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Stimulation of Incorporation of Precursors into Protein and Ribonucleic Acid of Rat Liver Slices by Bovine Growth Hormone *in vitro*

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Stimulatory effects of growth hormone on RNA and protein synthesis can be observed when it is added to the medium perfusing rat liver (Jefferson & Korner, 1967), but Jackson & Sells (1967) reported that growth hormone *in vitro* had no effect on RNA labelling in liver slices. The concentration of amino acids present during perfusion affects the response to growth hormone, so it seemed possible that the absence of an effect of growth hormone on liver slices was due to the absence of amino acids. This point was investigated and the effects of growth hormone on amino acid incorporation into protein in liver slices were also studied.

Liver slices from male Sprague-Dawley rats, weighing 100–120g., were incubated for 1 hr. in Krebs-Ringer bicarbonate medium containing 3% bovine serum albumin, 8mm-glucose and a mixture of twenty amino acids in the proportions in which they occur in rat serum (Schimassek & Gerok, 1965), with a constant specific radioactivity of [¹⁴C]leucine and 1 μ C of [³H]orotic acid/ml. The slices were homogenized in 0.9% NaCl at the end of the incubation period and after precipitation with 0.5M-HClO₄, the protein was freed of amino acids, RNA and lipids, and radioactivity was measured by the procedure of Munro, Jackson & Korner (1964). When labelling of RNA was studied, the homogenate was treated with 20% (w/v) trichloroacetic acid and RNA was extracted and counted as described by Jefferson & Korner (1967).

It was found that optimum amino acid incorporation into protein occurred when the concentration of amino acids in the medium was six times that in rat plasma, but that the labelling of RNA was virtually unaffected by changes in amino acid concentration. At six times plasma concentration of amino acids, addition of growth hormone at a concentration as low as 10ng./ml. stimulated incorporation of amino acids into protein by 30% and of labelled precursor

into RNA by 27%. No effect of the hormone on labelling of protein *in vitro* was observed when the amino acid concentration was at the normal plasma value. Preliminary observations suggest that RNA labelling is stimulated by the hormone under the latter conditions but not when amino acids are absent entirely.

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Studies on Drug-Metabolizing Activity in Rats Pretreated with Phenobarbitone and Compounds Related to Emetine

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Protein synthesis at the liver microsomal level *in vitro* can be stimulated maximally in incubations of preparations from female albino rats pretreated with sodium phenobarbitone at 315 μ moles/kg. daily for 3 days (Kato, Jondorf, Loeb, Ben & Gelboin, 1966), or with (–)-emetine dihydrochloride (Jondorf & Szapary, 1968) or ($\pm$)-2,3-dehydroemetine dihydrochloride (Jondorf, Drassner, Johnson & Miller, 1969) at 18 μ moles/kg. for 1 day.

Although the microsome-specific stimulation of protein synthesis in phenobarbitone-pretreated animals is also associated with stimulation of microsomal drug-metabolizing activity (Conney, 1967), this is not the case in experiments with microsomal material from rats treated with emetine or 2,3-dehydroemetine. These compounds inhibit drug metabolism *in vitro* (Jondorf, Johnson & Drassner, 1969) measured by liver microsomal *N*-demethylation of aminopyrine (Jondorf, Simon & Avnimelech, 1966) and azo-reduction of neoprontosil (Hernandez, Gillette & Mazel, 1967).

It therefore seemed worth while to test whether the co-administration of phenobarbitone and emetine or 2,3-dehydroemetine would lead to diminished drug metabolizing effects. Two injection schedules were devised, to differentiate between effects of emetine and 2,3-dehydroemetine on already existing, phenobarbitone-induced stimulation and effects on incipient induction. In the

first type, rats were pretreated with phenobarbitone for two days, and on the third day received phenobarbitone and emetine or 2,3-dehydroemetine. When liver microsomal fractions prepared 24hr. later under standard conditions were incubated *in vitro* with aminopyrine or neoprontosil, the drug-metabolizing enzyme activity in the phenobarbitone-stimulated rats was decreased by about 50% after co-administration of emetine or 2,3-dehydroemetine on the third day.

In the second type of injection schedule, animals were treated for 24hr. only, with phenobarbitone and emetine or 2,3-dehydroemetine alone or in combination. Both alkaloids blocked the stimulation of liver microsomal drug-metabolizing enzyme activity brought about by phenobarbitone.

The microsomes prepared from phenobarbitone-pretreated animals can *N*-demethylate *N*-methylated (–)-emetine *in vitro* at the rate of 50nmol/mg. of microsomal protein/½hr. No such *N*-demethylating activity is seen in corresponding experiments with control microsomal preparations and *N*-methylemetine as substrate (Jondorf, Johnson & Drassner, 1969). This would explain why animals pretreated with phenobarbitone tolerate rather lower doses of *N*-methylemetine than control animals. Lethal concentrations of metabolically formed emetine may build up in rats with *N*-demethylating drug-metabolizing enzyme activity enhanced by phenobarbitone.

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The Metabolism of Caffeic Acid in Humans: the Dehydroxylating Action of Intestinal Bacteria

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Previous reports that caffeic acid (3,4-dihydroxycinnamic acid), administered orally to humans, gave rise to urinary *m*-hydroxyphenyl derivatives by a dehydroxylation process (Booth, Emerson, Jones & De Eds, 1957; Shaw, Gutenstein & Jepson, 1963) have been confirmed and extended. Oral caffeic acid rapidly causes the urinary appearance of *O*-methylated phenolic acids (including ferulic acid, isoferulic acid and vanillic acid and their conjugates), whereas the dehydroxylation process is delayed many hours after ingestion. The relationship between the methylation (a tissue reaction) and the dehydroxylation (due solely to the action of intestinal bacteria) has been established by suppressing the gut flora with neomycin, or by delaying caffeic acid absorption by administering it in specially-coated capsules, or both. In addition to the acid, each capsule contained quinine sulphate, which acted as a readily excreted fluorescent marker to indicate when the capsule released its contents. The capsule technique was also used to release *m*-hydroxybenzoic acid directly into the small intestine, to show that neomycin treatment really had prevented dehydroxylation and was not just interfering with the absorption of *m*-hydroxy acids that were still being formed.

The bacteria responsible for this dehydroxylation have not yet been investigated, but Perez-Silva, Rodriguez & Perez-Silva (1966) claimed that a strain of *Pseudomonas* in rat faeces is capable of performing the reaction.

All the feeding experiments were performed with the patient on a diet containing the minimum of plant and fruit foodstuffs, with rigorous exclusion of foods known to contain or give rise to caffeic acid and related phenolic acids.

The importance of considering the part played by the varied and largely unidentified species of intestinal bacteria in the metabolism of minor dietary components, including therapeutic agents, is emphasized.

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