dicarboxylic acids. This was best resolved for *m*-phthalate at both pH values, as indicated by biphasic plots, and was characterized by K_a approx. 1 mM.

Significantly different values for the absorption at the long-wavelength maximum of the enzyme at infinite ligand concentration are obtained from the intercepts of the plots. Thus at pH 6.5, relative to glutarate as 1.0, the values are 0.85 for α -oxoglutarate and 0.8 for *o*-, *m*- and *p*-phthalate; at pH 7.5 these values are 0.73 for α -oxoglutarate and 0.2, 0.35 and 0.4 for *o*-, *m*- and *p*-phthalate respectively. These differences may be in part due to differences in protonic dissociation constant of the protonated enzyme-ligand complex, $E-H-L^+$, since conversion of all the enzyme into this form does not necessarily occur. In addition, contributions to these differences from conformational or environmental factors in the region of the pyridoxal coenzyme may be inferred from the fine differences in the characteristic points of the spectrum of the $E-H-L^+$ complex.

The affinity for a given ligand, the change in apparent pK of the enzyme, the relative disposition of the binding sites for the two carboxyl groups and the conformational adaptability of the enzyme are evidently interrelated properties. The analogy between these properties and those of the enzyme-substrate complex provides the basis for obtaining structural information about the active site.

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Inhibitors of Shikimate Dehydrogenase as Potential Herbicides

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The shikimate biosynthetic pathway, which occurs in higher plants but not in animals, leads to several essential amino acids and also to a variety of other metabolites. It was hoped therefore that an inhibitor

of the pathway might have a damaging effect on plant metabolism and possibly be a useful commercial herbicide with low mammalian toxicity. Shikimate dehydrogenase (EC 1.1.1.25), the enzyme that catalyses the conversion of dehydroshikimate (I) into shikimate (II), was chosen as the target for inhibition because it occurs early in the pathway and because of the accessibility of synthetic substrate analogues.

It was our intention to prepare active-site-directed irreversible enzyme inhibitors for the enzyme, and as a starting point for this we prepared the novel dehydroshikimate analogue 1,6-dihydroxy-2-oxoisonicotinic acid (III). When tested against shikimate dehydrogenase from pea (assayed in the direction shikimate $\rightarrow$ dehydroshikimate) this had K_i 0.13 mM (K_m 0.17 mM). A hydrophobic area exists near the active site of the enzyme, since a series of 6-*O*-alkyl derivatives of compound (III) bound as well as or better than the parent compound. Maximum binding was obtained with the isopropyl ether (K_i 0.07 mM). We think this area may also have been instrumental in helping to complex the 6-*O*-phenyl analogue (K_i 0.12 mM) and the related potential active-site-directed inhibitors.

It is of interest that these compounds, all of which are more like dehydroshikimate than shikimate, are very weak inhibitors when the enzyme is assayed in the direction dehydroshikimate $\rightarrow$ shikimate (Dowsett *et al.* 1971).

Although chemical synthesis was difficult, we have prepared some iodoacetamidophenoxy derivatives of compound (III) that appear to cause progressive irreversible inhibition directed at the active site. The most powerful of these caused 50% inhibition after 48 min when tested at 1 mM.

The compounds were inactive as herbicides *in vivo* when screened on a range of representative plants, and possible reasons for this will be discussed.

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Metabolism and some Biochemical Effects of *N*-Nitrosomorpholine

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The hypothesis that the carcinogenic and other biological activities of *N*-nitroso compounds could be mediated by their conversion *in vivo* into alkylating agents has received support from studies on structure-activity relationships by Druckrey *et al.* (1967) and from direct evidence of alkylation of nucleic acids or proteins *in vivo* by several nitrosamines and nitrosamides (see Magee, 1969). However, Argus *et al.*

(1965) have claimed that heterocyclic nitrosamines, several of which are potent carcinogens, would probably not be metabolized, and that their carcinogenic action could be mediated by the unchanged molecule. Lijinsky & Ross (1969) reported a lack of alkylation of liver nucleic acids in rats treated with some heterocyclic nitrosamines that might be explained by lack of metabolism to form an alkylating agent.

The cyclic nitrosamine *N*-nitrosomorpholine induces a very similar pattern of pathological changes to that induced by dimethylnitrosamine and diethylnitrosamine. Each compound causes acute liver injury and may induce kidney tumours in the rat after appropriate large single doses. Liver tumours result from continued administration of each compound at smaller doses. The results of a comparative study of the metabolism of these compounds and of some of their effects on macromolecular synthesis *in vivo* will be described.

N-Nitrosomorpholine (400mg/kg body wt.) was given to rats by intraperitoneal injection. The concentration of the compound, determined polarographically, was similar in most organs, with no obvious accumulation. Administration of nitroso-[¹⁴C]morpholine resulted in exhalation of less than 5% of the injected radioactivity as ¹⁴CO₂, whereas about 80% of the radioactivity was recovered in the urine during the first 32h after injection. The same dose of nitrosomorpholine inhibited the incorporation of [¹⁴C]orotate and [¹⁴C]leucine into hepatic RNA and proteins respectively between 2h and 4h after administration, and caused an increase in the monoribosome fraction of liver polyribosomes analysed by sucrose-density-gradient centrifugation. No such effects were observed on RNA or protein synthesis in the kidney. These effects of nitrosomorpholine were similar to those caused by dimethylnitrosamine but were considerably smaller. Implications of these findings for possible mechanisms of action of the nitrosamines will be discussed.

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(-)-Emetine and Macromolecular Biosynthesis in Ehrlich Ascites-Tumour Cells

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It has been shown that (-)-emetine is an effective chemotherapeutic agent in prolonging the life-span in tumour-bearing mice (Jondorf *et al.*, 1971; Johnson & Jondorf, 1971) and has potential for clinical use (Panattiere & Coltman, 1971).

We have now studied the anti-tumour effects of (-)-emetine in greater detail. We were able to show that, in groups of male ICR mice (20–30g body wt.) inoculated with suspensions of Ehrlich ascites cells (1×10^6 cells/mouse) and treated under optimal conditions with (-)-emetine dihydrochloride (8mg intraperitoneally/kg body wt.) daily for 5 days (Jondorf *et al.*, 1971), the tumour yields (packed cell volumes) on the seventh day were 0.2 ± 0.05 ml/mouse compared with 2.2 ± 0.3 ml/mouse for saline-treated controls.

When mice bearing the Ehrlich ascites tumour were injected intraperitoneally with a pulse of L-[¹⁴C]-leucine or [*Me*-³H]thymidine at various times after a single injection of (-)-emetine dihydrochloride (16mg intraperitoneally/kg body wt.) and assayed for incorporation of labelled precursors, we found that tumour-cell protein and DNA biosynthesis *in vivo* were inhibited for 15h by 80–95% and 65–75% respectively. The synthesis of protein and DNA reverted to control values at 24h after treatment with (-)-emetine at this dosage.

In experiments with Ehrlich ascites-cell suspensions *in vitro* (5×10^5 cells/ml) the incorporation of radioactive precursors into macromolecules was determined by the membrane filtration technique of Brown *et al.* (1963). The incorporation of L-[¹⁴C]-leucine into protein *in vitro* was inhibited by 50% in the presence of (-)-emetine in the incubation medium at a concentration of $0.3 \mu\text{M}$. On incubation of cell suspension in the presence of (-)-emetine ($10 \mu\text{M}$), washing and reincubation in (-)-emetine-free medium, we find that inhibition of protein synthesis *in vitro* is irreversible.

The incorporation of [2-¹⁴C]thymidine into DNA *in vitro* was inhibited by 50% by including (-)-emetine ($5 \mu\text{M}$) in the incubation medium. The maximal inhibiting effect of (-)-emetine (0.1 mM) on DNA synthesis (70–75%) could not be reversed on reincubation of washed cells with labelled precursor in the absence of (-)-emetine. These effects are similar to those observed in HeLa cells in culture (Grollman, 1968), though protein synthesis and

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