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

an active-site-directed alkylation of the activating enzymes, owing to their structural resemblance to the substrate. The synthesis was achieved by reaction of *N*-t-butyloxycarbonyl-amino acids with dicyclohexylcarbodi-imide to form the corresponding anhydrides, which on treatment with diazomethane gave the diazoketones. The latter compounds reacted with HCl in ether to furnish the chloromethyl

ketone hydrochlorides $\text{Cl}^+ \text{H}_3\text{N}(\text{CHR})-\text{CO}-\text{CH}_2\text{Cl}$ (R =amino acid side chain). The chloromethyl ketone analogue of leucine was found to inhibit the action of leucine-activating enzyme in a time-dependent fashion at a concentration of 10mM, and the activity was not recovered on dialysis, showing the irreversible nature of the inhibition. It was found that the chloromethyl ketone analogues of both DL- and D-alanine were also effective irreversible inhibitors, as were the chloromethyl ketone analogues of *N*-trifluoroacetylalanine and bromopyruvate, which lack the specificity requirement of a free amino group.

The alkylation observed with these compounds and with classical thiol-group inhibitors (Boyko & Fraser, 1964; De Luca & McElroy, 1965, 1966; Baldwin & Berg, 1966; Harvey & Meister, 1967; Iaccarino & Berg, 1969), which occurs at or near the active site, suggests the presence of a nucleophile at the active centre. This appears to be sufficiently exposed for alkylation to occur without the prior formation of a Michaelis complex. This highlights the need for the synthesis of new types of inhibitors that can react to a significant extent only after the formation of a Michaelis complex.

The chloromethyl ketone analogues of DL-leucine and alanine proved to be powerful reversible inhibitors of the activity of leucine aminopeptidase, both producing 70% inhibition at a concentration of 17 μM . The chloromethyl ketone analogue of D-alanine showed no inhibition at concentrations up to 300 μM . This and other evidence will be presented to suggest that chloromethyl ketone analogues of L-alanine and leucine may inhibit the action of leucine aminopeptidase through a specific active-site-directed property.

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Computer Handling of Radioactive-Isotope Counting Data for Competitive Binding and Enzyme Kinetic Studies

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Three programs have been developed for processing radioactive-isotope counting data in connexion with competitive binding and enzyme kinetic studies. They are written in the BASIC programming language for use with the Honeywell G265 computer time-sharing system.

Radioactivities of the radioactive samples are counted in a Packard Tri-Carb liquid-scintillation spectrometer with punched-tape output. This tape is read into the computer from a remote terminal located in the laboratory, telephone lines being used for data transmission. Each program incorporates loops to locate any errors in the data arising either from tape-punch malfunction or transmission faults, and to ensure that the correct sequence of data is maintained.

Program 1 is used to determine the concentration of oestradiol receptors in uterine and breast-biopsy samples (Feherty *et al.*, 1970; Feherty & Kellie, 1971). The equation of the best-fitting line for the Scatchard-plot analysis is calculated for each set of data (Scatchard, 1949), and the receptor concentration and dissociation constant are evaluated from the gradient and intercept of this line, together with their confidence limits.

Program 2 is used to assay plasma oestradiol concentrations by competitive binding with uterine oestradiol receptor (Mester *et al.*, 1971; Robertson *et al.*, 1971). The experimental values are interpolated into the Scatchard plot of the interaction of the uterine receptor preparation with known amounts of oestradiol. An assessment of errors and determination of the dissociation constant and receptor concentration are thus possible; this is of value in assessing interassay variation.

Program 3 is used for enzyme kinetic studies, both of dopa decarboxylase (3,4-dihydroxy-L-phenylalanine carboxy-lyase, EC 4.1.1.26) (D. A. Bender & W. F. Coulson, unpublished work) and phenylalanine 4-hydroxylase (Coulson & Hughes, 1971). Data are corrected for counting efficiency by means of the channels-ratio method (Bush, 1963), and the enzymic activity is calculated from the radioactivity present in the substrate and the product. The double-reciprocal plot ($1/s$ versus $1/v$) is computed and extrapolated to determine the K_m and V_{max} of the enzyme preparation.

These programs have allowed objective assessment of results and achieved a very considerable saving of