Lack of Correlation between RNA Synthesis and Hormonal Induction of Liver Enzymes in Carbon Tetrachloride Treated Mice

The early, precise and the orderly manner in which biogenesis of selected proteins is amplified, following a corticosteroid hormone administration, affords an opportunity to understand the mechanisms underlying the expression of genetic information in mammalian liver. In recent years, this induction of enzymes by corticoids is believed to be secondary to the hormonal stimulation of hepatic RNA sythesis[1-3]. An appraisal of the nature of association between these two processes may be made by determining whether induction of enzymes can still be selectively modified under those conditions where: a) hormonally increased RNA synthesis should remain unimpaired, and b) hormonal influence on RNA may be eliminated. In previous studies it had been observed that carbon tetrachloride (CCl_4), an agent that altered liver Kupffer cell function, was capable of selectively modifying the response of some of the hormonally inducible enzymes[4]. This provided the lead to explore the influence of this chemical on RNA synthesis as related to hormonal enzyme induction in liver.

Data in Table I demonstrate that, as early as 3 h after CCl_4 administration, 5-^{3}H-orotic acid incorporation into hepatic RNA was 30% below control and that this was still suppressed 19 h after the hepatotoxin. Since there was no concordant decrease in precursor pool specific activity, it is clear that there was an actual inhibition in the rate of precursor incorporation into RNA. It is most significant that, at both time periods, cortisone did not augment RNA synthesis in CCl_4-poisoned mice. In fact, incorporation of precursors into RNA was less in mice given the hepatotoxin and cortisone than in those given the toxin alone. The reason for this is not understood, but it is clear that the results were independent of changes in the precursor pool size. Data in this table also show that cortisone alone increased RNA synthesis in normal mice.

The response of 2 hormonally inducible enzymes under these conditions is shown in Table II. It is evident that, within 4 h, CCl_4 did not significantly lower the activity of tryptophan oxygenase (TO) but increased significantly the level of tyrosine transaminase (TT) in normal, intact mice. 20 h after the toxin, TT levels were near normal but TO was almost completely eliminated. Thus, at least in the first few hours, when the rate of native RNA synthesis was 30% below the control level, CCl_4 mediated release of endogenous, corticoids permitted normal homeostasis of TO and some induction of TT; upon prolonged depression in the rate of RNA synthesis, however, both these processes were adversely affected. Data in the latter part of this table establish that the induction of TT and TO proceeds normally in mice given both CCl_4 and exogenous cortisone 4 h prior to assay. However, cortisone did not induce either enzyme in animals that had been pretreated with CCl_4 for 16 h. Induction of enzymes by exogenously administered cortisone was abolished, therefore, when RNA synthesis had been previously kept suppressed for 16 h but, at an earlier time point, biogenesis of selected proteins could proceed without reversal in the lowered rate of precursor incorporation into RNA.

Time course of the induction of TT and TO in CCl_4-poisoned mice, where incorporation of precursors into RNA is suppressed, provides a clear and direct lead into the nature of the relationship between the induction of enzymes by the corticoids, on the one hand, and synthesis of RNA, on the other. The results presented here argue against the possibility that biogenesis of specific enzymes proceeds via a mass action or 'pushing' mechanism coming into effect due to grossly increased level of total RNA. The general, nonspecific increase in total RNA synthesis effected by cortisone, therefore, does not seem to be necessary for the induction of specific enzymes by the hormone. Earlier studies with actinomycin-D[5], and the fact that enzyme induction did not occur in mice previously treated with CCl_4 for 16 h, all show that some selected synthesis of RNA, however, may be required for the inductive process.

Mammalian transfer RNA turns over with a half-life time of approximately 5 days[6] and this profile is not generally influenced by cortisone[7]. So it would appear that

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Table I. Inhibitory effect of carbon tetrachloride on RNA synthesis in the mouse liver

	Time with CCl_4 prior to hormone			
	0 h		16 h	
	Acid soluble	RNA	Acid soluble	RNA
Control	329 ± 21	655 ± 50	2469 ± 87	5700 ± 117
Cortisone	341 ± 53	786 ± 113	2108 ± 66	5970 ± 731
CCl_4	368 ± 33	430 ± 45	2218 ± 124	3547 ± 212
CCl_4 + Cortisone	351 ± 50	313 ± 12	2781 ± 254	3987 ± 56

Swiss, male mice (25 ± 2 g) were starved overnight and injected s.c. with 0.1 ml CCl_4 (Prolabo). Cortisone acetate suspension (5 mg/100 gm body wt.) was given either concurrently with or 16 h after CCl_4. In the former case 3 h, and in the latter 60 min, prior to sacrifice 10 μCi/100 g body weight of 5-^{3}H-orotic acid (16 Ci/m*M*) were given i.p. All assays were done 3 h after hormone administration. Isolation and quantitation of RNA and the acid soluble pool were carried out by the previously described procedures[7,12]. All samples were mixed with 10 ml of Instagel (Packard) and corrected for background and quenching as previously described[7,12]. The values represent average of at least 4 separate determinations ± the standard error of the mean and are expressed as counts per min/mg for RNA and CPM/A_{260} for the pool.

Table II. Induction of enzymes by cortisone in livers of carbon tetrachloride treated mice

	Time with CCl_4 prior to hormone			
	0 h		16 h	
	TT	TO	TT	TO
Control	5.17 ± 0.04	1.44 ± 0.02	5.17 ± 0.04	1.44 ± 0.02
Cortisone	9.53 ± 0.30	2.29 ± 0.02	9.53 ± 0.30	2.29 ± 0.02
CCl_4	7.67 ± 0.20	0.97 ± 0.10	4.40 ± 0.30	0.19 ± 0.07
CCl_4 + Cortisone	11.63 ± 0.02	2.06 ± 0.03	5.10 ± 0.70	0.17 ± 0.05

Details of the experimental design are given in Table I, except that all assays were carried out 4 h after hormone administration. The procedures for determination of liver tyrosine transaminase (TT) and tryptophan oxygenase (TO) activities are well established and have been described in detail previously[4,11]. The enzyme activities are expressed as μg *p*-hydroxyphenyl-pyruvic acid/mg liver/10 min and μg kynurenine/mg liver/h for TT and TO, respectively. All values represent average of 5–6 separate determinations ± the standard error of the mean.

enhanced transcriptional activity is a prerequisite for enzyme induction and this may be selectively amplified by the steroid even when RNA synthesis in general is otherwise suppressed. As the latter remains lowered for protracted periods of time, and as the available messenger RNA is presumably exhausted, both the endogenous homeostasis of enzymes, and their induction by the hormone, are gradually impaired. It has previously been established that TO is not inducible by the endogenously released corticoids at any time after CCl_4 administration[4]. So induction of TT in such animals under selected conditions (Table II; 4) could reflect differences in the relative stabilities of mRNAs for these 2 enzymes. This thesis explains the observation that synthesis of uracil-rich, DNA-like RNA (presumably mRNA) can be seen within 10–20 min after cortisone administration in rats and therefore precedes both the enzyme induction and the general ribosomal RNA synthesis[8], leading eventually to liver hypertrophy and increased RNA content. A similar relationship has previously been observed in hepatoma cell cultures[9,10]. In any event, these studies form the first evidence that, in mammalian liver, induction of selected enzymes by a corticosteroid can occur at a time when the stimulatory effect of the hormone on the synthesis of total RNA is completely eliminated.

It should be remembered that CCl_4 rapidly and markedly alters Kupffer cell function in the liver[4]. Since the cortisone inducible enzymes are found in liver parenchymal cells, it had previously been suggested that initial information processing may occur in the reticuloendothelial system of the animal (for a brief review see[11]). The manner in which this is accomplished remains to be determined, but it is clear that the information thus gathered would be of unusual singificance in delineating the control, organisation and expression of genetic information in mammalian liver.

Résumé. Chez des souris traitées par le tétrachlorure de carbone l'induction de quelques enzymes sélectives par la cortisone s'est produite à un moment où la synthèse de l'ARN total était inférieure au niveau observé chez les témoins.

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Biochemical Changes of Brain and Liver in Neonatal Offspring of Rats Fed Monosodium-L-Glutamate

Much attention has recently been focused on some of the possible hazards of high levels of monosodium-L-glutamate (MSG) given by injection or fed to several species of experimental animals. These effects range from obesity and neuroendocrine disturbances to sterility and brain lesions[1–8]. In a previous study[9] we attempted to establish a biochemical basis for the action of MSG by feeding it to rats at levels up to 20% of the diet and then measuring the concentrations of a number of brain and liver constituents. Analysis of liver indicated that dietary MSG had no effect on protein, RNA, DNA, glutamate, lactate, malate or α-glycerophosphate. Concentrations of glutamate, glutamine, aspartate, DNA and protein and activity of glutamic decarboxylase (GAD) in brain remained constant while γ-aminobutyric acid (GABA) concentrations were significantly decreased in animals fed MSG. The rats ingesting MSG exhibited increased irritability, which may be related to decreased levels of brain GABA[9,10].

The purpose of the present investigation was to study the effects of dietary MSG on some selected brain and liver metabolites of second generation neonatal rats born to parents fed a diet supplemented with 10% MSG.

Materials and methods. Holtzman weanling rats were fed Purina laboratory chow alone or supplemented with 10% MSG for 100 days. The rats were mated on a one-to-