identical with the elongation factors of the supernatant, are present in the ribosomal fraction. These factor(s), presumably, are involved in initiation: however, further investigation is required to elucidate this point. Moreover, preliminary results indicate that a fraction with stimulatory activity similar to that described above can be removed from ribosomes with 0.25 M-ammonium chloride. The nature of this fraction is also currently under investigation.

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Glycogen Synthesis in the Perfused Liver of Starved Rats

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In order to investigate the factors that control the reaccumulation of hepatic glycogen after starvation we have measured net rates of glycogen deposition in perfused livers from starved rats. Livers from 170–190 g male rats, starved for 48 h, were perfused with bicarbonate–albumin–saline, essentially as described by R. Hems, Ross, Berry & Krebs (1966), except that washed erythrocytes from fresh defibrinated rat blood (Baron & Roberts, 1963) were employed, so that haemoglobin was 4% (w/v). Liver samples (0.5 g) were taken at 20 min (ventral notched lobe) and 50 min (large main lobe).

When livers from 48 h-starved rats were perfused with glucose alone (30 mM) glycogen deposition was 0–0.4 $\mu\text{mol/min per g wet wt. of liver}$, but if pyruvate, glycerol and serine (each 5 mM) were added after 15 min perfusion with 30 mM-glucose the glucose concentration in the medium was stabilized and the rate of glycogen accumulation was 0.68 ± 0.05 (10) $\mu\text{mol/min per g wet wt. of liver}$. Unless the glucose concentration in the medium was 25–30 mM, glycogen accumulation was less than

0.5 $\mu\text{mol/min per g wet wt. of liver}$ and glucose was released into the medium.

When liver was perfused under the present conditions with glycerol, pyruvate and serine (each 5 mM) in the absence of glucose gluconeogenesis was 1.4 $\mu\text{mol/min per g wet wt. of liver}$ for 20–35 min, after which the rate decreased. In this experiment average glucose formation between 20–50 min was 0.76 $\mu\text{mol/min per g wet liver}$. Rapid glucose synthesis from substrate combinations including glycerol has been reported previously (Ross, R. Hems, Freedland & Krebs, 1967).

The present experiments suggest that the initial phase of post-starvation hepatic glycogen deposition after ingestion of glucose may largely be a consequence of gluconeogenesis; such a possibility was suggested from the results of experiments *in vivo* carried out by Olavarria, Godeken, Sandruss & Flawia (1968). This would resolve the difficulty that hepatic glucokinase activity in the post-starvation situation is not sufficient to support conversion of administered glucose into glycogen at the observed rate of glycogen deposition (for references and discussion see Friedmann, Goodman & Weinhouse, 1967; Olavarria *et al.* 1968). Initiation of glycogen accumulation apparently may occur solely as a result of a rise in blood glucose concentration, perhaps due to activation of glycogen synthetase (see Hers, de Wulf, Stalmans & Van den Berghe, 1970).

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The Physiological Role of L-Tyrosine Methyl Ester Sulphotransferase

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A sulphotransferase has been obtained from rat livers that appears to be specific for simple derivatives of L-tyrosine (Mattock & Jones, 1970). The physiological role of this enzyme is unknown,

but it may be involved in the incorporation of sulphated tyrosine residues into proteins. Since free L-tyrosine *O*-sulphate is not incorporated into protein and in view of the specificity of this sulphotransferase for derivatives of L-tyrosine having a modified carboxyl group and a free amino group, it has been suggested that the sulphation of certain tyrosine residues may occur at the L-tyrosyl-tRNA stage of protein biosynthesis (Mattock, Barford, Basford & Jones, 1970).

When a crude preparation of sulphotransferases from rat livers is purified by chromatography on DEAE-cellulose at pH 8.5 and by isoelectric focusing, it yields three enzyme fractions with pI values 6.6, 5.8 and 5.3 and all possessing L-tyrosine methyl ester sulphotransferase activity. The last-mentioned enzyme fraction is the major component of the mixture and corresponds to enzyme B described previously (Barford & Jones, 1970). An enzyme preparation containing all three of these isoenzymes fails to catalyse the transfer of sulphate from adenosine 3'-phosphate 5'-sulphatophosphate to L-tyrosyl-tRNA from rat livers. Again a suitably fortified enzyme preparation from rat livers failed to synthesize the *O*-sulphate ester of L-tyrosyl-tRNA under conditions that allowed the sulphation of L-tyrosine methyl ester and the synthesis of L-tyrosyl-tRNA.

Rat liver sulphotransferases catalyse an exchange of sulphate between adenosine 3'-phosphate 5'-sulphatophosphate and *p*-nitrophenyl sulphate or tyramine *O*-sulphate, but not the L-tyrosine *O*-sulphate residues of rat fibrinogen. An investigation of a large number of compounds related to L-tyrosine confirms the narrow specificity of these sulphotransferases for compounds with a free amino group and a modified carboxyl group. The best substrates were found to be L-tyrosine amide, L-tyrosine methyl ester, tyramine and 5-hydroxytryptamine (serotonin). Mixed-substrate experiments indicate that the three last-named compounds are all substrates for the same sulphotransferase.

It therefore appears unlikely that L-tyrosine methyl ester sulphotransferase is involved in the biosynthesis of proteins containing L-tyrosine *O*-sulphate residues, but it is probably important in the sulphation of physiologically active amines. These results are noteworthy in view of the demonstration (Hidaka, Nagatsu, Takeya, Matsumoto & Yagi, 1969) that the sulphation of 5-hydroxytryptamine diminishes its effect on isolated aorta and of the finding (Smith, Kellow, Mullen & Hanington, 1971) of a correlation between a diminished excretion of tyramine *O*-sulphate and susceptibility to dietary migraine in humans.

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The Fate of the Sulphate Esters of Diethylstilboestrol in the Rat

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Previous work in these laboratories on the metabolism and modes of excretion of a number of aryl sulphate esters has shown that only those aryl sulphates that are further conjugated with glucuronic acid appear in the bile to any significant extent (Hearse, Powell, Olavessen & Dodgson, 1969). These findings are consistent with current ideas on the molecular properties that render compounds suitable for biliary elimination (Millburn, 1970), for although the aryl sulphate esters satisfied the anionic and lipophilic requirements it was only after further conjugation with glucuronic acid that the molecular-weight requirement was satisfied.

Studies on the excretion of aryl sulphate esters have been extended to include investigations with diethylstilboestrol disulphate and diethylstilboestrol monosulphate, since both compounds satisfy the criteria for biliary elimination without further metabolic modification.

Mature free-ranging male and female rats received injections (either intraperitoneal or intravenous) of diethylstilboestrol disulphate (1mg/200g body wt.), and urine and faeces were collected over 48h. Of the administered radioactivity 55–80% was recovered in the urine as inorganic [³⁵S]-sulphate. Between 5 and 15% of the radioactive dose appeared in the faeces. In other experiments wholly anaesthetized rats with bile-duct and ureter cannulae received intravenous injections of diethylstilboestrol disulphate (1mg/200g body wt.). Up to 52% of the injected radioactivity was recovered in the urine in 6h as inorganic [³⁵S]sulphate, and significant amounts (12–28%) were eliminated in the bile as ester sulphate. Only traces (less than 1%) of unchanged parent ester were detected in the bile, and the major metabolite in bile was identified as diethylstilboestrol monosulphate monoglucuronide.

When diethylstilboestrol [³⁵S]monosulphate (1mg/200g body wt.) was administered to anaesthetized rats with bile-duct and ureter cannulae, between 45 and 60% of the radioactivity was recovered in the bile within 6h. The major metabolite