Possible Effect of Histamine on Protein Synthesis by the Perfused Rat Liver

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Increases in serum glycoproteins after injury (Keyser, 1949, 1950, 1952; Shetlar, Bryan, Foster, Shetlar & Everett, 1949) are accounted for largely by seromucoid and were tentatively attributed to stimulation of the liver (Keyser, 1960). Increased secretion of adrenocortical hormones is apparently not responsible (Weimer & Redlich-Moshin, 1953; Shetlar & Shetlar, 1955; Keyser, 1959; Keyser, Shuster & Stephens, 1963), though there is evidence for a 'humoral factor' of some sort (Sarcione, Bohne & Krauss, 1965; Bogden, Gray & Fuss, 1966).

Our experiments formed part of a series designed to investigate the effect of physiologically active substances on glycoprotein production. Livers from adult male Wistar rats were perfused with 80 ml of heparinized rat blood plus 90 ml of solution containing NaCl (118.5 mm), KCl (4.75 mm), CaCl₂ (2.54 mm), KH₂PO₄ (1.19 mm), MgSO₄ (1.19 mm) and NaHCO₃ (24.9 mm), 2 g of crystallized bovine plasma albumin (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K.), 200 mg of glucose, 10 mg of penicillin and 10 mg of streptomycin, in apparatus essentially as described by Brauer, Pessotti & Pizzolato (1951). [¹⁴C]Glycine infusion was started at 30 min and blood samples were taken after a further 2½ h (specimen A) and 5 h (specimen B). Except in the controls histamine infusion (35 µg-50 mg/h) was started when specimen A was taken. Radioactivity per unit volume and specific radioactivity, and the ratio B/A, were calculated for seromucoid and non-seromucoid.

Histamine did not significantly affect incorporation of [¹⁴C]glycine into seromucoid; however, a slight decrease ($P < 0.05$) occurred in non-seromucoid specific radioactivity. Since the shifts in the means for seromucoid and non-seromucoid were in opposite directions, the ratio of these (i.e. seromucoid/non-seromucoid) should provide a more sensitive index of any change. Statistical analysis showed a highly significant difference ($P < 0.001$) between histamine and control experiments with respect to both radioactivity per unit volume and specific radioactivity. However, since the values derive from a set of ratios and there is little indication of the form of the underlying frequency distribution, any conclusions must be tentative.

These preliminary observations, together with

those described in the following abstract (Keyser & Cawson, 1970), suggest that the possible role of histamine in serum protein changes is worth further investigation.

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Effects of Histamine and of Bradykinin on Incorporation of [¹⁴C]Leucine by Rat Liver Microsomal Fraction

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Rat liver microsomal suspension was incubated with 'pH 5 enzymes', pyruvate kinase, phosphoenolpyruvate, ATP, GDP and L-[¹⁴C]leucine, as described by Keller & Zamecnik (1956). Addition of histamine (down to 0.016 mm or lower) or bradykinin (down to 0.035 mm or lower) significantly decreased the incorporation of labelled leucine into the precipitated proteins. Results with the perchloric acid-soluble ('seromucoid') fraction were erratic, presumably owing to variable contamination with albumin and perhaps other proteins. Fractionation with trichloroacetic acid-ethanol as described by Majumdar, Tsukada & Lieberman (1967) showed that histamine diminished the incorporation of leucine into both albumin and globulin. The effect of histamine was not inhibited by an antihistamine (mepyramine maleate) in molar concentrations up to 80% of that of the histamine.

It is known that the concentration of some serum proteins is increased after trauma whereas that of

others is diminished. This might result from an increase in both synthesis and breakdown, the former predominating with some proteins and the latter with others. Our findings suggest that histamine, bradykinin or some other hormone may influence the hepatic production of proteins after trauma. Kahlson (1960) has reported that in the rat the histamine-forming capacity of the liver is about 100 times as great as that of other tissues. Relatively high concentrations of histamine might thus occur locally and influence protein synthesis.

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Molybdate Toxicity in *Salmonella typhimurium*

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The gross features of molybdate toxicity in *Salmonella typhimurium* are expressed as a decrease in growth rate and, in some cases, a decreased final cell density. When the organism is grown on sulphate as the sole sulphur source, the degree of toxicity is more severe than on other sulphur compounds tested. The inhibition can be overcome by supplying competitive amounts of sulphate. In substrate amounts, reduced sulphur compounds (thiosulphate and sulphite), which repress some of the enzymes of sulphate reduction but allow molybdate uptake, are effective in reversing molybdate toxicity. Those reduced sulphur compounds (L-cysteinesulphinate and L-djenkolate) that de-repress the sulphur-reduction pathway do not completely prevent toxicity. Mutant *cys* CD-519, which lacks ATP-sulphate adenylyltransferase, is not inhibited by molybdate when grown on djenkolate, suggesting that the residual toxicity in the wild-type grown under similar conditions is due to molybdolysis of ATP (Wilson & Bandurski, 1958) with the consequent depletion of cellular energy. The sulphate-transport mutant *cys* Aabc-20 is not affected by high concentrations of molybdate even under conditions of maximum de-repression of the transport system. L-Cysteine and GSH prevent toxicity by extracellular complex-formation with molybdate, and the complete reversal of toxicity by L-glutamate is due to its metabolism to α -oxoglutarate, which also complexes molybdate.

No evidence for the involvement of copper (cf. Dick, 1956) in molybdate toxicity in *S. typhimurium* has been obtained.

Molybdate, sulphate and thiosulphate appear to be transported by the same system in *S. typhimurium*. The transport of the ions in the presence of each other is competitive; the sulphate-transport-negative mutant fails to transport molybdate or thiosulphate; and the transport of each of the ions is similarly affected by the various degrees of metabolic repression and de-repression.

The ability of inorganic and organic sulphur compounds to reverse or ameliorate the toxicity of molybdate to *S. typhimurium* is consistent with explanations involving the following factors: (a) competition between molybdate and sulphate at the transport site; (b) competition between molybdate and sulphate for ATP-sulphate adenylyltransferase, thus preventing sulphate assimilation and also depleting cell ATP; (c) the entry into the cells of sulphur compounds at a more reduced level than sulphate; (d) the extent of the repression/de-repression of sulphate transport and the sulphate-reduction pathway; (e) detoxication of molybdate by co-ordination with organic ligands either extra- or intra-cellularly.

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Particulate Malate Oxidation in Strictly Aerobic Bacteria

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Disintegrated cells of strictly aerobic bacteria oxidize fumarate far more readily than do disintegrated cells of facultatively anaerobic bacteria (Jones & King, 1968; Jones, 1969).

The most important single factor is the presence of a particulate malate oxidase system in the strict aerobes studied, whereas the facultative anaerobes were found to contain only soluble NAD-linked malate dehydrogenase (Jones, 1969).

Experiments to determine other factors that might account for the differences between the two types of bacteria showed that dilution of cofactors (e.g. NAD and CoA) was a significant factor causing the very low fumarate and malate oxidase activity in the facultative bacteria, and lower enzyme-substrate affinities also contributed to the differences observed; but these studies did not fully explain the