some population into polyribosomes (Marcus & Feeley, 1965). The conversion takes place in the absence of transcription (Dure & Waters, 1964; Marcus & Feeley, 1966; Chen, Sarid & Katchalski, 1968), suggesting that the process involves 'activation' of preformed messenger. In support of this idea, a messenger-containing subcellular fraction (fraction MF) has been isolated from dry wheat embryo. By comparison of the characteristics of fraction-MF-directed amino acid incorporation with the reactions catalysed by tobacco-mosaic-virus RNA on one hand and by preformed polyribosomes on the other, it could be shown that a messenger-ribosome attachment reaction is a prerequisite for fraction-MF activity. In addition, direct demonstration of such a reaction is provided by experiments in which fraction MF is allowed to react *in vitro* with messenger-free ribosomes. Sucrose-density-gradient analyses of this reaction shows a progressive conversion of monoribosomes into functional polyribosomes.

When fraction MF is stored at 0°C its activity decays with a half-life of 12–13h. The loss of activity is not due to ribonuclease, as shown by the complete stability of tobacco-mosaic-virus RNA stored in the presence of fraction MF. These findings, as well as experiments demonstrating the sensitivity of fraction MF to low concentrations of pronase and *N*-ethylmaleimide, suggest that a component other than RNA is necessary for fraction-MF activity and that the functional form of fraction MF may be a ribonucleoprotein. The low template activity of purified fraction-MF RNA is consistent with this conclusion.

Studies *in vivo* show a rapid decrease in messenger fraction concomitant with polyribosome formation during early germination. Cumulatively these findings suggest an important physiological role for fraction MF in the initiation of the protein synthesis that occurs at the outset of germination.

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Protein Synthesis and Viability in Rye

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Embryos of non-viable rye grains do not make protein, but protein synthesis can be demonstrated in viable embryos within 60 min of imbibition (see Marcus & Feeley, 1965). Ribosomes and soluble components have been isolated from the embryos of both types of grain, and incorporation of amino acids into trichloroacetic acid-precipitable proteins determined in the presence of either endogenous or synthetic message [poly(U)]. Comparisons of homologous and heterologous ribosomal and soluble constituents from the viable and non-viable embryos indicate a functional lesion in the non-viable ribosomes. Although the two kinds of ribosomes show no visible differences with the electron microscope either in sectioned material or when isolated and negatively stained, sucrose-density-gradient analysis shows that the non-viable ribosomes are lighter with a greater heterogeneity of density. The possibility is considered that loss of protein-synthetic activity in non-viable grains is associated with loss of long-life stored mRNA.

A second modification in the non-viable embryo is associated with the DNA. Although spectrophotometric determinations of total Feulgen-staining nuclear DNA indicates no difference between viable and non-viable embryos before or after imbibition, less DNA can be recovered by extraction (Marmur, 1961) from non-viable embryos. Electron microscopy of comparable tissue of viable and non-viable embryos shows differences in fine structure in the dry grain before imbibition and dramatic and progressive changes during the early hours of imbibition.

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COMMUNICATIONS

Formation and Utilization of Ketone Bodies during Development of the Male Rat

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It is now clear that hepatic lipogenesis and gluconeogenesis in the rat adapt to the nutritional changes that occur during development. However,

little is known about changes in lipid breakdown during development despite the fact that the neonatal rat derives most of its energy from the fat content of milk (Drahota, Hahn & Honova, 1966). The results of Drahota *et al.* (1966), Lockwood & Bailey (1970) and Augenfeld & Fritz (1970) suggest that during the suckling period rat liver has an increased capacity for fatty acid degradation and ketone-body formation. The present paper is concerned with developmental changes in blood

acetoacetate and β -hydroxybutyrate concentrations, in the activities of enzymes concerned with hepatic acetoacetate formation and the activity of a key enzyme involved in ketone-body utilization by brain and heart.

Highest blood concentrations of acetoacetate are found at 5 days of age, after which time the concentration falls to reach the adult value by 30 days of age. Blood concentrations of β -hydroxybutyrate follow a developmental pattern similar to those of acetoacetate but increase to a greater extent during suckling. Both mitochondrial and cytoplasmic hydroxymethylglutaryl-CoA synthase (EC 4.1.3.5) activities were detected, with highest activities being found in the mitochondria at all stages of development. Activity of the mitochondrial enzyme increases rapidly immediately after birth, thereafter exhibiting a similar developmental pattern to blood ketone bodies. The cytoplasmic enzyme, on the other hand, increased steadily in activity after birth to reach a maximum at 40 days of age, after which time activity fell to adult values. Both mitochondrial and cytoplasmic acetoacetyl-CoA thiolase (EC 2.3.1.9) activities were detected, with the mitochondrial enzyme having considerably higher activities at all stages of development. The developmental patterns for both enzymes were very similar to those for the corresponding hydroxymethylglutaryl-CoA synthases. The activity of heart acetoacetyl-CoA transferase (3-oxo acid CoA-transferase, EC 2.8.3.5) remains constant from late foetal life until the end of the suckling period, after which time there is a gradual threefold increase in activity to reach the adult values. The activity of brain acetoacetyl-CoA transferase increases steadily after birth, reaching a maximum at 30 days of age, thereafter decreasing to adult values, which are similar to foetal activities.

Page, Krebs & Williamson (1971) have reported results for blood ketone bodies and brain and heart acetoacetyl-CoA transferase that are similar to those presented here.

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Significance of the Caffeine-Like Effect of Various Purines, Pyrimidines and Derivatives on Adipose-Tissue Phosphodiesterase

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Mammalian tissues are exposed to a variety of purines, pyrimidines and derivatives as endogenous metabolites, food constituents and drugs. The disturbances of metabolism brought about by one such group of substances, the methylxanthines, can largely be related to the elevation of tissue concentrations of cyclic AMP (adenosine 3':5'-cyclic monophosphate) due to inhibition of the enzyme 3':5'-cyclic nucleotide phosphodiesterase (Brodie, Davies, Hynie, Krishna & Weiss, 1966). We have compared the inhibitory effect of caffeine on this enzyme with that of other purines, pyrimidines and derivatives to which tissues may be exposed.

The phosphodiesterase of rat epididymal adipose tissue was shown to be probably heterogeneous, since it yielded K_m values that differed according to the range of substrate concentration used. Of the nucleosides and corresponding free bases tested only caffeine appreciably inhibited the fresh enzyme. However, after a 16 h dialysis the enzyme was more susceptible to inhibition by inosine, guanosine or deoxyadenosine than by caffeine. This transformation was reversed by mercaptoethanol.

Isolated fat-cells derived from epididymal adipose tissue yielded a phosphodiesterase with $K_m 1 \times 10^{-5} M$. Under circumstances where caffeine (1 mM) caused 51% inhibition, this enzyme preparation was inhibited by various nucleosides and free bases, e.g. deoxyadenosine (1 mM), 55%, adenosine (1 mM), 30%, adenine (1 mM), 29%, and guanosine (0.5 mM), 24% (substrate concentration, 1 μM). The actions of inosine and adenosine were shown to have competitive kinetics.

The inhibitory effect of caffeine on phosphodiesterase activity is well correlated with its effect on lipolysis in the intact fat-cell (Brodie *et al.* 1966). This correlation could be extended in some but not all instances to the selection of nucleosides and free bases tested (Davies, 1968).

Preliminary studies have been carried out to determine the extent to which the caffeine-like effects of these nucleosides and free bases could be manifested *in vivo*. Administration by stomach tube into rats (100 mg/rat) led to responses in plasma non-esterified fatty acids and glucose that were generally small compared with those due to caffeine (50 mg/rat). Work is in progress to determine the extent to which this is due to the detoxifying activity of the gut, liver etc.

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