(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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DNA synthesis particularly in Ehrlich ascites-tumour cells are less sensitive to inhibition by (–)-emetine than are the corresponding biosynthetic activities in HeLa cells.

We find that much higher concentrations of (–)-emetine are needed to interfere with RNA biosynthesis. The incorporation of [2-¹⁴C]uridine into RNA *in vitro* is inhibited by 50% in the presence of (–)-emetine (0.7 mM) in the incubation medium. However, the inhibition of RNA synthesis brought about by incubation with (–)-emetine (1 mM) could be reversed when cell suspensions were washed free of (–)-emetine and reincubated with labelled uridine in fresh medium.

We find, further, that inhibitory concentrations of (–)-emetine have no effect on the release of radioactivity from Ehrlich ascites-tumour cells with prelabelled DNA, RNA or protein. This implies that the inhibitory effects above are not related to increased breakdown of macromolecules.

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Measurement of Liver Protein-Synthetic Rate

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The rate of protein synthesis in rat liver has been measured by constant intravenous infusion of radioactively labelled amino acids. The results from [¹⁴C]glycine and [¹⁴C]tyrosine infusions are compared.

This technique enables the protein-synthetic rate to be determined from measurement of the specific radioactivity of free and protein-bound amino acid in the tissue at a single point of time. Although the specific radioactivity of the amino acid in the blood and tissues reaches a plateau, except for very long infusions, the early time-course must be known. In practice the early time-course of specific radioactivity of the precursor amino acid is assumed to approximate to a single exponential (Waterlow & Stephen, 1968). The rate constant for this exponential need only be known approximately. It depends very largely on the turnover rate of the intracellular pool of

free amino acid, which in turn depends, not only on the rate of protein synthesis occurring within the tissue, but also, to an important extent, on the ratio of protein bound to free amino acid (*R*). This ratio differs greatly for different amino acids. For glycine *R* lies close to 25, whereas for tyrosine it is near to 400. This means that the nature of the approximation for the exponential rate constant will differ for these two amino acids.

It will be demonstrated how the value of this rate constant may be determined for glycine and tyrosine in rat liver and how this may be used to determine the protein-synthetic rate.

Rats (100 g body wt.) were infused for periods of 6 h into the internal jugular vein with either [¹⁴C]glycine or [¹⁴C]tyrosine, after which time their livers were removed. The protein-bound and free amino acids were separated by homogenizing the tissue in 10% trichloroacetic acid and by centrifugation. The specific radioactivity of glycine was determined by separation of its 2,4-dinitrophenyl derivative by t.l.c. (Garlick, 1969) and tyrosine by enzymic decarboxylation and fluorimetry (Garlick & Marshall, 1972).

Glycine gave a protein synthetic rate of 40 ± 7%/day and tyrosine 45 ± 12%/day; the difference between these two values is insignificant. Variations in synthetic rate between animals occurred, but different lobes from the same liver gave the same value. Infusions of different amino acids with widely differing properties therefore can be used to determine protein-synthetic rate in rat liver, giving very similar results.

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The Inhibition of Amino Acid-Activating Enzymes and Leucine Aminopeptidase by Chloromethyl Ketone Analogues of Amino Acids

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The amino acid-activating enzymes catalyse the following reaction:

They show a high substrate specificity, in that each enzyme will react only with the corresponding amino acid. This makes a study of their active sites of particular interest. We have now synthesized chloromethyl ketone analogues of amino acids that retain the free amino group. Such compounds may exhibit