the kinetics of the 'two activities' are competitive rather than additive, indicating that only one enzyme system is involved. At a biliverdin concentration of 25 μM , NADPH is by far the more effective coenzyme, with a K_m value (about 5 μM) very much lower than that for NADH (above 500 μM). Higher concentrations of biliverdin (e.g. 60 μM) disturb the kinetics in a complex manner.

In the light of our results the discrepancies between the results of Singleton & Laster (1965) and Tenhunen *et al.* (1970) are readily explainable in terms of the different concentrations of coenzymes and biliverdin in their assay systems.

The specificity with respect to different biliverdins, previously undetermined, was also studied. The rates of reduction of mesobiliverdin IX α and deuterobiliverdin IX α were comparable with that of natural biliverdin (protobiliverdin IX α). The four isomers of protobiliverdin IX were prepared as described by Ó Carra & Collieran (1970). With either NADH or NADPH as coenzyme, the rate of reduction of both the IX β and IX δ isomers was less than 4% that of the IX α isomer, and the IX γ isomer was not reduced at all.

This degree of specificity for the 'natural' IX α isomer seems to rule out conclusively the early view of Lemberg & Wyndham (1936) that enzymic biliverdin reduction is a non-specific process. It also suggests that the extremely low proportion of the IX β and IX δ isomers, and the complete absence of the IX γ isomer, in biliary bilirubin (Ó Carra & Collieran, 1970) may reflect the specificity of biliverdin reduction as well as the specificity of haem breakdown (see Ó Carra & Collieran, 1969).

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Capsid Proteins of Two Bovine Enteroviruses

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A comparison has been made of the capsid proteins from two bovine enteroviruses VG/5/27 and VC/65/182.

The growth and purification of the viruses has been described (Martin, Johnston & Clements,

1970). Purification consists of differential centrifugation of the virus harvest to remove gross cellular contamination and to sediment a crude virus pellet.

Residual contamination was removed by gel filtration through Sepharose 2B and sedimentation through 15–45% (w/v) sucrose gradients. The virus obtained from the sucrose gradient was disrupted to yield the constituent polypeptides by incubation at 45°C for 1 h with 1% sodium dodecyl sulphate, 0.5 M-urea and 1% 2-mercaptoethanol.

The capsid proteins obtained were separated by polyacrylamide-gel electrophoresis on 12.5% acrylamide gels containing 0.1% of sodium dodecyl sulphate by the method of Dimmock & Watson (1969). The gels were stained with Coomassie Blue.

Enteroviruses VG/5/27 and VC/65/182 both gave similar gel patterns showing three major bands VP1, VP2 and VP3 and one fast-moving minor band VP4.

Estimates of the molecular weights of the virus proteins have been made by the use of sodium dodecyl sulphate-containing polyacrylamide gels calibrated with standard-molecular-weight proteins (Shapiro, Viñuela & Maizel, 1967). The proteins from both viruses have been found to be indistinguishable on the basis of molecular weights and these are: VP1, 34000; VP2, 28000; VP3, 26000; VP4, 9000.

From an estimate of the molar ratios of each protein it is possible to determine the number of polypeptide chains in each structural subunit of the virus coat.

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Electron Transport and Uptake of Calcium Ions by Sarcoplasmic Reticulum

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Muscle mitochondria accumulate large amounts of Ca^{2+} when the energy source is either oxidizable substrate or ATP (Brierley, Murer & Bachmann, 1964). This transport system is inhibited by azide, dinitrophenol and oligomycin. On the other hand the uptake of Ca^{2+} by sarcoplasmic reticulum, the physiological relaxing event, is driven only by ATP and is not affected by the mitochondrial inhibitors

(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

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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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