dependent fashion, in addition to forming hydrazones with the aldehyde substrates. Iproniazid (*N*-isonicotinoyl-*N*-isopropylhydrazine) is not inhibitory.

Ethanol in concentrations up to 500mM is not inhibitory towards pig brain aldehyde dehydrogenase, although the thiol reagents iodoacetic acid and iodoacetamide are inhibitory.

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Subcellular Distribution of Rat Liver L-Alanine-Glyoxylate Aminotransferase

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Rat liver was homogenized in 0.4M-sucrose at 0–5°C. Centrifugation at 7.5×10^6 g-min gave supernatants with 88–97% of lactate dehydrogenase (values are percentages of activity in original homogenate), <5% of glutamate dehydrogenase, glucose 6-phosphatase, uricase or acid phosphatase and 19–25% of L-alanine-glyoxylate aminotransferase (assayed as described by Rowsell, Snell, Carnie & Al-Tai, 1969). Of the particle-marker enzymes (as indicated above) more than 95% were recovered in the sedimented particles with 75–82% of the aminotransferase.

For subfractionation of the particles differential centrifugation procedures were then applied, based on methods used by Appelmans, Wattiaux & de Duve (1955). Preparations of nuclei contained 80–90% of the DNA, 8–10% of the glutamate dehydrogenase or acid phosphatase, 5% of the uricase or glucose 6-phosphatase, 4% of the lactate dehydrogenase and 10% of the aminotransferase. Microsomal preparations contained 48% of the glucose 6-phosphatase, 56% of the arylsulphatase C, 11% of the lactate dehydrogenase, no measurable glutamate dehydrogenase and 7% of the aminotransferase. Crude mitochondrial preparations contained 90% of the glutamate dehydrogenase, 50% of the acid phosphatase, 40% of the uricase, 10% of the lactate dehydrogenase and 75% of the

aminotransferase. It was concluded that one could narrow the field for investigation to mitochondria, lysosomes and peroxisomes.

Lysosomes were prepared by flotation after intraperitoneal injection of rats with Triton WR1339 (Leighton *et al.* 1968). Of the acid phosphatase, 26–30% was recovered with <3% of the glutamate dehydrogenase, uricase or aminotransferase.

For application to a density gradient a particle fraction was prepared by differential centrifugation, designed to discard nuclei and microsomes. In a representative experiment percentage recoveries of aminotransferase, glutamate dehydrogenase, rhodanese, lactate dehydrogenase, acid phosphatase and uricase were respectively 70, 65, 68, 6, 70 and 82. A resuspension of these particles in 48% (w/v) sucrose (density 1.18g/ml) was layered over a discontinuous sucrose gradient (1.18–1.25g/ml) and after centrifugation at 2.9×10^6 g-min tube contents were recovered as four separate density-range fractions. In the topmost fraction percentage recoveries (compared with activities in the layered particles) for aminotransferase, rhodanese, uricase and acid phosphatase were respectively 73, 75, 12 and 32; corresponding values for the bottom fraction were 2, 2, 42 and 15. Altogether the profile for the aminotransferase closely matched that of rhodanese but clearly differed from those observed for uricase or acid phosphatase.

We conclude that a large part, at least, of the particulate alanine-glyoxylate aminotransferase is mitochondrial.

The aminotransferases in the cytosol and in solubilized particles are different; K_m values for glyoxylate are respectively 1.2 and 0.24 mM and pH-activity curves showed marked differences.

Triton WR1339 was a gift from Hilton Davis Chemicals Ltd., Newcastle upon Tyne, U.K.

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A Prostaglandin Isomerase in Cat Plasma

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Prostaglandin A_1 [15-hydroxy-9-oxoprost-10,13-(*trans*)-dienoic acid] is a natriuretic agent in the dog

(Herzog, Johnston & Lauler, 1967) and an arteriolar dilator in the cat and dog (Horton & Jones, 1969) at blood concentrations of 0.1–10 ng/ml. During passage through the pulmonary circulation of the cat or dog substantial loss of the biological activity of the prostaglandins E but not the prostaglandins A occurs, and consequently the possibility that the prostaglandins A may act as circulating hormones has been suggested (Horton & Jones, 1969; McGiff *et al.* 1969).

The metabolism of prostaglandin A₁ by cat blood plasma has been investigated. Prostaglandin A₁ was incubated with cat plasma at 37°C for 1 h and the plasma extracted for polar fatty acids. Examination by u.v. spectrophotometry of fractions from silicic acid chromatography revealed a compound with the same elution volume as prostaglandin A₁ and with an extinction maximum at 278 nm. This compound was separated from any residual prostaglandin A₁ by g.l.c. and identified by mass-spectrometric analysis as prostaglandin B₁ [15-hydroxy-9-oxoprost-8(12),13(*trans*)-dienoic acid], a structural isomer of prostaglandin A₁.

Isomerization of prostaglandin A₁ to B₁ is known to occur rapidly under mild alkaline conditions (Bergström, Ryhage, Samuelsson & Sjövall, 1963). However, prostaglandin B₁ was not formed when prostaglandin A₁ was incubated with Krebs-Henseleit solution at pH 7.4. Further, the isomerization system present in the plasma was destroyed by heating at 70°C but not at 55°C for 20 min. These experiments suggested that the conversion was enzymic.

To obtain a purified preparation, cat plasma was subjected to ammonium sulphate precipitation and the isomerase activity of the dialysis residues was estimated by measuring the rate of increase of extinction at 283 nm after the addition of prostaglandin A₁. Some 80% of the isomerase activity and 30% of the protein content of the plasma was found in the 50–75% saturation fraction. Ion-exchange chromatography of this fraction on DEAE-Sephadex A-50 resulted in a further fivefold purification. The isomerase has an optimum pH in the region of 8.5.

The isomerization of prostaglandin A₁ to B₁ results in the considerable loss of biological activity. For example, prostaglandin B₁ has only 2% of the potency of prostaglandin A₁ on the peripheral blood vessels of the cat.

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The Role of Haemocytes in the Biosynthesis of Protocatechuic Acid in the Cockroach Colleterial System

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L-Tyrosine is the precursor of protocatechuic acid found in the oothecae of *Periplaneta americana* (Brunet, 1963). Haemocytes from this cockroach have been shown to contain tyrosine decarboxylase (Whitehead, 1969). Unexpectedly, tyramine can also be converted into protocatechuic acid *in vivo* by colleterial glands. The monoamine oxidase responsible for this deamination is located in haemocytes (see Shambough, 1969) and not in the gland cells. The products of monoamine oxidase activity found in the haemolymph of adult female cockroaches 1 h after a 'pulse' of [1-¹⁴C]tyramine or [G-³H]tyramine have been separated by high-voltage electrophoresis and chromatography.

Protocatechuic acid is found in the gland but not in the haemolymph. Of the substances in the blood that are derived from tyramine, an electrically neutral (pH 6.5) fraction gives rise to protocatechuic acid when injected into female *P. americana*.

Inhibitors of tyrosine decarboxylase and monoamine oxidase have been used with [U-¹⁴C]tyrosine 'pulses' *in vivo* to show the dependence of the tyrosine- to -protocatechuic acid pathway on these haemocyte enzymes.

The results of an experiment with allotectomized cockroaches 'pulsed' with [U-¹⁴C]tyrosine suggest that the pathway in the colleterial system is controlled by juvenile hormone (see Willis & Brunet, 1966) and inhibited by α -ecdysone. Isolated haemocytes treated with juvenile hormone and [U-¹⁴C]-tyrosine shown an enhanced ability to deaminate tyrosine.

Control of protocatechuic acid biosynthesis is therefore most likely exerted in the haemocytes associated with the colleterial glands. In the absence of the haemocytes, protocatechuic acid cannot be synthesized from tyrosine by the isolated gland.