dehydrogenase (Rose et al., 1969). The $^2$H labelling of $[^1H]$ lactate in $^2$H$_2$O and the $^1$H labelling of $[^1H]$lactate in $^1$H$_2$O has been studied. A comparison of the exchange in whole cells and lysates has also been undertaken. The detailed measurement of exchange kinetics permitted by the n.m.r. technique has revealed the dependence of the exchange on the different enzyme activities and on the triose phosphate concentrations.

The isotope-exchange reaction involving the C-2 position of lactate can be inhibited by iodoacetate and acetamide. The locus of this inhibition is glyceraldehyde phosphate dehydrogenase and is due to the alkylating effect of its essential thiol group (Harris & Waters, 1976). If the exchange takes place in the presence of these inhibitors, then a decrease in the velocity of C-2 exchange is observed. At sufficiently high concentrations of inhibitor (50–2000µM) the exchange no longer goes to completion. This behaviour can be assigned to the loss of glyceraldehyde phosphate dehydrogenase activity as the other enzymes involved are insensitive to these reagents (Webb, 1966).

By using a steady-state treatment of isotope exchange (Oster et al., 1971), it can be shown that:

$$-\frac{d[^1H]Lactate}{dt} = V \cdot [[^1H]Lactate]_0 \cdot \exp(-\alpha t)$$

where $V$ is the equilibrium velocity for the exchange and $\alpha$ is the pseudo-first-order rate constant for the inactivation of glyceraldehyde phosphate dehydrogenase.

Both of these rate laws are found to be applicable to the exchange and its inhibition. The variation of $\alpha$ with inhibitor concentration is hyperbolic for iodoacetate and linear for iodoacetamide (MacQuarrie & Bernhard, 1971). Triose phosphates are found to protect the enzyme against inactivation in haemolysates and further investigations are needed to probe the environment of the enzyme in whole cells.


Studies of transport in suspensions of whole cells by using nuclear magnetic resonance

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Activation of the zymogen of collagenase by Ehrlich-ascites-tumour-cell-surface trypsin-like enzyme

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It has been observed that the degradation of collagen fibrils in vivo takes place pericellularly (Tarin, 1976; Dingle, 1979; Woolley et al., 1978). The cell culture fluid obtained from such cells is well known to contain latent collagenase, which is considered to be an enzyme–inhibitor complex (Sellars et al., 1977).

The cell surface of Ehrlich ascites cells grown in mice (Itzhaki, 1972) has been shown to possess a trypsin-like enzyme (Steven et al., 1980). This trypsin-like enzyme was inhibited by active-site titrants for trypsin and low-molecular-weight active-site-directed inhibitors of trypsin but not by soya-bean trypsin inhibitor, trasyloyl, serum or the high-molecular-weight trypsin inhibitor exported from these cells into the extracellular fluid (Steven et al., 1980). Ehrlich ascites cells have also been shown to contain a granule-derived zymogen of collagenase that requires proteolytic cleavage for activation, and this may be carried out with trypsin (Steven & Itzhaki, 1977). We have been able to demonstrate the role of this cell-surface enzyme in the activation of the zymogen exported from these cells.

Tropocollagen was prepared from rat tail tendons and reconstituted collagen-fibril gels prepared in sterile Petri dishes, as described by Gross & Kirk (1958). The collagen in these gels was demonstrated to have no trypsin-like activity associated with the fibrils by employing the very sensitive fluorimetric assay of β-naphthylamide activity (MacDonald et al., 1966). Tumour cells were harvested from mice and washed in iso-osmotic NaCl by centrifugation six times before use, in order to remove traces of trypsin inhibitor associated with the extracellular fluid. The cells were treated in the following manner before placing on the collagen gels in Petri dishes at 37°C: (a) control cells, which had no treatment; (b) cells treated with soya bean trypsin inhibitor, 20µg/ml; (c) cells treated with 100µM-Tos-Lys-CH$_2$Cl in 2% (w/v) NaHCO$_3$, pH 8.0; and (d) cells treated with 4-methylumbelliferyl 4-guanidinobenzoate hydrochloride in 2% (w/v) NaHCO$_3$, pH 8.0. After 10 min treatment of the cells at 16°C, the supernatant fluid was removed by centrifugation and the cells were ready for study with respect to their ability to activate the zymogen of collagenase.

Control cells and cells treated with soya-bean trypsin inhibitor exhibited collagenase activity as shown by the appearance of a clear zone of collagen lysis surrounding the cells after 12h (Gross & Lapière, 1962). Inclusion of soya-bean inhibitor in the collagen solution before gel formation did not inhibit the export of collagenase away from the site of cell application on the gels. Cells treated with Tos-Lys-CH$_2$Cl exhibited no collagenase

* Abbreviation: Tos-Lys-CH$_2$Cl, 7-amino-1-chloro-3-L-lysylamido-heptan-2-one ('TLCK').
activity after 12h: however, on being left for a further 24–36h, traces of collagenase activity were observed surrounding the cells. It would appear that this inhibitor diffused away from the cells, and, as a consequence, the cells slowly regained trypsin-like activity. When Tos-Lys-CH₂Cl was included in the collagen gel, the inhibition of collagenase activity was maintained for 48h. With cells treated with 4-methylumbelliferyl 4-guanidino-benzoate hydrochloride, no collagenase activity was observed over 48h, indicating irreversible inhibition of the cell-surface enzyme required to activate the zymogen of collagenase. This latter result would be expected from an active-site tiritant that forms an irreversible complex with the enzyme (Chase & Shaw, 1967).

It was further demonstrated that, with collagen gels containing Tos-Lys-CH₂Cl plus added chymotrypsin, cells pretreated with Tos-Lys-CH₂Cl exhibited collagenase activity after 12h. This result, taken with the results described above, is clear proof that the cells export a zymogen of collagenase requiring proteolysis by chymotrypsin or the cell-surface trypsin-like enzyme before activation and the demonstration of collagenolysis. Since none of the inhibitors described above has any effect on collagenase itself, the effect of these agents must be on the activation process. We believe that the cell-surface enzyme, which is not inhibited by high-molecular-weight inhibitors of trypsin, has been shown to be capable of carrying out this activation.

The presence of α₁-macroglobulin and other extracellular inhibitors of collagenase in in vitro would prevent collagenolysis taking place distant from cells exporting collagenase or its zymogen. The role of the cell-surface trypsin-like enzyme (which is not affected by serum inhibitors) in zymogen activation would explain the pericellular degradation of collagen fibrils observed in connective-tissue damage.

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Transverse asymmetry of the enzymes that catalyse methylation of phosphatidylethanolamine to yield phosphatidylcholine in rat liver microsomal fraction

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The site of incorporation of the 14C-labelled methyl group from S-adenosylmethionine into phospholipids of rat liver microsomal fraction has been investigated by two experimental approaches. The site of labelled phospholipids in the membrane bilayer has been determined by using phospholipase C as a probe of the transverse asymmetry of the enzymes that catalyse methylation of phosphatidylethanolamine and phosphatidylcholine, and in each case the site of incorporation of the 14C-labelled methyl group from S-adenosylmethionine into phospholipids of rat liver microsomal fraction ('microsomes') was incubated with unlabelled S-adenosylmethionine, sequestered from any known enzyme catalysing methylation has been investigated by using trypsin to hydrolyse proteins of the outer leaflet.

Methyl groups were rapidly incorporated into N-methylphosphatidylethanolamine, N,N-dimethylphosphatidylethanolamine and phosphatidylethanolamine, and in each case the inner leaflet (unhydrolysed by phospholipase C) was labelled initially (1–2 min) followed by labelling of the outer leaflet (hydrolysed by phospholipase C) at later times. In a typical experiment the specific activity of unhydrolysed phosphatidylethanolamine was 4100 c.p.m./μmol at 2 min and 4300 c.p.m./μmol at 60 min, whereas the specific activity of the total phosphatidylethanolamine was 2912 c.p.m./μmol at 2 min and 9805 c.p.m./μmol at 60 min. The incorporation of the 14C-labelled methyl group into phosphatidylcholine of the inner leaflet is rapidly completed, therefore, and phosphatidylcholine pools of inner and outer leaflets do not equilibrate. Labelled N,N-dimethylphosphatidylethanolamine occurred initially in a pool sequestered from hydrolysis by phospholipase C in either unopened microsomal vesicles or vesicles opened by taurocholate (0.4%). At later times a large fraction of the labelled N,N-dimethylphosphatidylethanolamine was available for hydrolysis. Most of the labelled N-methylphosphatidylethanolamine was in a sequestered pool at all times investigated.

When microsomal fraction ('microsomes') was incubated with S-[Me-14C]adenosylmethionine for 2 min followed by incubation with unlabelled S-adenosylmethionine, sequestered labelled N-methylphosphatidylethanolamine and N,N-dimethylphosphatidylethanolamine were transferred and methylated to produce phosphatidylcholine in the outer leaflet. This transfer of labelled phospholipids is inhibited by addition of S-adenosylhomocysteine, and is therefore linked to methylation.

To investigate directly the site of methytransferases, microsomes were treated with trypsin before incubation with S-adenosylmethionine. Incorporation of methyl groups into phosphatidylcholine was inhibited over 90%, into N,N-dimethylphosphatidylethanolamine 65% and into N-methylphosphatidylethanolamine 10%. When the vesicles were opened with taurocholate before treatment with trypsin, incorporation of methyl groups into phosphatidylcholine, N,N-dimethylphosphatidylethanolamine and N-methylphosphatidylethanolamine was inhibited 75–85%. In intact microsomes incorporation of 14C-labelled methyl groups into the inner leaflet of the bilayer (not hydrolysed by phospholipase C) was unaffected by trypsin treatment, whereas incorporation into the outer leaflet (hydrolysed by phospholipase C) was inhibited over 95%. Labelled N-methylphosphatidylethanolamine and N,N-dimethylphosphatidylethanolamine sequestered in the membrane, therefore, appear to be synthesized by enzymes located at the inner surface of the microsomal membrane.

These observations suggest that the first methylation of phosphatidylethanolamine occurs at the inner surface of the microsomal membrane and that N,N-dimethylphosphatidylethanolamine produced is sequestered. The second and third methylation steps take place on both sides of the membrane resulting in two separate pools of phosphatidylcholine, which do not equilibrate. There is therefore an asymmetric distribution of methytransferases and translocation of N,N-dimethylphosphatidylethanolamine and/or N,N-dimethylphosphatidylethanolamine across the bilayer takes place. A similar model has been suggested by Hirata & Axelrod (1978) for the methylation of phosphatidylethanolamine in erythrocyte membranes. However, in liver microsomes, enzymes catalysing the second and