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Serine dehydratase activity in livers of phlorrhizin-treated rats and the hepatic serine *plus* threonine concentration

The activities of serine and threonine dehydratase are known to increase in rat liver in situations involving intensive protein catabolism, such as starvation, alloxan-diabetes or a high-protein diet, and furthermore the same protein reacts with both substrates[1–3].

Since the administration of phlorrhizin to starved rats results in a rapid increase (within 2.5 h) in the hepatic concentration of serine *plus* threonine, whereas the concentrations of certain other glucogenic amino acids decrease[4], it seemed of interest to examine the activity of serine dehydratase (L-serine hydro-lyase (deaminating), EC 4.2.1.13) in this situation where the amino acids arising from increased protein catabolism in extra-hepatic tissues are expected to contribute to the high rate of hepatic gluconeogenesis.

Male rats of the Wistar strain, weighing 120–170 g, were fed a diet of commercial rat cubes containing about 15% protein, 3% fat and the remainder consisting mainly of carbohydrate. Adrenalectomized rats received 1% NaCl in the drinking water and were used 4–5 days after the adrenalectomy. Phlorrhizin (75 mg) was administered

intramuscularly as a suspension in arachis oil (1.0 ml) to rats which had been starved for 48 h.

The rats were killed by cervical fracture; a portion of liver was rapidly removed (within 10 sec of death of animal) and immediately frozen with metal tongs previously cooled in liquid nitrogen[5]. The frozen liver was powdered in a mortar with frequent additions of liquid nitrogen. A portion of liver powder was then deproteinized with perchloric acid as previously described[6], but with omission of the florisil treatment. Serine *plus* threonine were determined in the deproteinized extract by a combined chemical and enzymatic method[4].

Another portion of the powdered liver was homogenized with 9 vol. of 0.2 M phosphate buffer (pH 7.4) containing 10 mM mercaptoethanol. After centrifuging for 20 min at 30 000 × *g*, the supernatant fluid was used directly for determination of the

TABLE I

EFFECT OF STARVATION AND PHLORRHIZIN TREATMENT ON HEPATIC SERINE DEHYDRATASE ACTIVITY

Normal rats were starved for 48 h and then given an intramuscular injection of either 1.0 ml of arachis oil or 1.0 ml of arachis oil containing a suspension of 75 mg phlorrhizin. The rats were starved for a further 16 h after injection and then killed. The serine dehydratase activity is expressed in μmoles of pyruvate formed per min per g fresh wt. of liver at 25° and the results are mean values (± S.D.).

Treatment	*Number of rats*	*Serine dehydratase activity (μmoles/min per g fresh wt.)*
Fed	6	0.85 ± 0.36
Starved	10	2.89 ± 1.50
Starved *plus* phlorrhizin	13	8.32 ± 2.86

serine dehydratase activity. The activity was measured by a spectrophotometric assay in which the pyruvate formed from L-serine was reduced with NADH and lactate dehydrogenase[2]. The cuvettes contained in a final volume of 3.0 ml: L-serine, 80 mM; NADH, 0.4 mM; Tris buffer (pH 8.5), 75 mM; pyridoxal phosphate, 0.07 mM; and lactate dehydrogenase (25 μg protein). The cuvette was equilibrated at 25° for 5 min and then the liver extract (0.1 ml) was added. After rapid mixing, the reaction was followed by the rate of decrease in absorbance at 340 mμ. Control experiments omitting the serine were carried out with each liver sample and suitable corrections applied to the reaction rates with substrate. The activity is expressed in μmoles pyruvate formed per min per g fresh wt. of liver at 25°.

In agreement with previous workers, starvation resulted in a 3-fold increase in hepatic serine dehydratase activity (Table I). Administration of phlorrhizin to starved rats caused a further 3-fold rise in activity within 16 h (Table I). Thus the overall increase with phlorrhizin *plus* starvation (approx. 10-fold) was comparable to that obtained on feeding a high-protein diet[2]. Adrenalectomy did not prevent the rise in serine dehydratase activity after phlorrhizin treatment.

There was no appreciable increase in dehydratase activity until 7 h after administration of phlorrhizin (Fig. 1a), whereas the serine *plus* threonine concentration initially rose (by about 100% at 2.5 h) and then slowly declined, until 16 h after

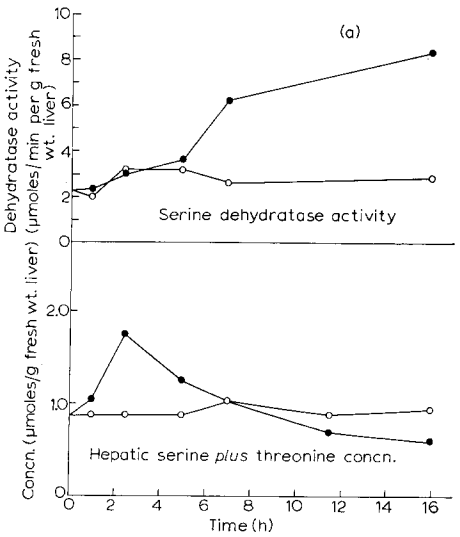

Fig. 1. Time course of changes in serine dehydratase activity and serine *plus* threonine concentration in rat liver on administration of phlorrhizin. Starved rats (48 h) were given an intramuscular injection of either 1.0 ml of arachis oil or 1.0 ml of arachis oil containing a suspension of 75 mg of phlorrhizin. The rats were then killed at timed intervals. The values are the mean for 4–8 animals at each time. a. Serine dehydratase activity. b. Serine *plus* threonine concentration. ○, control rats; ●, phlorrhizin-treated rats.

phlorrhizin the concentration was about 35% lower than that of the control rats (Fig. 1b). The mean values for the serine dehydratase activity and serine *plus* threonine concentration in the control rats did not change significantly throughout the experimental period (Figs. 1a and 1b). To test the possibility that the elevated activity of the dehydratase was caused by substrate induction, L-serine (1 mmole) was administered to starved rats. No change in serine dehydratase activity occurred, although the hepatic concentration of serine *plus* threonine 30 min after injection was 4 μmoles/g and it remained higher than that of the controls for at least 5 h.

The initial elevation of the serine *plus* threonine concentration in the liver of the phlorrhizin-treated rat is presumably due to the fact that increased extra-hepatic protein catabolism[7] results in an increased flux of amino acids to the liver and since the activity of serine dehydratase probably limits the rate of serine and threonine removal under these conditions, the concentrations of the substrates rise. However, the low affinity of the dehydratase for serine and threonine (K_m about $5 \cdot 10^{-2}$ M) means that over the physiological range, an increase in substrate concentration can result in increased enzyme activity without the need for a concomitant change in the amount of enzyme. The parallelism between the decrease in the concentration of serine *plus* threonine and the increase in the amount of dehydratase in the later stages of phlorrhizin treatment (Fig. 1) is further support for this suggestion.

The increase in hepatic serine dehydratase activity on administration of phlorrhizin to starved rats is another example of the response of this enzyme to increased protein catabolism.

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Comparative study of mitochondrial and soluble rat liver protein kinase

The presence of a protein kinase which transfers the terminal phosphate from ATP to phosphoproteins in mitochondria has been described by several authors[1–6].

In a previous paper from this laboratory[7], it was reported that two forms of protein kinase are present in liver mitochondria preparations: (1) a fraction tightly bound to the mitochondrial structure, and (2) a form readily soluble in water upon sonication of mitochondria.

It was suggested that the latter enzyme activity could be accounted for by cytoplasmic contamination, since a very active protein kinase was found to be present in soluble liver cytoplasm.

The present paper deals with a comparison between liver cytoplasmic and mitochondrial protein kinases under comparable conditions, made possible by removing, almost quantitatively, ATPase activity from both fractions. The low ATPase activity still present in both fractions does not interfere critically with the rate of protein kinase reaction, since ATP breakdown at the end of incubation was never found to exceed 10%.

As can be seen in Table I, the protein kinase activity, tested both with phosvitin and with casein, is much higher (up to 80-fold) in the soluble cytoplasm than in the mitochondria.

The relatively low protein kinase activity in mitochondria cannot be completely released by sonication and several washings with water[7]. However, the activity can be extracted by ionic solutions without any previous disorganization of the lipid–protein structure of mitochondrial membranes (Table II).