The Relationship Between the Effect of Endotoxin on Inducible Enzymes and Biosynthetic Activity in Rat Liver

It has previously been shown that specific enzyme alterations are inflicted by endotoxin, and other agents capable of altering the Kupffer cell function, by a direct action on rat liver[1]. The possible involvement of the reticuloendothelial system (RES), as contributing to liver parenchymal cell homeostasis, has such fundamentally important implications in assessing the nature of liver enzyme regulation that it seemed important to relate the aforementioned changes with liver biosynthetic activity. This is especially relevant since glucocorticoid enzyme regulation in liver is believed to be secondary to increased RNA and protein synthesis[2], and since all the indirect evidence had suggested that the repressive action of endotoxin on liver tryptophan oxygenase(TO) is a reflection of decreased amounts of the enzyme protein in vivo[1,3], although proof of actual decrease is still wanting. These effects were not evident on liver tyrosine transaminase (TT), so endotoxin was not acting as a generalized hepatotoxin[1,3]. The experiments reported here are part of a large study designed to assess the nature of competition between cortisone and RES-active agents on lymphatic and nonlymphatic organs: specifically, to explore whether the early derangements in selected liver enzyme function are manifest at the level of general RNA and protein synthesis in this organ during the first few hours of endotoxicosis.

Data in the first part of the Table establish that, in intact rats, the incorporation of ^{3}H-orotic acid into total liver RNA increased 4 h after endotoxin and that this was not due to augmented precursor pool specific activity. With ^{14}C-glycine, however, the assessement of RNA biosynthesis was equivocal, due to increase in the pool size of this precursor – as previously reported for intact rats[4]. At this and other time points, small increase in ^{14}C-glycine incorporation into liver proteins occurred, but it could not be ascertained whether this was a reflection of altered pool size since no efforts were made to isolate free glycine. In other experiments, total RNA and protein synthesis in animals given endotoxin and cortisone were similar to those seen after treatment with the toxin. From such studies it could not be determined whether these effects in the intact rat were innate to endotoxin or secondary to the release of endogenous glucocorticoids as a consequence of endotoxic stress.

The second part of the table shows that in adrenalectomized rats given endotoxin, the 13% decrease and the 34% increase in the ^{3}H and ^{14}C acid soluble pools, were associated, respectively, with the concurrent 17% decrease and 5% increase in the incorporation of the precursor ^{3}H-orotate and ^{14}C-glycine into RNA. Similar results were obtained at other incorporation and/or time intervals in the first few hours after endotoxin. Thus, RNA synthesis was measured by incorporation of both a purine and a pyrimidine precursor and found to remain unaffected during the first 4 h after endotoxin. It is also clear that cortisone increased the incorporation of both the isotopes into liver RNA and, considering the large and consistent alterations in the precursor pool size after endotoxin in this and other experiments, this was not further influenced by endotoxin. Expressed as % change, the RNA synthesis was 30% above control, as judged by ^{3}H-orotate incorporation (and less with ^{14}C-glycine), after cortisone and endotoxin. Accordingly, it would appear that prevention of glucocorticoid induction of TO (but not of TT) by the toxin at this time period, as shown in the last part of the

[1] M. K. AGARWAL, W. W. HOFFMAN and F. ROSEN, Biochim. biophys. Acta *177*, 250 (1969).
[2] M. FEIGELSON and P. FEIGELSON, in *Advances in Enzyme Regulation* (Ed. G. WEBER, Pergamon Press, New York 1965), vol. III, p. 11.
[3] M. K. AGARWAL and L. J. BERRY, J. reticuloendoth. Soc. *4*, 490 (1967).
[4] J. J. BETHEIL, M. FEIGELSON and P. FEIGELSON, Biochem. biophys. Acta *104*, 92 (1965).

Effect of endotoxin on hepatic enzyme induction and RNA synthesis in rat liver

		Control	Endotoxin	Cortisone	Endotoxin + cortisone
Intact					
Acid soluble	^{3}H	6,640 ± 08	6,620 ± 120		
	^{14}C	632 ± 56	848 ± 40		
	^{3}H	29,430 ± 2,100	34,195 ± 986		
RNA	^{14}C	1,645 ± 126	1,995 ± 85		
Adrenex					
Acid soluble	^{3}H	8,341 ± 540	7,369 ± 398	6,386 ± 291	5,661 ± 1,248
	^{14}C	930 ± 92	1,245 ± 74	958 ± 90	1,216 ± 220
	^{3}H	20,794 ± 2,460	17,697 ± 138	23,410 ± 1,487	17,244 ± 3,885
RNA	^{14}C	1,999 ± 297	2,109 ± 221	2,329 ± 157	2,623 ± 470
Tryptophan oxygenase		43.9 ± 2.9	31.3 ± 2.6	143.9 ± 4.4	46.6 ± 1.7
Tyrosine transaminase		0.64 ± 0.04	2.08 ± 0.18	4.41 ± 0.21	5.20 ± 0.37

Holtzman rats (150–200 g) were starved overnight and injected i.p. with either 500 μg of endotoxin (Difco), 5 mg cortisone acetate (Roussel) or both. 2 h later, and 2 h prior to sacrifice, a saline mixture of 10 μCi/100 g body wt. of 5-^{3}H-orotic acid (16 Ci/m*M*) and 5 μCi/100 g body wt. of U-^{14}C-glycine (34 mCi/m*M*) was injected i.p. With adrenalectomized rats, only 5 μg of endotoxin was used and the isotope was injected 90 min prior to sacrifice. The livers were homogenized directly in 10 volumes of cold 0.3 *M* $HClO_4$; the acid soluble, the RNA and protein fractions were isolated and quantitated. Corrections for background, quenching and spilling of ^{14}C into the ^{3}H window were carried out as previously described [7,9,10]. All values are average of 3–4 separate determinations ± the standard error and are expressed as counts/min/mg for RNA and cpm/A_{260} for the pool. The values for TT and TO, obtained 6 h after the toxin and 4 h after the hormone, are taken from a previous publication (1) and expressed in units of activity per unit weight of tissue.

Table, is not due to gross impairment of general RNA and protein synthesis effected by the hormone. How these relationships may be influenced by the known permeability changes following endotoxin administration[5], and possibly reflected here as altered pool sizes, can only be conjectured. It is clear, in any event, that lowering of TO (but not of TT) in intact and adrenalectomized rats is not a reflection of general biosynthetic derangement as a consequence of endotoxin administration. In fact, as with cortisone, hepatomegaly follows endotoxin administration[6]. So it is likely that an increase in biosynthetic activity would ensue later in endotoxicosis. To understand selected enzyme alterations in the early hours of endotoxemia, therefore, one could imagine either formation or release of inhibitors for selected enzymes or gene modulation, among others, via specific events of transcription or translation which would probably escape detection in the background of general synthetic activity.

As regards the mechanism of glucocorticoid enzyme induction, there has been much speculation on the role of RNA synthesis in this process (for brief review see[7]) and there is a tendency to treat the subject in a collective manner. Since TT and TO behave in the opposite fashion at a time when the hormone mediated stimulation of RNA synthesis remains apparently unaffected (see the Table), it seems logical to conclude that relationship between modification of RNA synthesis, and induction of an enzyme by the corticoid, cannot be applied to another enzyme system per se. In other words, hormonally increased RNA synthesis, on the one hand, and induction of enzymes, on the other, are not an all-or-none phenomenon. In hepatoma cell system, synthetic corticoids can induce some enzymes in absence of gross stimulation of RNA synthesis[8]. The results presented here form the first clear indication that, in rat liver, general non-specific increase in RNA synthesis effected by the corticoids is probably not causally related to the induction of specific enzymes but some selective synthesis of RNA may be required for the inductive process.

The general utility of employing a mixture of ^{3}H-orotic acid and ^{14}C-glycine within the same animal is emphasized. This allows: a) assessment of RNA biosynthesis in liver as a function of incorporation of both a purine and a pyrimidine precursor, b) in organs other than liver, such as thymus and spleen, glycine is a better precursor for RNA than orotate (this was actually observed in the present set of experiments, though not reported here) and c) an indication of the rate of protein synthesis may also be gained at the same time.

Résumé. Étude du mécanisme d'action de la cortisone. L'induction de tryptophane oxygénase hépatique peut être complètement abolie par l'injection d'endotoxine à un moment où la synthèse hormonale de l'ARN total n'est apparemment pas modifiée.

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[5] A. Nowotny, Bact. Rev. *33*, 72 (1969).
[6] M. K. Agarwal and L. J. Berry, J. reticuloendoth, Soc. *5*, 353 (1968).
[7] M. K. Agarwal, J. Hanoune, F. L. Yu, I. B. Weinstein and P. Feigelson, Biochemistry *8*, 4806 (1969).
[8] G. Tomkins, L. D. Garren, and B. Peterkofsky, J. Cell. comp. Physiol., Suppl. *66*, 137 (1965).
[9] M. K. Agarwal and I. B. Weinstein, Biochemistry *9*, 503 (1970).
[10] M. K. Agarwal, J. Hanoune and I. B. Weinstein, Biochim. biophys. Acta *224*, 259 (1970).
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Stabilization and Enhancement of Glutamate Decarboxylase Activity in Extracts of the Slime Mold, *Physarum polycephalum*

Success in isolating enzymes from the slime mold *Physarum polycephalum* has been quite limited. The most notable purification achieved to date has been that of a ribonuclease which was extracted from the culture medium of the organism and not directly from the plasmodium[1]. A previous report of efforts to purify slime mold glutamate decarboxylase (E.C.4.1.1.15) suggested that the instability of this enzyme may be due to its inactivation by endogenous phenols or their oxidation products[2]. The purpose of the present study has been to test certain H-bonding additives for their ability to enhance and stabilize the glutamate decarboxylase activity of plasmodial supernatants. Cysteine and ascorbic acid, which inhibit protein phenol interactions stabilize the enzyme while caffeine and alumina (aluminium oxide-G, Merck) enhance its activity, probably by disrupting preformed H-bonded complexes. The enzyme is stable in the absence of additives when separated from $FeCl_3$-positive components and its activity is not then enhanced by caffeine or alumina.

Techniques for the preparation of lyophilized plasmodial powders, assay of glutamate decarboxylase activity and protein determination have been detailed elsewhere[2,3]. One unit of enzyme is that amount which mediates the decarboxylation of 1 µmole of glutamate in 1 min at 36 °C. Specific activity denotes milliunits (mU) of enzyme per mg of protein. The stabilizing effects of soluble additives (5 mM in 0.1 M phosphate buffer, pH 5.8) were tested by combining 10 ml of each with 10 ml of the supernatant obtained by homogenizing 3 g dry plasmodium in 50 ml buffer and centrifuging at $12{,}700 \times g$ (20 min, 10 °C). Temperature was adjusted to 25 °C and samples were assayed at 1 h intervals for 3 h. Insoluble additives, 3 g/100 ml, were hydrated in buffer overnight and added during homogenization[4]. First-order rate constants of inactivation (k) were obtained from: $-dA/dt = kA$, where A is specific activity and t is time in min.

Supernatants from 240 mg samples of plasmodium in 10 ml of each reagent or in 10 ml buffer plus 400 mg insoluble additive (dry weight) were assayed for initial activity. Concentrations of the test solutions were increased to as high as 0.1%. The possible stabilizing effects of those substances found to enhance enzyme activity (caffeine, 0.1% and alumina) were then tested as described above except that the additives were included in the homogenizing medium.

A pigment-free preparation was obtained from plasmodial supernatants and glutamate decarboxylase stability in the absence of $FeCl_3$-positive components was investigated. 1 g of dry plasmodium was homogenized in 100 ml of a medium consisting of $MnCl_2$, 0.1 M and caffeine 1 mM