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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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

in 0.1 M NH_4Cl buffer, pH 4.7, and centrifuged for 30 min. The $(NH_4)_2SO_4$ fraction sedimented between 40–55% saturation was dialyzed[2] for 3 h against 500 ml 0.1 M potassium phosphate buffer plus caffeine 1 mM, and the centrifugation repeated. The preparation was again fractionated with $(NH_4)_2SO_4$ from 200 ml of the phosphate buffer minus caffeine, dialyzed 16 h against 1 l of 0.01 M phosphate buffer, centrifuged for 1 h and lyophilized. All operations were at 0–4 °C. The centrifugal force was 28,700 $\times g$ in all cases except that active $(NH_4)_2SO_4$ fractions were sedimented at 3000 $\times g$. Phosphate buffer pH was 5.8. The lyophilized powder was resuspended in 20 ml 0.1 M phosphate buffer and enzyme stability tested as described above for supernatants over a period of 5 h.

The additives which altered enzyme stability are listed in Table I. With additives other than cysteine and ascorbate, the glutamate decarboxylase activity of supernatants decreased sharply during the first hour. Thereafter, inactivation rates were relatively low and linear as indicated by the k-values. This supports a previous suggestion that some inactivation is by a preformed slime mold component[2]. No inactivation occured in the presence of cysteine. Complete stabilization is conferred by this reagent for as long as 8 h. Its effectiveness over longer periods was not measured. Cysteine can protect the enzyme against inactivation by inhibiting: protein-phenol interactions, quinone formation via its copper-chelating and reducing properties, and quinone-oxidation of essential SH groups. Cysteine also forms thioether linkages with quinones[4].

Table I. Additives affecting the stability of glutamate decarboxylase activity of slime mold supernatants[a]

Additive[b]	0-h specific activity (mU/mg protein)	1-h inactivation (%)	3-h inactivation (%)	k_{1-3}[c]
Buffer (control)	34.6	49.1	85.3	5.9×10^{-3}
Cysteine	34.5	0	0	0
Ascorbate	34.6	21.4	56.0	3.6×10^{-3}
Phenylthiourea	30.2	56.2	96.5	7.6×10^{-3}
KCN	1.1	100.0	–	–

[a] Values are means of results from 3 repetitions. [b] 2.5 mM in 0.1 M phosphate buffer, pH 5.8. [c] Inactivation-rate constant for 1–3 h incubation in units of reciprocal min.

Table II. Effects of alumina and caffeine on initial activity and stability of glutamate decarboxylase in slime mold supernatants

Initial activity[a]	Additives			
	Control	Alumina (40 mg/ml	Alumina +caffeine	Caffeine (0.1%)
Activity (ml)	16.0	27.4		25.9
Protein (mg/ml)	2.0	2.1		2.1
Specific activity	35.7	57.7		52.0
Increase in specific activity (%)	–	61.6		45.7
Stability[b]				
0-h specific activity	26.3	51.7	60.1	50.2
1-h inactivation (%)	47.7	43.2	44.2	41.2
3-h inactivation (%)	75.7	76.4	78.1	70.1
k_{1-3}	4.5×10^{-3}	4.7×10^{-3}	5.1×10^{-3}	4.2×10^{-3}

[a] Supernatants from 240 mg/10 ml homogenates. Values are means of repetitions. [b] Supernatants from 3 g/50 ml homogenates.

Ascorbate did not prevent but reduced the rate of enzyme inactivation. This rate was first-order over the 3 h observation period. EDTA has previously been identified as a weak stabilizer of the enzyme[2]. However, effects of the phenolase inhibiting additives tested in this study were negative. KCN totally disrupted initial activity, as expected[5]. Phenylthiourea increased the inactivation rate.

Hydrated alumina and caffeine significantly enhanced initial activity of the enzyme (Table II). These additives release proteins[6] and viruses[7] from complexes with phenols suggesting that H-bonded complexes are formed during the freezing and lyophilizing of plasmodia. Browning, characteristic of such interactions, is observed in frozen plasmodia following prolonged storage. Neither alumina nor caffeine stabilize the enzyme appreciably. The pigment-free enzyme preparation obtained had a specific activity of 118 and the yield was 41.7%. This activity was constant throughout each of the 1-h assay periods whether the gas phase was air or nitrogen. At this stage of purification the enzyme was completely stable over the 5 h observation period in the absence of cysteine and ascorbate. Neither caffeine nor alumina enhanced its activity and no browning occurred. This suggests that the observed effect of stabilizing additives is a protection of the enzyme against endogenous slime mold components or their derivates and not a shielding of essential groups on the enzyme against autoxidation.

The above findings are the result of current efforts to develop a medium for stabilizing and purifying slime mold glutamate decarboxylase in an unmodified form. Treatments applied by other workers for reversing or inhibiting enzyme inactivation by phenolic compounds were also tested. Pharmaceutical grade and cross-linked polyvinylpyrrolidone, Tween-80, borate, urea, and untreated Al_2O_3 used eleswhere[4] with occasional or frequent benefits were ineffective. Treating plasmodia with acetone or defatting[8] with butanol and acetone increased browning and inactivation rates. Glutamate decarboxylase is also active in slime mold spores and in microsclerotia prepared in media described by BREWER et al.[9]. Although sufficient material for valid comparisons is not yet available, activity of the spore enzyme has been observed to proceed at a constant rate when assayed for 1 h in an atmosphere of air whereas that of its counterpart in sclerotia declines with time.

Zusammenfassung. Aktivität und Stabilität der Glutamat-Decarboxylase von *Physarum polycephalum* in Gegenwart von Zusätzen, welche die Wechselwirkung zwischen Proteinen und Phenolen hemmen oder aufheben, wird beschrieben. Cystein und Ascorbinsäure stabilisieren das Enzym, während hydratisiertes Aluminiumoxyd-G und Koffein die spezifische Aktivität erhöhen.

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