Cholinesterase activity was defined as hydrolysis of butyrylthiocholine inhibited more than 96% by physostigmine sulphate (10 μ mol/l) at 37°C. Initial enzyme velocities were determined with the dithiobisnitrobenzoate reagent of Ellman, Courtney, Andres & Featherstone (1961).

Young male rats show a significant ($0.01 > P > 0.001$) decrease in plasma cholinesterase activity (to 76% of initial activity) within 1 day of starvation but no further fall over the next 3 days. In contrast Harrison & Brown (1951) showed that no change occurred in the plasma cholinesterase activity of adult male rats over 6 days of starvation.

In the liver we found that whether the cholinesterase activity was expressed on the basis of initial body weight, final body weight or liver weight there was a dramatic decrease in enzyme activity. Thus there was a 50% decrease within 24 h of starvation when liver activity was expressed per 100 g initial body weight and there was a further significant decrease between days 2 and 4. In the adult Harrison & Brown (1951) found a much slower decrease (per 100 g initial body weight), finally levelling off after 4 days of starvation. Thus the response of the young rat to starvation is much more dramatic, resulting in large decreases in activity in both liver and plasma.

Freedland (1967) observed that young rats starved for up to 4 days showed several patterns in loss of enzyme activity. The activities of four enzymes decreased with the same biphasic response as cholinesterase. Mg^{2+} -activated DNA-dependent RNA polymerase activity also decreases on starvation (Henderson, 1970) and has a biphasic response (Rickwood & Klemperer, 1970), but Mn^{2+} -ammonium sulphate-activated RNA polymerase decreases only after several days of starvation. These findings suggest a crucial role for rRNA and mRNA, but rates of protein synthesis and degradation may also alter during starvation (Schimke, 1970).

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The Effect of Feeding on the Apparent Kinetic Constants and Activation Energy of the Magnesium Ion-Activated Deoxyribonucleic Acid-Dependent Ribonucleic Acid Polymerase of Rat Liver

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The activity of the rat liver nuclear Mg^{2+} -activated DNA-dependent RNA polymerase (EC 2.7.7.6) decreases during starvation (Rickwood & Klemperer, 1970) and increases during re-feeding (Henderson, 1970). This increased activity is extremely rapid, reaching a maximum 1 h after feeding, and then it falls off slowly. This response could be prevented by pretreatment of the animals with puromycin or cycloheximide.

Because the RNA polymerase in the liver of the starved animal is not responsive to these drugs, it was tentatively suggested that the enzyme synthesized after feeding differed from this basal enzyme by its more rapid synthesis and degradation. However, no other evidence could be found showing that the RNA polymerase of the starved and the fed animals differed.

We have now determined K_m and V for each nucleoside triphosphate involved in the RNA polymerase reaction for rat liver nuclei isolated from 18 h-starved animals and animals fed for 1 h with a complete amino acid mixture. The regression technique of Wilkinson (1961) were used to obtain the constants. The precisions of the K_m values were relatively poor, and because of this no differences could be found between the values for the two dietary groups. In contrast, all values of V differed significantly between the dietary groups. As it had been suggested that this difference was probably due to synthesis of polymerase *de novo* (Henderson, 1970) some other means of differentiating between the enzymes was attempted.

The effect of temperature on enzyme activity can sometimes be used to differentiate enzyme variants from the same tissue, and we accordingly determined the RNA polymerase activity in both dietary groups over the range 15–60°C. Arrhenius plots of these results tend to suggest that there is a component present in the fed rats that is not present in the starved animals.

Chesterton & Butterworth (1971) have demonstrated that rat liver Mg^{2+} -activated RNA polymerase can be separated on phosphocellulose columns into two active components, which are present in equal proportions. We tentatively suggest that our results could be interpreted as an increase in one of the components in the fed animals, and that these components differ in their rates of synthesis and degradation.

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Stimulation of the Release of Unesterified Fatty Acids in Synaptosomes by Noradrenaline and 5-Hydroxytryptamine

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Intraventricular injection of eserine or carbamoylcholine into rat brain caused up to 40-fold increases in cerebral unesterified fatty acid (Lunt & Rowe, 1971), suggesting that unbound acetylcholine stimulates fatty acid release *in vivo*. Noradrenaline and 5-hydroxytryptamine are concentrated in nerve endings in brain (see Michaelson & Whittaker, 1963) and are putative transmitter substances. The question arises as to whether these compounds also stimulate the release of unesterified fatty acid. The effects were investigated of noradrenaline and 5-hydroxytryptamine on the concentration of unesterified fatty acid in isolated nerve endings (synaptosomes).

Synaptosomes isolated from guinea-pig cortex and cerebellum (Eichberg, Whittaker & Dawson, 1964) were incubated in Krebs-Ringer bicarbonate medium, containing glucose (11 mM), at 37°C for 90 min with shaking. Unesterified fatty acids were extracted (Dole, 1956) and determined as their methyl esters by g.l.c. (Rowe, 1964). Noradrenaline (1.0 mM) caused up to threefold increases in the yields of unesterified palmitate, stearate, oleate and arachidonate. Similar increases were obtained with 5-hydroxytryptamine and adrenaline, but not with acetylcholine in the presence of eserine, or with methylamine. Fluorimetric determinations of noradrenaline (Miller, Cox, Snodgrass & Maickel, 1970) and 5-hydroxytryptamine (Curzon & Green, 1970) indicated that concentrations of these substances in the synaptosomes were considerably less than in the incubation media.

Synaptosomes were ruptured by osmotic shock (Whittaker, Michaelson & Kirkland, 1964) and the suspension was incubated in 50 mM-tris buffer, pH 7.4. Iproniazid phosphate (100 µg/ml) increased the yield of acid (from 17%, w/w, to 101%, w/w). This increase was maximal after 15–20 min. In the presence of iproniazid phosphate relatively low

concentrations of noradrenaline (0.01 mM) could stimulate further release of fatty acids. Higher concentrations (1.0 mM), however, depressed fatty acid yields. An analogous depression was also obtained with 5-hydroxytryptamine.

Synaptosomal membrane fractions and vesicle fractions were isolated from an osmotically shocked crude mitochondrial pellet (Lapetina, Soto & De Robertis, 1967). After incubation in tris buffer for 30 min unesterified fatty acids were determined as their [³H]methyl esters. Noradrenaline (0.01 mM) and 5-hydroxytryptamine (0.01 mM) stimulated the release of fatty acids in both these fractions.

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Synthesis of Fatty Acids from [1-¹⁴C]Acetyl-Coenzyme A in Subcellular Particles of Rat Epididymal Adipose Tissue

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Fatty acid synthesis from [1-¹⁴C]acetyl-CoA was studied in mitochondria and microsomes of adipose tissue. We also studied the relationship between particulate fatty acid synthesis and triglyceride formation, which has been reported to be a major function of subcellular particles of this tissue (Roncari & Hollenberg, 1967; Angel & Roncari, 1967).

Yield of particles (means ± S.E.M.) was small (mitochondria: 1.33 ± 0.07 mg of protein/g wet wt. of tissue; microsomal fraction: 0.75 ± 0.05 mg of protein/g wet wt. of tissue), but represented about 20% of protein obtained in a defatted homogenate.

The two types of particle were clearly distinguished (1) by fatty acid composition of total lipids and (2) by the distribution of various enzyme