Acetylation of Ribosomal Proteins in Regenerating Rat Liver
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It has been shown that rat liver ribosomal proteins can be acetylated both in vivo and in vitro (Liew & Gornall, 1973). After purification of the ribosomal proteins to remove any possible contamination by lipids, nucleotides and cytoplasmic protein, the radioactivity still remained in these proteins. When the [3H]acetate-labelled ribosomal proteins were hydrolysed in strong acid at 110°C overnight, more than 50% of the radioactivity was removed. These results are regarded as evidence that the labile acetyl groups were incorporated into the ribosomal proteins. Acetylation of ribosomal proteins was found to occur also in rat heart, kidney and thymus gland and in rabbit reticulocytes.

The present report deals with the possible direct relationship between acetylation and protein biosynthesis in regenerating rat liver. It is known that protein synthesis is increased after partial hepatectomy and reaches a maximum rate after approx. 15-24 h (Tsukada et al., 1968). Partial hepatectomy or sham operation was performed in groups of rats according to the following experimental design. [3H]Acetate (5 mCi) was given to rats that had been partially hepatectomized for 1, 3, 6, 14 or 24 h. Then, 15 min after the administration of the tracer, the animals were killed and the ribosomes were isolated as previously described (Liew & Korner, 1970). Ribosomal proteins were obtained by extraction with LiCl–urea. An increased incorporation of [3H]acetate into ribosomal proteins occurred at 3, 6, 14 and 24 h as compared with the sham-operated animals, but not in the partially hepatectomized rats after 1 h. A maximum twofold increase in acetylation of ribosomal proteins over the sham-operated controls was observed 6 h after partial hepatectomy. Ribosomes obtained 3 and 6 h after partial hepatectomy were separated into ‘total’, ‘free’, and ‘bound’ fractions (Redman, 1969). It was found that acetylation of the ribosomal proteins was significantly affected by partial hepatectomy in all three fractions.

In double-isotope-labelling experiments [3H]acetate and [14C]serine were given to rats that had been partially hepatectomized for 3, 6, 14 and 24 h and ribosomes were isolated 15 min after the administration of the tracers. It was found that the incorporation of [14C]serine into the ‘total’ ribosomal proteins was increased to 167% of sham-operated control values at 24 h and that [3H]acetate incorporation was greatest 6 h after partial hepatectomy. These observations suggest that acetylation of ribosomal proteins occurs before an increase of protein synthesis.

Further analysis was carried out to separate nascent chains that were attached to the ‘total’, ‘bound’ and ‘free’ ribosomes by treatment in vitro with either puromycin or EDTA. Ribosomal subunits sedimented through 1 M-sucrose during 198 000g centrifugation overnight. Both the nascent chains and the ribosomal subunits were then treated with NaOH before Millipore filtration to remove any possible contamination by RNA. It was found that [3H]acetate and [14C]serine were incorporated into both the nascent chains and ribosomal subunits. The labelled ribosomal proteins were then treated with trypsin and proteases. The amino acid residues in the hydrolysate were separated by exclusion chromatography and identified by ion-exchange chromatography as described previously (Liew et al., 1970). Three labelled amino acid residues were identified as Nε-acetylserylserine, Nε-acetyllysine and Nε'-acetyllysine.

In vivo the administration of puromycin to rats 6 h after partially hepatectomy, before the injection of the tracers, resulted in a 90% inhibition of [14C]serine incorporation into the ribosomal proteins and a concomitant 60% inhibition of the incorporation of [3H]acetate into ribosomal proteins. These results suggest that acetylation of ribosomal proteins may be closely associated with the regulation of protein synthesis.

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Urinary Enzymes and the Detection of Kidney Damage in the Dog

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The kidney nephron is divided into distinct morphological and functional regions, each having a characteristic complement of enzymes (Mattenheimer, 1968). Schonfield (1965) has suggested that the output of urinary enzymes reflects the anatomic integrity of the kidney and that, because the kidney has a large functional reserve capacity, urinary enzyme excretion should vary before any change in physiological function.

On the basis of previous experiments on rats (Robinson et al., 1967a; Price et al., 1971) we believe that measurement of enzyme excretion in the urine is in fact a more sensitive and an earlier detection of kidney damage than the present commonly used tests such as clearances of various specific substances or the blood concentrations of certain excretory products.

Two forms of kidney damage have been clearly described and can be induced by chemical agents. Tubular damage is produced after dosing with HgCl₂, and administration of ethyleneimine produces papillary damage with resultant malfunction of the collecting tubules and loops of Henle.

In the present series of experiments the following enzymes were monitored in dog urine after a continued dosage programme of one or other of the two agents. β-Galactosidase, N-acetyl-β-glucosaminidase and β-glucosidase were measured as typical lysosomal glycosidases, easily measured by the well-known fluorescent methods (Robinson et al., 1967a), and acid phosphatase (Robinson & Wilcox, 1969) was also used as a standard lysosomal enzyme. Alkaline phosphatase (Fernley & Walker, 1965), mainly a membrane enzyme localized in the brush border, was also measured, along with lactate dehydrogenase (Wacker & Dorfman, 1962), which has been followed by other authors for similar purposes. These enzyme activities were compared with urinary protein excretion and the concentration of serum urea, and the overall results were set against the common clearance tests with inulin, creatinine and p-aminohippuric acid.

Over a period of 7 days during which a regular daily dose of 0.1 mg of HgCl₂/kg body wt. was administered intravenously to the dogs, the activities of the lysosomal enzymes showed hardly significant rises to no more than twofold their normal values. There was no significant increase in urinary protein or lactate dehydrogenase activity, but alkaline phosphatase activity showed a marked increase to about threefold normal during the process of the experiment. All the enzyme activities returned to normal values within 3 days of stopping the injections. After this return to normal a further dosing, this time of oral HgCl₂ (2.0 mg/kg body wt. daily for 10 consecutive days), produced a similar cycle of events. Of the lysosomal enzymes only acid phosphatase and β-glucosidase activities showed significant rises up to threefold normal, those of lactate dehydrogenase and the other glycosidases not being greatly affected. Alkaline phosphatase activity once more showed the most marked rises, up to six times the normal. At the end of this period the