with glycine-HCl or sodium iodide solution consisted of 50–90% of precipitable antibody; the quantity of precipitable antibody eluted depended mainly on the ratio of antibody to immunosorbent used. By adsorption on insoluble human immunoglobulin G, all proteins eluted with solutions of sodium iodide were shown to be antibody. By immunoelectrophoresis only γ -globulins were detected in the purified antibody preparations. Electrophoresis in polyacrylamide-agarose gel gave comparable results.

When antibody preparations eluted with glycine-HCl buffer or solutions of sodium iodide were stored at 4° for 2 months, it was found that the glycine-HCl-eluted antibody contained two additional precipitation lines by immunoelectrophoresis and six additional bands in polyacrylamide-agarose gel electrophoresis. With antibody eluted by 5.5M-sodium iodide, degradation was less pronounced, since only one additional precipitation line was found by immunoelectrophoresis and four additional bands by polyacrylamide-agarose electrophoresis. With antibody eluted by 3.5M-sodium iodide only a change in electrophoretic mobility was observed, whereas with antibody eluted with 2M-sodium iodide no observable change was detected; the latter antibody retained fully its capacity to react with antigen.

Treatment of human serum, containing either anti-A or anti-B antibodies, for 1 hr. with 5M-sodium iodide destroyed its isoagglutinating activity. However, no change in the isoagglutinin titre of human serum was noted when it was treated with 2M-sodium iodide for as long as 24 hr.

The purpose of this study was not to find out whether iodide salts dissociate the antigen and antibody of immune precipitates or whether they simply dissolve the complex without separating antigen from antibody. However, although in the

present experiments with immune precipitates it was not possible to discriminate between solubilization and dissociation, the experiments with immunosorbents demonstrated that, in this case, dissociation had occurred.

Antibodies isolated from immunosorbents with 2M-sodium iodide are considerably more stable than those eluted with acid. In addition, antibodies isolated with 2M-sodium iodide do not adsorb non-specifically to tissue sections as is the case with acid-eluted antibodies. It is also of importance that fragile antibodies such as isoagglutinins are not noticeably affected by a 2M solution of iodide salts.

It would thus appear that iodide salts can be used to dissolve protein-antibody complexes and to isolate antigen or antibody from immunosorbents with a reduced risk of denaturation.

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Activation of Short-Chain Fatty Acids by Sheep-Liver Mitochondria

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Four enzymes capable of activating some short-chain fatty acids have been described in ruminant tissues, and each of them shows some breadth of substrate specificity. Thus the acetate-CoA ligase

(AMP) (EC 6.2.1.1) isolated from ox-heart particles by Hele (1954) activated propionate at about the same rate as acetate, whereas a butyrate-CoA ligase obtained from ox-heart mitochondria by

Webster, Gerowin & Rakita (1965) activated straight-chain fatty acids of chain length C_3 – C_7 with maximum activity at C_4 . Two activating enzymes have been described in ox liver that appear to be responsible for activation of intermediate-chain-length fatty acids. Mahler, Wakil & Bock (1953) purified an acid-CoA ligase (AMP) (EC 6.2.1.2) from ox-liver particles that activated straight-chain fatty acids from C_4 to C_{12} , showing maximum activity at C_7 , and Rossi & Gibson (1964) reported the presence of an acid-CoA ligase (GDP) in ox-liver mitochondria that activated octanoate at about twice the rate of butyrate when GTP was the energy source.

Sheep liver appears to be the chief site of metabolism of propionate absorbed into the portal blood from the rumen (Reid, 1950), and studies with sheep-liver slices (Leng & Annison, 1963) have shown that propionate is metabolized at higher rates than either acetate or butyrate. Sheep-liver mitochondria readily metabolize propionate (Smith & Osborne-White, 1965) and the rate is stimulated 50% or more by low concentrations of glutamate or α -oxoglutarate (Smith, Osborne-White & Russell, 1965a,b). The possibility was therefore envisaged that propionate activation might be specific for GTP or ITP, and that this might arise from the substrate-level phosphorylation accompanying the oxidation of α -oxoglutarate. Such an explanation was proposed by Rossi & Gibson (1964) for the stimulation of octanoate oxidation by α -oxoglutarate in rat-liver mitochondria. The present work was undertaken to examine the relative rates of activation of acetate, propionate and butyrate by enzymes in sheep-liver mitochondria and to gain some information about the nucleoside triphosphate specificity.

CoA and nucleoside triphosphates were obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A. Twice-washed mitochondria were prepared from the livers of freshly killed Australian Merino ewes as described by Smith *et al.* (1965a). They were finally suspended in 0.25M-sucrose to a concentration of 1.0–1.5mg. of N/ml. and frozen in 2ml. lots in polystyrene vials at -15° . For estimation of activating enzyme mitochondrial preparations were thawed after adding 10ml. of ice-cold water, by shaking by hand. Activity was determined by the substrate-dependent disappearance of CoA thiol in the presence of excess of nucleoside triphosphate (Mahler *et al.* 1953). The composition of the reaction medium is given in the legend to Table 1. Unchanged CoA thiol was maintained in the reduced state by passing oxygen-low nitrogen through each reaction tube at 200ml./min. both during and after incubation (Meites & Meites, 1948). Incubations (final vol. 0.3ml.) were stopped by addition of 1.7ml. of 2% (w/v) metaphosphoric acid in saturated sodium chloride solution, and residual thiol was estimated by the method of Grunert & Phillips (1951). Uptakes were calculated from control tubes incubated without substrate but with the appropriate nucleoside triphosphate. For comparison of nucleoside triphosphate specificity, activation of succinate by succinate-CoA ligase (GDP) (EC 6.2.1.4) was also measured. Nitrogen in mitochondrial preparations was determined by the Kjeldahl procedure of McKenzie & Wallace (1954).

With propionate as substrate and ATP as energy source, the rate with whole mitochondria was less than 20% of that with frozen-and-thawed mitochondria. When fresh mitochondria were exposed to ultrasonic energy [Dawe Soniprobe type 1130A (Dawe Instruments Ltd., London, W.3), 60w

Table 1. *Rates of activation of short-chain fatty acids and succinate by frozen-and-thawed sheep-liver mitochondria*

Frozen-and-thawed mitochondria were incubated in duplicate for 15 min. at 37° with nucleoside triphosphate, CoA and one of the acids shown. Residual thiol was measured in entire tube contents and net consumption determined from duplicate control tubes incubated without the acid. Incubations were performed in 0.3ml. volumes with the following final composition: potassium glycyglycine buffer, pH 8 (66.7mm), $MgCl_2$ (5.0mm), KCl (85mm), CoA (1.67mm), ATP, GTP or ITP (3.33mm), potassium acetate, propionate, butyrate or succinate (16.7mm), sucrose (14mm) and frozen-and-thawed mitochondria (17–24 μ g. of N). Final pH was 7.6 and a gas atmosphere of oxygen-low nitrogen was used. Mitochondrial preparations (1.0–1.5mg. of N/ml.) in 0.25M-sucrose were frozen (-15°) for 3–109 days (mean 55 days) before thawing with 5 vol. of ice-cold water and immediate estimation of enzyme activity. Results are given as means $\pm$ s.e.m. of the numbers of experiments shown in parentheses.

Nucleoside triphosphate	CoA thiol fixed (μ moles/mg. of mitochondrial N/hr.)			
	Acetate	Propionate	Butyrate	Succinate
ATP	17.3 $\pm$ 3.1 (6)	48.9 $\pm$ 2.9 (6)	36.0 $\pm$ 3.7 (6)	12.3 $\pm$ 2.9 (5)
GTP	11.7 $\pm$ 3.1 (6)	8.0 $\pm$ 3.0 (6)	10.1 $\pm$ 3.4 (6)	37.8 $\pm$ 2.9 (6)
ITP	7.9 $\pm$ 3.5 (6)	8.6 $\pm$ 3.5 (6)	8.6 $\pm$ 3.3 (6)	59.6 $\pm$ 5.3 (4)

capacity, 20keyc./sec. for 1 min. at 0°] 80%–90% of this activity was released, but further treatment of frozen-and-thawed mitochondria with ultrasonic energy (1 min. at 0°) led to loss of up to 25% of the activity. To obtain maximum activity it was necessary to leave the mitochondria frozen for about 2 days, but thereafter no change in activity was detected in samples left frozen for 4 months. A second cycle of freezing and thawing (without further dilution) was accompanied by loss of 50–80% of the activity. Increasing the pH of incubation from pH 6.7 to pH 7.4 caused measured activity to increase by about 20%. Between pH 7.4 and pH 8.3 a further approximately linear increase of 9% occurred. No activity was obtained with boiled (2 min.) preparations, or when GSH replaced CoA.

The relative rates of activation of acetate, propionate, butyrate and succinate (all 16.7 mM) by ATP, GTP and ITP with frozen-and-thawed mitochondria are shown in Table 1. Of the short-chain fatty acids, propionate was activated by ATP at the highest rate, followed by butyrate and acetate. GTP and ITP were less effective than ATP and led to similar rates with all three acids. This observation suggests a common rate-limiting step in the activation by GTP or ITP. It is consistent with activation being specific for ATP, the effects of GTP and ITP being due to synthesis of ATP by transphosphorylation of catalytic concentrations of adenosine phosphates present in the mitochondrial preparations. A nucleoside triphosphate-AMP phosphotransferase of suitable specificity has been described in calf liver by Heppel, Strominger & Maxwell (1959). The ADP formed in this way could then have given rise to ATP by the action of adenylate kinase (EC 2.7.4.3) known to be present in mammalian mitochondria (Siekevitz & Potter, 1953). The rates observed with GTP and ITP could then have been limited either by the activity of one of these enzymes or by the concentration of endogenous adenosine phosphates.

The results with succinate show the reverse effect, GTP or ITP resulting in higher rates than ATP. Succinate-CoA ligase from mammalian sources is believed to be specific for GTP or ITP (Sanadi, Gibson, Ayengar & Jacob, 1956; Hager, 1962). The activation of succinate observed with ATP may have been due to activation by endogenous inosine phosphates or guanosine phosphates after transphosphorylation by ATP-nucleoside

diphosphate phosphotransferase (EC 2.7.4.6).

With ATP as energy source the rate of activation of propionate was almost equal to the sum of the rates with acetate and butyrate. This suggests the presence in sheep-liver mitochondria of a CoA ligase relatively specific for propionate, rather than the sum of incidental propionate-CoA-ligase activity from both acetate-CoA ligase and butyrate-CoA ligase. Such a conclusion, however, must await the isolation of the enzyme.

The relative rates of activation of the three acids are consistent with their relative rates of oxidation both by sheep-liver mitochondria (Gallagher & Buttery, 1959) and by sheep-liver slices (Leng & Annison, 1963). The rate of metabolism of propionate by fresh sheep-liver mitochondria in the presence of 1.67 mM- α -oxoglutarate is 17.9 ± 0.9 μ moles/mg. of N/hr. (mean of six experiments) (R. M. Smith & G. R. Russell, unpublished work). The rates obtained in Table 1 show that, though the rate of activation of propionate with ATP is adequate to explain these results, those with GTP or ITP are not.

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