Starvation-Induced Adaptation of Rat Liver Tyrosine Transaminase and Serine Dehydrase

Current studies have indicated two separate mechanisms which help to explain the adaptive formation in vivo of various rat liver enzymes[1,2]. The first, the hormonal type, includes agents such as glucocorticoids as inducers and the second, the substrate type, comprises the substrate of an enzyme as its inducer. Effective enzyme increases which occur adaptively in response to non-specific stimulus, such as starvation, are generally thought to be caused secondarily by the corticoids (first mechanism) released by the adrenals of the stressed animal[3]. Because of its physiological importance, the nature of the starvation-induced adaptation was examined with regard to rat liver tyrosine transaminase (L-tyrosine 2-oxoglutaric aminotransferase, EC 2.6.1.5) and serine dehydrase (EC 4.2.1.13), both of which[2,4] are increased following glucocorticoid administration as well as starvation.

Male Wistar rats (Centre de Sélection des Animaux de Laboratoire du C.N.R.S.), fed a commercial diet (17% crude protein) at 4 weeks of age, were grouped according to the schedule shown in the Table. Bilateral adrenalectomy was performed under ether anaesthesia. The adrenalectomized rats were offered 1% NaCl solution for drinking and sacrificed on the 7th day after the date of operation. 33% homogenates, prepared in the cold from fresh liver in 0.25 M sucrose, were centrifuged at 105,000 g for 45 min in a refrigerated Spinco Model L Ultracentrifuge, and enzyme activities were determined in the supernatant under conditions in which the reaction rates were linear with time and enzyme concentration. For tyrosine transaminase the reaction mixture containing enzyme, 100 μg pyridoxal phosphate, 150 μm phosphate buffer, pH 7.0, 10 μm diethyldithiocarbamate, 12 μm L-tyrosine, and 50 μm α-ketoglutarate, was made to final volume of 3 ml (pH 7.1) and incubated at 37 °C for 15 min. For serine dehydrase 2 ml reaction mixture (borate buffer, final pH 8.1) containing enzyme, 100 μg pyridoxal phosphate and $10^{-1}M$ L-serine was incubated at 37 °C for 20 min in N_2 atmosphere. The reactions were stopped by 0.5 ml of 50% trichloroacetic acid. For transaminase, p-hydroxyphenylpyruvate formed was routinely estimated in an aliquot of the supernatant by a modified Brigg's reaction[5] and occasionally checked by the enol-borate procedure[6]. For serine dehydrase, the pyruvate formed was measured in the supernatant as the phenylhydrazone[7].

The data on enzyme activities, shown per g liver and 100 g body weight (changes in enzyme activities in different groups, when calculated per mg protein and DNA content of the liver, followed the same patterns respectively as those per g liver and 100 g body weight), are summarized in the Table. It is clear that 48 h starvation induced an increase in liver tyrosine transaminase and serine dehydrase in non-adrenalectomized as well as adrenalectomized rats. The degree of increase was even higher after adrenalectomy, mainly due to a lowering of the basal enzyme activity. Thus liver serine dehydrase, in agreement with a previous report[4], was depressed by as much as 50% in the adrenalectomized rats, and in these animals the effect of starvation was manifest by 6- to 7-fold increases of the enzyme level compared to only 2- to 3-fold in the non-adrenalectomized rats. These enzyme elevations, as shown for adrenalectomized rats in the Table, were effectively suppressed (about 40%) by puromycin, suggesting the possible formation of new enzymes in the process. In any event, since the adrenal was not an obligatory requirement for eliciting the starvation-induced adaptation, the suggestion that corticoids acted as inducers (first mechanism) in this case may be excluded. The alternative possibility of the substrates acting as inducers (by their accumulation in the liver) was also tested. Free amino acids extracted[8] from the livers of the adrenalectomized rats (fed and starved 48 h) were assayed in a Technicon Autoanalyzer after passing the extract through a Dowex 50 column[9]. The results revealed no appreciable change in the level of free tyrosine, tryptophane, or phenylalanine (substrates of the

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Adaptive increase of hepatic tyrosine transaminase and serine dehydrase of 5- to 8-week-old rats (about 150 g body weight) in starvation and its inhibition by glucose administration

	Group	Tyrosine transaminase (μm p-hydroxyphenylpyruvate/h)		Serine dehydrase (μm pyruvate/h)	
		per g liver	per 100 g body weight	per g liver	per 100 g body weight
Adrenals intact	Fed	40 ± 14[a] (12)	192 ± 6	399 ± 25 (6)	1717 ± 21
	Starved 48 h	87 ± 4 (6)	277 ± 14	1052 ± 66 (6)	3388 ± 239
	Starved 48 h + glucose[b]	55 ± 6 (5)	168 ± 15	418 ± 11 (6)	1369 ± 21
Adrenalectomized	Fed	34 ± 3 (9)	133 ± 11	195 ± 30 (7)	755 ± 94
	Starved 48 h	85 ± 7 (7)	256 ± 22	1431 ± 92 (10)	3966 ± 202
	Starved 48 h + glucose[b]	34 ± 2 (7)	99 ± 6	685 ± 91 (7)	1955 ± 255
	Starved 48 h + puromycin[c]	54 ± 2 (6)	160 ± 9	743 ± 17 (6)	2436 ± 298

[a] Average ± S.E. for the number of animals in parenthesis. [b] 1–1.3 g of D-glucose in water were given daily to each rat by intraperitoneal route in two divided doses; the animals were sacrificed 4–5 h after the last dose of 0.6 g. [c] 3 doses, each of 10 mg puromycin aminonucleoside (in 0.9% NaCl)/rat/day, were injected intraperitoneally. The rats were killed 4 h after the last dose.

transaminase[10]), about 50% decrease of threonine plus serine (substrate of serine dehydrase) and approximately 25% reduction in the level of total free amino acids. There was no indication that the enzyme elevation caused by starvation was associated with elevation of their substrates in the liver. The above findings, then, suggest that starvation initiated the elevation of the two enzymes in the liver by a mechanism independent of either glucocorticoid or substrate induction.

The reason for the adaptive increases of the hepatic transaminase and the dehydrase in starvation is not clear, but the physiological relevance of the changes is understandable. The critical need for energy in starvation is ultimately contributed by tissue protein catabolism. The elevation of specific amino acid catabolic enzymes in such a situation is an appropriate adaptation towards homeostasis. Accordingly when an external energy source, D-glucose, was provided in gram quantity to the rats with the onset of starvation, the enzyme elevation, as shown in the Table, was either abolished or suppressed. An inhibitor in the enzymes of the starved rats given glucose could not be detected. The sugar or its metabolites (glucose-6-phosphate, glucose-1-phosphate, lactate) when added in vitro in millimolar amounts did not inhibit the enzyme activities. Also the administration of the sugar to fed rats did not lower the basal transaminase and dehydrase activities of the liver. Such an in vivo inhibitory effect of injected glucose was previously reported[11] with the starvation-induced adaptive increase of cysteine desulfhydrase activity in the chick liver. It is of interest that similar inhibition by glucose of the adaptive increases of a microsomal enzyme, liver dimethylaminoazobenze reductase induced by starvation[12] and liver threonine dehydrase and ornithine transaminase by casein feeding[13] has also been reported in recent publications.

Résumé. Les activités de la tyrosine transaminase et de la sérine déhydrase sont augmentées dans le foie de rats normaux ou surrénalectomisés soumis au jeûne. Ces augmentations, qui sont supprimées par la puromycine ou le glucose, ne sont dues ni à une sécrétion d'hormones surrénaliennes ni à une accumulation des substrats des enzymes.

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Role of Heavy Water as Solvent in the Antigen-Antibody Precipitin Reaction

Mice drinking 30% heavy water (D_2O) have been observed to manifest depressed responsiveness to antigenic stimuli[1]. The mechanism of this suppressive action, however, has not been elucidated. In this regard, experiments have been designed to distinguish between the effects of D_2O acting as solvent and source of readily exchangeable hydrogen isotope and D_2O acting as a source of non-exchangeable isotope incorporated by synthesis into C–D bonds. The present report is a description of such an experiment carried out in vitro, in which the effects of D_2O as a solvent system for the quantitative precipitin reaction were studied. To maximize such effects, the level of D_2O employed (50%) was twice that extant in the body fluids of mice on a 30% D_2O regimen[2].

Serum from a normal albino rabbit immunized by a single subcutaneous injection of 2.0 ml complete Freund's adjuvant containing 10 mg bovine serum albumin was collected by heart puncture on the 50th day post-immunization. Serum was filtered and inactivated at 56 °C for 30 min. One volume was then added to an equal volume of isotonic 99.7% D_2O saline. As a control, a sample of the same serum was similarly diluted with isotonic saline and both samples incubated at room temperature for 74 h. At the end of the incubation period, pH measurements were made on both samples. Optical densities were determined in the Beckman DB spectrophotometer between 240 and 320 nm on 1:100 dilutions of each sample, using the corresponding aqueous or deuterated saline as diluent. Antigen-antibody reactions using $^1/_2$ ml of the 1:1 diluted antisera were carried out in an isotonic pH 7.4 saline borate buffer, in a volume of 2.5 ml. These systems were maintained aqueous or 50% with respect to D_2O to correspond respectively with the aqueous or deuterated antisera employed. After addition of antigen, samples were incubated for 1 h at 37 °C and refrigerated at 4 °C for 7 days. Specific precipitates in both sets were then washed twice with 3 ml of 0.15 *M* NaCl near 0 °C, digested and nitrogen analyses carried out in duplicate for each point by a micro-Kjeldahl method.

As seen in the Figure, the quantitative precipitin behavior of normal rabbit antibody in 50% D_2O was observed to coincide with that of the control in the antibody excess zone, although an increased formation of precipitate was noted in the region of antigen excess. The pH and UV-spectral characteristics of the D_2O and H_2O dissolved antisera employed in this assay were found to be similar. Normal precipitin behavior in the antibody excess zone indicated that no loss of combining ability had occurred. Increased precipitate observed in the antigen excess zone might be ascribed to diminished solubility of normally soluble antigen-antibody complexes, since the solubility of a variety of polar compounds in heavy water has been shown to be markedly less than in

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