the assay system of shaking cultures aerobically for 2 days, and extracting the steroid substrate from the broth. Activity was demonstrated with five out of 12 strains of lecithinase-negative clostridia and relatively weakly with five out of 11 strains of lecithinase-positive clostridia but not with nine strains of enterobacteria, nine strains of enterococci or 16 strains of the non-sporing strictly anaerobic bacteria.

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The Glyceraldehyde 3-Phosphate Dehydrogenase of *Azotobacter beijerinckii* and its Possible Significance in Poly- β -hydroxybutyrate Biosynthesis

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Azotobacter beijerinckii can accumulate the storage polymer poly- β -hydroxybutyrate up to 70% of its dry weight when grown on glucose as the sole source of carbon and energy in the absence of a fixed nitrogen source. The enzymes β -ketothiolase, acetoacetyl-CoA reductase and polyhydroxybutyrate polymerase effect the synthesis of one hydroxybutyrate monomer from 2 molecules of acetyl-CoA, with the concomitant release of 1 CoA molecule and the reoxidation of 1 molecule of either NADPH or NADH. An acyl-carrier protein is not involved in polyhydroxybutyrate biosynthesis (Ritchie & Dawes, 1969). The reductive step is catalysed by acetoacetyl-CoA reductase, which reacts with NADPH at five times the rate with NADH (Ritchie, 1968). Our interest in the glyceraldehyde 3-phosphate dehydrogenase of the organism stems from a survey of reactions generating the large amounts of NADPH necessary for the massive synthesis of polyhydroxybutyrate which occurs when the organism is fixing nitrogen, a process that itself makes considerable demands on the production of reducing equivalents.

A glyceraldehyde 3-phosphate dehydrogenase has been isolated and partially purified by ammonium sulphate fractionation and ion-exchange cellulose chromatography. The enzyme reacts preferentially with NADPH ($V_{\max}$ 63.1 and 11.1 nmol/min with NADPH and NADH respectively) and thus resembles that more commonly encountered in plants,

which, however, operates in the direction of reduction. Johnson & Johnson (1961) recorded a similar coenzyme preference by the enzyme from *Azotobacter agilis*. The *A. beijerinckii* enzyme requires a thiol group for activity, as indicated by the inhibitory action of *p*-chloromercuribenzoate, *N*-ethylmaleimide, iodoacetate and mersalyl. The pH optimum is 7.5 and with phosphate concentrations higher than 100 mM there is an inhibitory effect at pH values above 6.5. The equilibrium of the reaction at pH 7.5 and 30°C greatly favours oxidation (K_{eq} approx. 10^7) and we believe that this reaction may play an important role in furnishing reducing equivalents for polyhydroxybutyrate biosynthesis.

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Conversion of Pyruvate into Citrate by Mitochondria Isolated from Rat Epididymal Fat-Cells

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From a previous study of the total activities and location of enzymes in isolated fat-cells and the permeability properties of intact fat-cell mitochondria (Martin & Denton, 1970) we have concluded that acetyl units for cytoplasmic fatty acid synthesis are transferred from mitochondria as citrate (as postulated by other workers largely on the basis of the parallelism between ATP citrate lyase activity and fatty acid synthesis rates), that transfer of citrate requires malate, presumably for counter-transport, and that the intramitochondrial metabolism of citrate and malate is restricted to allow transfer of acetyl units to the cytoplasm and to limit the loss of cytoplasmic reducing power into mitochondria.

Support for these conclusions has come from an investigation of the metabolism of pyruvate by isolated fat-cell mitochondria. The mitochondria were incubated with pyruvate, ADP and P_i and the effects of additions of malate, hydroxymalate (a non-oxidized malate analogue) and bicarbonate on the time-courses of pyruvate uptake, citrate output, malate uptake (or output) and oxygen consumption were determined. On addition of bicarbonate alone there was an appreciable lag in citrate production presumably because in the absence of

added malate (or hydroxymalonate) citrate transport was inhibited until malate formed from pyruvate had reached sufficient concentration outside the mitochondria. On addition of bicarbonate plus malate or hydroxymalonate production of citrate was linear and accounted for 70% or more of the pyruvate uptake whereas metabolism of citrate through the tricarboxylate cycle was negligible; under these conditions oxaloacetate for citrate synthesis was largely formed from pyruvate through pyruvate carboxylase. In the absence of bicarbonate malate uptake was markedly increased and was sufficient to account for the oxaloacetate required for citrate synthesis. In fat-cells under physiological conditions (in the presence of bicarbonate), oxaloacetate for citrate synthesis would thus be expected to be formed largely from pyruvate through pyruvate carboxylase rather than from malate through malate dehydrogenase.

In parallel experiments on liver mitochondria prepared from fed rats, rates of citrate production were much smaller (in terms of mitochondrial protein), and in the presence of bicarbonate malate rather than pyruvate was the main source of oxaloacetate for citrate synthesis.

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Kinetics of 5-Hydroxytryptamine Exchange in Rabbit Blood Platelets and its Analysis by Digital Computer

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Digital-computer simulation of the effects of reserpine on the rate of 5-hydroxytryptamine uptake and distribution in rabbit blood platelets has been performed as part of a programme to assess the value of the technique in biochemical pharmacology.

Platelet-rich plasma was incubated either alone or with reserpine (50 µg/ml) for 20 min. Tritium-labelled 5-hydroxytryptamine was added, raising the plasma 5-hydroxytryptamine concentration to 4 µg/ml, and incubation was continued for additional periods up to 40 min. The platelets were spun down immediately at 0°C, washed and treated ultrasonically in 0.25M-sucrose. Soluble, mitochondrial and 5-hydroxytryptamine-storage-granule fractions were isolated from the ultrasonically treated material by centrifugation on sucrose density gradients. The specific radioactivity of the 5-hydroxytryptamine in each subcellular compartment relative to plasma at zero time was deter-

mined. Volumes of the compartments were found from the distribution of K⁺ after storage of platelets at 0°C for 1 h and were approx. 0.2 ml/mg of platelet protein for soluble material (cytoplasmic compartment) and 0.05 ml/mg of protein for storage granules.

The concentrations of 5-hydroxytryptamine in the soluble and granule compartments were 8 and 80–100 µg/ml respectively, remaining almost constant for the duration of the experiments. The exchange of 5-hydroxytryptamine between platelet contents and plasma proceeded rapidly; in the absence of reserpine the soluble fraction had a relative specific radioactivity of 0.11 at 10 min and 0.35 at 40 min and the granular material 0.08 and 0.22 at these times. Reserpine decreased these values to 0.09, 0.26 and 0.05, 0.17 respectively.

The complete kinetics of 5-hydroxytryptamine uptake and exchange were analysed on an ICL 1905 computer, with a program written by myself. The program combined in a novel way principles of simulation and curve-fitting described by Garfinkel (1968), Kibby (1969) and Powell (1965).

Models describing the transport of 5-hydroxytryptamine within the platelet were tested and increased in complexity, starting from the very simplest possible system, until an acceptable fit between the observations and predicted values was obtained. The final model contained zero-order and first-order transport mechanisms at both the platelet and granule membranes, and had four parameters which were estimated by the computer program. Reserpine was found to decrease the diffusion constant across the platelet membrane from 0.0045 ± 0.0017 to $0.0016 \pm 0.0006 \text{ min}^{-1}$ (mg of protein)⁻¹, and to decrease the zero-order constant for uptake into the platelet granule from 1.1 ± 0.7 to $0.7 \pm 0.2 \text{ µg min}^{-1}$ (mg of protein)⁻¹. (Results are given as value $\pm$ S.E.M.)

These quantitative results agree with qualitative observations on the reserpine-induced depletion of biogenic-amine stores in platelets (Pletscher, Da Prada & Tranzer, 1968). They also appear to be the first numerical expression of the effect of reserpine on amine kinetics of the intact cell. The simulation technique leads to conclusions not hitherto obtainable without much greater experimentation, and by its flexibility should have a number of applications.

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