of mitochondrial oxidative phosphorylation by bongkrekic acid was measured. Again, the presence of CoA considerably shortened the time required for bongkrekic acid to act.

A possible explanation for these observations is that bongkrekic acid must first be converted into a CoA derivative before it can inhibit adenine nucleotide translocation. Further work is aimed towards the isolation of such a derivative and identification of an enzyme (probably an acyl-CoA synthetase) responsible for its formation,

We thank Mrs. Cheryll Crosby for her excellent technical assistance.

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Ureolysis in the Ovine Rumen

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The rumen contents of sheep and cattle on all diets possess ureolytic activity. Although this activity was shown to be of bacterial intracellular origin, so confirming earlier work (Gibbons & McCarthy, 1957), only a few ureolytic bacteria have so far been isolated and positively identified (Gibbons & Doetsch, 1959). In the present work only ten ureolytic strains out of over 1000 bacteria were isolated from a sheep fed on hay and dried grass.

The bacteria were isolated by two methods. In the first, 15 different media varying from the non-selective counting media to several selective media were used in the standard roll-tube method. The media should provide growth conditions for all types of rumen bacteria, but the only ureolytic bacteria isolated were five strains of *Staphylococcus* (group II), two strains of group VI and a strain of *Lactobacillus casei* var. *casei*. These were isolated from the 10^6-dilution cultures.

In the second method, media that enriched the ureolytic bacteria of undiluted rumen fluid were used to enrich serial dilutions of rumen fluid, and *Klebsiella aerogenes* and a *Streptococcus* sp. (intermediate in properties between *Streptococcus faecium* and *Streptococcus faecalis*) were isolated from dilutions 10^5 and 10^7 respectively.

No ureolytic strains of obligate anaerobes were isolated from the rumen, and the results of this survey suggest that ureolytic activity is associated with bacteria present in comparatively small numbers and not with those bacteria present in high numbers and considered important in rumen function.

The *Streptococcus* sp. was ureolytic only under anaerobic conditions, and investigations of factors controlling the level of the activity per unit growth show that the activity would account for most of the ureolytic activity of the sheep rumen contents.

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Factors Affecting the Inhibition of Mitochondrial Adenosine Triphosphatase by Trialkyltin Compounds

By M. J. Selwyn, S. J. Dunnett, R. D. Philo and A. P. Dawson (*School of Biological Sciences, University of East Anglia, Norwich NOR 88C, U.K.*)

The 2,4-dinitrophenol-stimulated ATPase* of rat liver mitochondria is potently inhibited by trialkyltin compounds (Aldridge, 1958; Aldridge & Street, 1971). However, unless Mg^{2+} ions are present, at concentrations greater than or equal to the concentration of ATP, the apparent K_i for inhibition by trimethyltin is very much increased.

At pH6.8 the effectiveness of trimethyltin as an inhibitor of the 2,4-dinitrophenol-stimulated ATPase of whole mitochondria is diminished by the presence of 10mM-P_i. In whole mitochondria the situation is complicated by intramitochondrial pH changes resulting from the Cl^-–OH^- exchange mediated by trialkyltins (Selwyn *et al.*, 1970). At pH6.8 the effectiveness of trimethyltin as an inhibitor of the Mg^{2+}-stimulated ATPase of fragmented mitochondria is also diminished by the presence of P_i, but at pH7.5 the effect of P_i is much less. Rose (1969) has reported that trialkyltins are complexed by P_i and that the affinity decreases above pH7. In the absence of P_i the K_i for inhibition of the ATPase of broken mitochondria by trimethyltin is about one-quarter of the dissociation constant measured directly by Aldridge & Street (1970). However, Aldridge & Street (1970) measured the binding at pH6.8 in the presence of 15mM-P_i. When allowance is made for the effects of P_i there is little discrepancy between the two estimates of the dissociation constant.

The failure of atractyloside to inhibit the ATPase of broken mitochondria supports the view (Stockdale

*Abbreviation: ATPase, adenosine triphosphatase.

et al., 1970) that atractyloside and trialkyltins act at different sites. The ATPase of broken mitochondria is inhibited by oligomycin, unlike the solubilized ATPase (Selwyn & Chappell, 1962). The soluble ATPase from acetone-dried ox heart mitochondria (Selwyn, 1967) is not inhibited by trimethyltin at concentrations up to 250μM. Kagawa & Racker (1966) have shown that the soluble ATPase is inhibited by tributyltin only in the presence of the factor that confers oligomycin-sensitivity on the ATPase. These observations leave little doubt that the site of action of trialkyltins is the same as that of oligomycin.

We thank the Science Research Council for financial support; R. D. P. acknowledges the award of a Science Research Council Studentship.

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Inhibitory Actions of Trialkyltin Compounds on Mitochondrial Respiration

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In rat liver mitochondria, trialkyltin compounds inhibit ADP-stimulated respiration at pH7.5 and 6.8 (Aldridge, 1958; Sone & Hagihara, 1964; Stockdale *et al.*, 1970; Aldridge & Street, 1971), but inhibition of 2,4-dinitrophenol-stimulated respiration is significant only at pH6.8 (Coleman & Palmer, 1971). Investigation of the pH–activity curves for the dinitrophenol-stimulated oxidation of succinate by mitochondria reveals that the activity is shifted to a more alkaline region by trialkyltin compounds. The pH–activity curve for mitochondria broken by freeze-thawing is not affected by dinitrophenol or by trialkyltin compounds, but closely resembles the pH–activity curve for intact mitochondria in the presence of dinitrophenol and trialkyltin compounds. The inhibition of respiration by trialkyltins on intact mitochondria at pH6.8 in the presence of dinitrophenol is well correlated with their ability to catalyse the Cl^-–OH^- exchange. This exchange has been shown to allow rapid pH equilibration across the mitochondrial membrane (Selwyn *et al.*, 1970), in contrast with uncoupling agents, which mediate proton uniport (Mitchell & Moyle, 1967). These observations indicate that the respiratory inhibition caused by trialkyltin compounds at pH6.8 is a result of the equilibration of pH across the mitochondrial membrane changing the pH of the environment of respiratory enzymes to a pH where they have low activity. The absence of any inhibition on broken mitochondria is not in accord with the suggestion (Coleman & Palmer, 1971) of a direct inhibitory action on the respiratory chain, and Aldridge & Street (1971) have produced evidence that, at high substrate concentrations, depletion of intramitochondrial substrate is not an important factor in the inhibition.

In the presence of 10mM-P_i, trialkyltins show a decreased effectiveness as inhibitors of phosphorylation as the pH is lowered from 7.5 to 6.8 (Coleman & Palmer, 1971). At pH6.8, 10mM-P_i greatly diminishes the effectiveness of trialkyltins as inhibitors of dinitrophenol-stimulated respiration. In a sucrose medium containing 1.0mM-P_i, trialkyltin compounds are equally as effective as inhibitors of phosphorylation at pH6.8 and 7.6. The pH-dependence observed by Coleman & Palmer (1971) may be due to the binding of trialkyltins to P_i, which is in agreement with the observations made by Rose (1969) and Selwyn *et al.* (1972).

We thank the Science Research Council for financial support.

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