies (Cleland, 1963) were used in an attempt to identify the kinetic mechanism of the enzyme. Ammonia is not inhibitory and uridine, the other product, gave competitive inhibition. These results would be compatible with an apparent Uni Bi mechanism (Crypto Ping Pong) with the first substrate cytidine and the second water (which must be saturating to prevent ammonia acting as an inhibitor); the first product would be ammonia and the second uridine. This type of mechanism is common to many hydrolytic enzymes (Bender & Kézdy, 1965). The 'modified-

enzyme' intermediate in this mechanism would be a 'uridylyl-enzyme' complex, whose hydrolysis should not be the rate-limiting step in the reaction sequence.

Further studies have indicated that the reaction is not catalysed via a simple nucleophilic displacement.

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The Alcohol Dehydrogenase of *Clostridium acetobutylicum*

By W. M. FOGARTY and J. A. WARD (introduced by K. F. MCGEENEY). (*Department of Industrial Microbiology, University College, Dublin 4, Irish Republic*)

The principal neutral volatile metabolites of *Clostridium acetobutylicum* N.C.I.B. 8049 are butan-1-ol, acetone and ethanol. Formation of propan-1-ol and 2-methylpropan-1-ol has been induced by increasing the redox potential of culture media for a short period during the exponential phase of growth or by addition of certain amino acids (Ward & Fogarty, 1970). In the present study the alcohol dehydrogenase (EC 1.1.1.1) of *C. acetobutylicum* was investigated with respect to method of preparation and substrate specificity.

The bacterium was grown anaerobically in a glucose-peptone-salts broth at 37°C. The cells were harvested after 32h by centrifugation and were stored at -25°C until required. After being washed once, the cells were resuspended in 0.01M-phosphate buffer, pH 7.5, before disruption at 0°C. The following methods of cell disintegration and enzyme extraction were examined: ultrasonic sound waves in an MSE ultrasonic disintegrator, agitation with 0.1mm Ballotini glass beads in a Braun cell homogenizer and acetone-powdering followed by extraction with buffer. In addition, grinding with the following abrasives was investigated: glass powder, 1μm particles of aluminium oxide and aluminium sodium silicate. The cell homogenate was centrifuged at 20 000g for 30min at 0°C. The supernatant was retained for enzyme studies and for protein determination. Active enzyme preparations were obtained with each treatment. The alcohol dehydrogenase activity was particularly high in the cell-free extract prepared by grinding with aluminium sodium silicate.

The specificity of the alcohol dehydrogenase of