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synthesis in a duck lysate system, and also when injected into *Xenopus* oocytes (C. D. Lane & C. E. Drewienkiewicz, unpublished work).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also show little detectable difference between the bands (J. N. Mansbridge, T. McShane, R. Williamson & W. G. Lanyon, unpublished work).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

(Turner & Tipton, 1971). The K_m values for aldehydes ranged from 4×10^{-5} M to 5×10^{-3} M (K_m for *p*-nitrobenzaldehyde is 2.9×10^{-4} M). The pH optimum was 6.6.

The minor peak differed in several respects from both the 'high- K_m ' enzyme and bovine brain aldehyde reductase. The pH optimum was much lower and K_m values for the aromatic aldehydes tested were less than 2×10^{-6} M (K_m for *p*-nitrobenzaldehyde is approx. 8×10^{-7} M). The enzyme was also less sensitive to barbiturate inhibition. Both activity peaks could reduce indol-3-ylacetaldehyde, *p*-hydroxyphenylacetaldehyde and *p*-hydroxyphenylglycolaldehyde. 1 mM-Pyrazole and 50 mM-ethanol did not affect enzyme activity.

Under normal conditions brain aldehydes are presumably metabolized by aldehyde dehydrogenase and the less active 'low- K_m ' aldehyde reductase. Under conditions of rising aldehyde concentrations, the more active 'high- K_m ' aldehyde reductase could come into play, resulting in an increased proportion of reduced product. The differences observed between bovine and pig brain aldehyde reductases may be due to a species difference.

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The Effects of Catecholamines and Methylxanthines on Glucose Utilization in Isolated Fat-Cells Derived from the Rat

By D. G. PEERS and J. I. DAVIES (*Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caerns., U.K.*)

The catecholamines and the methylxanthines have similar stimulatory effects on fat-cell lipolysis, which almost certainly reflect their activities in elevating tissue concentrations of cyclic AMP (adenosine 3':5'-cyclic monophosphate). However, the influences of these substances on the rate and pattern of glucose utilization in this tissue are in many ways opposed to one another. Thus the rate of glucose uptake is enhanced by adrenaline but inhibited by caffeine. It appears, too, that the relative incorporations of 14 C-labelled substrate into the fatty acid and glycerol

moieties of cellular triglycerides differ in response to these two agents.

A more detailed analysis of the situation shows that the most direct effects of adrenaline and caffeine on the pattern of glucose utilization are in fact very similar. Both substances drastically decrease the proportion of glucose metabolized via the pentose phosphate pathway, probably because they enhance phosphofructokinase activity by elevating cellular concentrations of cyclic AMP.

The differences between the patterns of utilization that are attributable to the catecholamines and methylxanthines are entirely secondary to the effects of these substances on the rate of glucose utilization. Thus both create a situation where non-esterified fatty acids available for esterification are apparently in excess of the supply of α -glycerophosphate. Under these circumstances it appears that a high priority exists for the conversion of glucose into α -glycerophosphate and that the synthesis of fatty acids occurs secondarily where substrate requirement for the synthesis of this metabolite is largely satisfied. Thus the catecholamines increase the proportion of glucose incorporated into the fatty acid moiety of triglycerides because they enhance the rate of glucose uptake so that its supply surpasses more completely the minimal requirement for α -glycerophosphate formation.

Neither the site nor the mechanism of catecholamine and methylxanthine actions on the rate of glucose utilization is known with certainty, though the similarity of their effects on the pattern of glucose utilization implies that they influence some stage early in the pathway. In view of the findings by Bray & Goodman (1968) on the effect of adrenaline on glucose uptake, the membrane translocation phase appears a likely site of action for both agents. Despite the controversy about the role of cyclic AMP in regulating glucose uptake (Blecher *et al.*, 1969; Schimmel & Goodman, 1971), it appears unlikely that this nucleotide mediates the actions of both the catecholamines and the methylxanthines.

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