NADP + NADPH₂ (Slater, Sträuli & Sawyer, 1964); the mechanism underlying this decrease has been studied in this investigation.

At 1–2 hr. after dosing with CCl₄ there is no change in the total content of ATP in the liver or in total liver NAD + NADH₂, which are substrates for the NAD kinase reaction; NAD kinase is slightly increased in activity at this period of poisoning. These results suggest that there is no failure in the synthesis of NADP during the first 2 hr. of poisoning with CCl₄, but this interpretation is complicated by intracellular shifts of ATP and the nicotinamide-adenine dinucleotides during this period.

The decrease in total liver NADP + NADPH₂ produced by CCl₄ *in vivo* is diminished by the concomitant administration of various free-radical scavengers with the CCl₄ (Slater, Sawyer & Sträuli, 1966). This indicates that the breakdown of the nucleotide is homolytic in character. This is further suggested by experiments *in vitro* that show that NADPH₂ is rapidly destroyed by u.v. irradiation in the presence of CBrCl₃ and, to a lesser extent, CCl₄. This rapid degradative reaction can be followed by fluorimetric or enzymic procedures, and is retarded by free-radical scavengers. The possibility will be considered that NADPH₂ is broken down *in vivo* as a result of the homolytic interaction of CCl₄ and the NADPH₂–cytochrome c reductase in the endoplasmic reticulum of rat liver.

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The Metabolic Fate of DL-[7-³H]Isoprenaline in Man and Dog

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After intravenous injection of DL-[7-³H]isoprenaline (90 μC, 0.5 μg./kg.) in the dog, plasma radioactivity fell biphasically, the initial rapid fall coinciding with a return of the pulse rate towards normal. Free amine represented about 85% of the total radioactivity, 3-*O*-methyloisoprenaline making up the remainder. The findings were similar in man, intravenous dosage (40 μC, 0.06 μg./kg.) resulting in mostly free isoprenaline and a small amount of *O*-methylated metabolite in plasma. It is thus possible that the rapid termination of the pharmaco-

logical effect derives from a mechanism such as tissue uptake (Vane, 1969) rather than rapid *O*-methylation.

Urine samples collected in the first 24 hr. after intravenous injection in man contained 80% of the administered radioactivity (40% free amine, 35% *O*-methylated amine). A progressively increasing proportion of 3-*O*-methyloisoprenaline was excreted with time, and its sulphate conjugation also increased.

In contrast, after oral administration (70 μC, 220 μg./kg.) in man, peak concentrations of total plasma radioactivity (95 min.) and maximum pharmacological effect (50 min.) did not correspond. The major plasma metabolite was isoprenaline sulphate, with a small amount of *O*-methylated amine; 89% of the dose was excreted in the urine in 24 hr., largely as isoprenaline sulphate conjugate, although 10% was the conjugated *O*-methylated derivative. A little free isoprenaline was also present in early urine samples. Intraduodenal administration in the dog (240 μC, 600 μg./kg.) gave a similar pattern; conjugated amine was largely responsible for plasma radioactivity, but a little 3-*O*-methyloisoprenaline was detected. The main metabolite in the urine was isoprenaline sulphate.

Thus the pattern of metabolic degradation of isoprenaline, some aspects of which have previously been studied in the rat (Hertting, 1964) and the dog (Conway, Minatoya, Lands & Shekosky, 1968), varies with the route of administration.

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Regulation of Cytidine Aminohydrolase

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A possible regulatory role in nucleic acid synthesis for cytidine aminohydrolase (EC 3.5.4.5) has been suggested (Wisdom & Orsi, 1967) that implicates it in base conservation after nucleic acid breakdown. Further work on this enzyme has confirmed these conclusions and seems to indicate some rather novel characteristics for cytidine aminohydrolase from sheep liver.

The enzyme has been purified by the method of Wisdom & Orsi (1969) with slight modifications. The most important of these is the substitution of dithiothreitol (1 mM) for 2-mercaptoethanol throughout the purification: this greatly increases the yield and stabilizes the enzyme enough to permit it to be freeze-dried or frozen for storage.

Preparative polyacrylamide disc electrophoresis (Davis, 1964; Ornstein, 1964) has been added as a final step in the purification to give an electrophoretically homogeneous preparation. The electrophoresis is carried out in an acrylamide gel with a high concentration of triethanolamine (0.2M) as both polymerization catalyst and cationic buffer component. The anionic buffer components are phosphate and diethylbarbituric acid, which give a running pH of 8.5.

Removal of the dithiothreitol from the enzyme preparations by dialysis renders the enzyme sensitive to inhibition by thymidine triphosphate. The molecular weight of the enzyme has been studied by Agarose gel filtration to give a value of 37000 for both the crude and the purified enzymes, in both the presence and the absence of dithiothreitol.

The kinetics of the enzyme have been studied in the presence and absence of dithiothreitol. With deoxycytidine as substrate the enzyme shows no change in K_m (0.15mm) or V_{max} , whether in the S-form in the absence of dithiothreitol (thymidine triphosphate-sensitive), or in the D-form in the presence of dithiothreitol (desensitized to thymidine triphosphate). Nor is there evidence for any co-operativity with respect to substrate under these conditions. When thymidine triphosphate is added to the S-form the resulting inhibition is of a complex competitive type, with a parabolic relationship between K_m and thymidine triphosphate concentration. The Hill plot (Monod, Changeux & Jacob, 1963) for this inhibitor has a slope of 2 and a plot by the method of Dixon (1953) is non-linear. This seems to indicate co-operativity in the binding of thymidine triphosphate but none in the binding of the substrate.

Nucleotide inhibition patterns suggest that cytidine aminohydrolase is a low-molecular-weight enzyme with a single substrate site and separate interacting inhibitor sites.

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Melanin Production in *Aspergillus nidulans*

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Little is known of the structure and function of melanins, though they are ubiquitous polymers in the plant, animal and protist kingdoms. After the characterization of melanin production in *Aspergillus nidulans* by Bull & Faulkner (1965), we undertook a study of the process, since it offered a promising system for the application of modern culture techniques and genetical methods to investigate the biogenesis and nature of melanins. In submerged batch culture on glucose-nitrate-inorganic salts medium, pH 6.8, the organism grew in the unpigmented form, and then over a period of many hours, after growth had ceased, the dark brown melanin accumulated in the mycelium. By application of the chemostat continuous-culture technique, it was established that melanin production is immediately induced when growth is glucose-limited over the range of specific growth rates up to at least 0.07 hr.⁻¹ (doubling time 9.9 hr.). The specific rate of melanin production in the chemostat increased considerably when the growth rate was decreased. Electron microscopy showed that melanin is laid down in a microfibrillar form on the exterior of the fungal hyphae. Some melanin was present in the medium in soluble form.

By application of automatic control (MacLennan & Pirt, 1966) it was found that melanin production was dependent on dissolved oxygen tension. Mycelial melanin production was maximal when the dissolved oxygen tension exceeded 30mm.Hg. At lower tension, the mycelial melanin production decreased in a manner dependent on the previous history of the culture, although mycelial growth (with specific growth rate 0.04 hr.⁻¹) was unaffected. The production of the soluble melanin rose sharply severalfold above the basal level when the dissolved oxygen tension was decreased below 30mm.Hg, reached a peak when the tension was about 18mm.Hg, then sharply fell at lower tension.

Methods for the extraction and assay of melanin have been developed. Chemical analysis indicates that *Aspergillus nidulans* melanin is a co-polymer of dihydroxyphenylalanine residues and non-nitrogenous monomers. The absorption spectra of the mycelial and soluble forms of *A. nidulans* were, in the visible range, similar to those of melanin extracted from *Sepia* and that synthesized by aeration of dihydroxyphenylalanine.

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