precursor that labels intermediary products having small pools and high turnover rates. Attempts to achieve this condition (Feinendegen *et al.*, 1961; Owen, 1967), including those involving the use of antibiotics or changes in medium (see, e.g., Cooper, 1968), have proved largely unsuccessful or subject to criticism (Rovera *et al.*, 1970; Plagemann, 1971b).

A consideration of the utilization of [5-³H]uridine, which is a specific label for RNA, by fast-growing tumour populations, such as Ehrlich ascites cells, shows that it is the most likely of the RNA precursors to be useful in such studies. Important factors in relation to this are its rapid phosphorylation and incorporation into RNA (Plagemann, 1971a), which are characteristic of such systems having high rates of RNA synthesis. Thus Rovera *et al.* (1970) were able to pulse-label the RNA of a hypotetraploid subline of ascites cells by using [³H]uridine.

We have found that, when cells from a hyperdiploid line of Ehrlich ascites cells were incubated at 37°C *in vitro*, in phosphate-buffered saline, with tracer amounts of [5-³H]uridine, of the order of 10nM, incorporation into acid-precipitable material ceased after 10–11 min. Incorporation was assessed by a Whatman GF/C filter-disc technique, and investigation, prompted by recent criticism of such methods (Yu & Feigelson, 1971), showed that 98% of the acid-precipitable radioactivity was in RNA. With a higher concentration of [³H]uridine *in vitro* a similar plateau of incorporation was reached. That such a pattern was not caused by a loss of the ability to incorporate uridine was shown by an experiment in which a second addition of [³H]uridine after a period of incubation resulted in further incorporation of similar degree and pattern to the first. In the experiments carried out *in vitro* up to about 50% of the available uridine was incorporated into RNA.

A similar pulse-labelling of RNA was observed *in vivo* when [³H]uridine (100 µCi) was injected intraperitoneally into a tumour-bearing mouse. Again incorporation ceased after 10 min. Polyacrylamide-gel electrophoresis of cytoplasmic RNA showed that the label was incorporated into the major cytoplasmic species of RNA.

These results demonstrate that pulse-labelling capacity may be more general in lines of Ehrlich ascites cells than Rovera *et al.* (1970) originally suggested, and indicate the potential usefulness of these cells for studying the parameters of RNA synthesis.

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A Demonstration that Embryonic Mouse Liver Heterogeneous Nuclear Ribonucleic Acid Contains Globin Messenger Ribonucleic Acid Sequences

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It has been suggested that heterogeneous nuclear RNA contains mRNA sequences, though definitive and direct proof has so far been lacking (Britten & Kohne, 1969; Georgiev, 1969). With the isolation of large amounts of mRNA for globin (Williamson *et al.*, 1971), and the proof that it codes for haemoglobin in heterologous cell-free systems (Schapira *et al.*, 1968; Lockard & Lingrel, 1969) and on injection into *Xenopus* oocytes (Lane *et al.*, 1971), it is now possible to attempt a direct experiment testing for the presence of mRNA sequences in heterogeneous nuclear RNA.

Total RNA was isolated from 14½-day embryonic mouse livers, a tissue that contains greater than 70% nucleated erythropoietic cells. The RNA was sedimented on a sucrose density gradient with the zonal BXIV or 6×15ml swing-out rotor, and the fraction sedimenting at greater than approx. 35S was collected and precipitated with ethanol. This RNA was dissolved and injected at a concentration of 1–2mg/ml into *Xenopus* oocytes. ³H-labelled histidine was present in the incubation medium and all conditions were as given by Lane *et al.* (1971) and Lingrel (1972). After 16h incubation, the oocytes were removed from the incubation medium, washed and lysed in the presence of haemoglobin. Material that co-chromatographed with the carrier haemoglobin was isolated from Sephadex G-100, and then globin was prepared from the peak and chromatographed on Whatman CM-52 CM-cellulose (Lingrel, 1972). Label was found associated with the globin peaks, and was not found in the controls that were injected with medium alone. Mouse 9S polyribosomal globin mRNA was also translated in this system at a very much higher efficiency.

This demonstrates that large heterogeneous RNA (>35S) carries coding sequences for globin that can be translated in heterologous cytoplasm. RNA isolation and fractionation procedures were designed to minimize RNA aggregation, but the unlikely possibility that cytoplasmic mRNA is non-specifically bound to >35S RNA has nonetheless not yet been

rigorously excluded. Further experiments are in progress to determine if a precursor-product relationship to 9S mRNA exists.

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Studies on the Heterogeneity of Mouse Globin Messenger Ribonucleic Acid

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It now has been demonstrated beyond doubt that the 9S RNA that can be isolated from reticulocyte polyribosomes is the mRNA for globin (Lingrel, 1972; Lane *et al.*, 1971). We have prepared this RNA in milligram amounts (Williamson *et al.*, 1971), and have confirmed that our material is active in a duck lysate cell-free system and also on injection into *Xenopus* oocytes.

The fact that 9S RNA is active in directing globin synthesis does not give information as to the proportion of the RNA that is messenger; as has been pointed out (Lingrel, 1972), the results are compatible either with a very inefficient translation of the messenger or with the utilization of only a small proportion of the added 9S RNA. We have electrophoretically fractionated the mRNA for globin isolated from mouse reticulocytes into three components on 6 and 10% polyacrylamide gels. It has been found that only two of the bands, amounting to approx. 70% of the total, prime for globin synthesis. The band moving most quickly primes primarily for α -chain synthesis, whereas the intermediate band appears to code for the β -chain, although we have been unable to separate the messenger activities of these two bands completely. The fastest component primes more efficiently than the intermediate one on a weight basis. The component running nearest to the origin of the gel is inactive in directing globin

synthesis in a duck lysate system, and also when injected into *Xenopus* oocytes (C. D. Lane & C. E. Drewienkiewicz, unpublished work).

There is evidence that when the mRNA is run on 6% polyacrylamide gels in 8M-urea the positions of the bands relative to one another alter. This could indicate a conformational change. 'Fingerprint' results also show little detectable difference between the bands (J. N. Mansbridge, T. McShane, R. Williamson & W. G. Lanyon, unpublished work).

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The Separation of Two Aldehyde Reductases from Pig Brain

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The aldehydes derived by oxidative deamination of the biogenic amines in brain are further metabolized either by oxidation or by reduction (Rutledge & Jonason, 1967; Breese *et al.*, 1969). The reductive conversion is effected by an NADPH-linked aldehyde reductase (alcohol-NADP⁺ oxidoreductase, EC 1.1.1.2) (Eccleston *et al.*, 1966). Brain aldehyde reductases have been partially purified from bovine brain (Tabakoff & Erwin, 1970) and pig brain (Turner & Tipton, 1971). These enzymes are similar with respect to pH optima, K_m values for NADPH, inhibition by barbiturates and insensitivity to pyrazole inhibition. However, they show considerably different properties in terms of K_m values for aldehyde substrates. The aldehyde reductase content of pig brain has been re-examined.

The aldehyde reductase preparation used was obtained by ammonium sulphate fractionation of a brain high-speed supernatant. When the reduction of *p*-nitrobenzaldehyde was studied, markedly biphasic Lineweaver-Burk plots were obtained. This suggested the presence of two enzymes with widely different K_m values for *p*-nitrobenzaldehyde. Chromatography on DEAE-cellulose completely resolved two peaks of activity. When the major peak (90% of total activity) was rechromatographed, a single peak of activity was obtained. This major peak corresponded to the 'high- K_m ' enzyme already described