The Half-Life of Alanine Aminotransferase and of Total Soluble Protein in Livers of Normal and Glucocorticoid-Treated Rats

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SUMMARY

The half-lives of soluble protein pool and alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2) of rat liver were determined before and after prednisolone treatment by measuring the rate of disappearance of radioactivity from the labeled proteins. The half-life value of soluble protein is 4.2–4.3 days, and that of alanine aminotransferase is 3.0–3.5 days. Glucocorticoid treatment has little or no effect on the turnover rates.

INTRODUCTION

Half-lives of specific tissue proteins can be calculated by measurements of the rate of disappearance of radioactivity, a method which requires the isolation of the protein for each measurement, or from the rate of approach of activity to a new steady-state level. Application of the latter method is valid only when certain conditions have been met (1). The half-lives of several liver enzymes, such as tryptophan pyrrolase, tyrosine aminotransferase, and arginase, have been determined by both techniques (2–10). That of alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2) has been obtained only by the approach to steady-state method, and discrepant values arose in the approach from the normal steady-state level to the high glucocorticoid steady-state level, and vice versa (1). It was therefore considered important to derive comparative values for this parameter in normal and glucocorticoid-treated livers by the independent method of disappearance of radioactivity.

At the same time an average half-life for the total pool of soluble liver proteins in normal and glucocorticoid-treated animals was determined.

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MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 175–200 g were used. The body weight difference among the animals in an experimental group was less than 5 g. Animals were maintained on Wayne laboratory animal feed and water *ad libitum*. Animals in "normal" groups were given intraperitoneally 20 μC of either L-arginine-(guanido-^{14}C) (35 μC/μmole) or uniformly labeled L-leucine-^{14}C (305 μC/μmole). After the administration of radioactive amino acids, animals were killed by decapitation at intervals up to 5 days. The livers were rinsed thoroughly in cold 0.01 M potassium phosphate buffer, pH 7.4, containing 0.25 M sucrose, and then were blotted free from excess buffer solution,

weighed, and frozen in liquid nitrogen. The livers were stored at −30° until used. Animals in hormone-treated groups received subcutaneously 4 mg of prednisolone acetate every day for 5 days prior to the administration of 20 μC of L-leucine-^{14}C (305 μC/μmole). The hormone-treated animals were killed at successive 24-hr intervals, starting 30 min after the administration of radioactive amino acid. Surviving animals continued to receive the daily dose of prednisolone. It had been shown previously that alanine aminotransferase activity increased to a plateau level in 4–5 days of prednisolone treatment [the "high-glucocorticoid steady-state condition" (1)].

The frozen liver was homogenized, in the phosphate-sucrose buffer described above, in a Lourdes high-speed Multi-Mixer for 2 min, and the final concentration was adjusted to 1 g of liver per 10 ml of homogenate. The homogenate was centrifuged at 105,000 × *g* for 90 min. The supernatant fraction was used to prepare samples for the estimation of radioactivity in the total soluble protein and in the antiserum-precipitable enzyme as described previously (1, 11, 12). The method of determination of turnover rate has been described (13, 14).

The following experiment was performed in order to examine the time course of appearance of radioactivity in the circulating bloodstream. Two normal animals and two hormone-treated animals similar to those used in the preceding experiments were given intraperitoneally 20 μC of L-leucine-^{14}C (305 μC/μmole), and 0.5 ml of blood was drawn from the tail vein of each animal at intervals up to 3 hr. Each blood sample was mixed with 2.5 ml of 0.9% NaCl solution containing 0.01 M EDTA, and the mixture was centrifuged at 2000 × *g* for 10 min. The radioactivity of the supernatant protein (counts per minute per milligram) was estimated by the method used for the liver proteins.

RESULTS

The data for the turnover of liver alanine aminotransferase from normal and glucocorticoid-treated animals are presented in Fig. 1. The half-life values obtained were 3.5 and 3.0 days, respectively.

Figure 2 represents the corresponding measurements for the turnover of total soluble protein. Experiments with both L-arginine-(guanido-^{14}C) and uniformly labeled L-leucine-^{14}C were included to evaluate the possible effect of reutilization of amino acids (9, 15) on the value obtained. It can be seen that the disappearance of radioactivity was biphasic. Calculations from the second, long-term phase give half-lives for the normal animals of 4.3 days with guanido-labeled arginine and 4.2 days with uniformly labeled leucine, respectively, and 4.3 days for the glucocorticoid-treated animals.

The early, rapid decay phase, which has a half-life of approximately 1.5 hr, is pre-

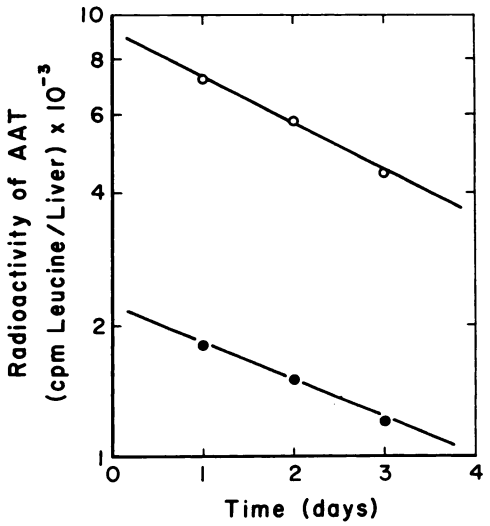

FIG. 1. *Turnover of liver alanine aminotransferase (AAT) in normal and glucocorticoid-treated animals*

○, Hormone-treated animals; ●, control animals. The radioactivity in 75–100 units of enzyme precipitated by an equivalent amount of antiserum was measured, and then the value was corrected to the radioactivity in the total amount of enzyme per liver on the basis of the enzyme activity in the 105,000 × *g* supernatant fraction. The specific enzyme activity of 105,000 × *g* supernatant fluid from the hormone-treated livers was always approximately 3 times greater than that from normal livers. Each point represents the mean value of three livers. The standard deviation was approximately ±10% of each mean.

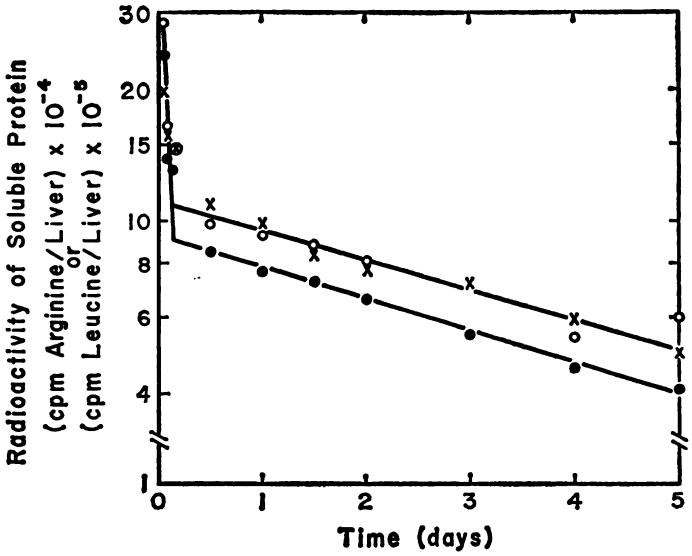

FIG. 2. *Turnover of total soluble protein pool of liver in normal and glucocorticoid-treated animals*

○, Hormone-treated animals given L-leucine-^{14}C; ●, control animals given L-leucine-^{14}C; ×, control animals given L-arginine-(guanido-^{14}C). Each point represents the mean value of at least three or four livers. The standard deviation was less than ±10% of each mean.

sumed to represent loss through secretion rather than turnover. Comparison of the radioactivity curves for liver proteins and plasma protein is consonant with this presumption (Fig. 3), as shown by the coincidence of disappearance of radioactivity from the liver and accumulation of radioactivity in the plasma proteins. It appears that, whereas glucocorticoid treatment increases the specific radioactivity of the soluble liver protein, hormone treatment does not increase the specific radioactivity of the plasma proteins, the major portion of which arise by secretion from the liver.

DISCUSSION

From the results presented here, it can be concluded that there is no significant

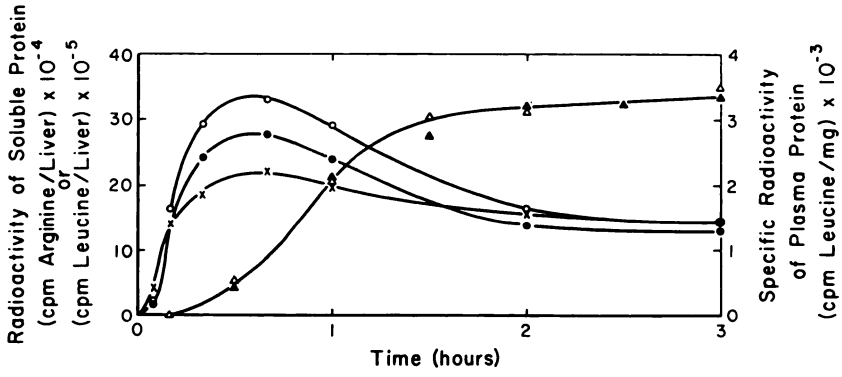

FIG. 3. *Changes in radioactivity of blood plasma protein and of soluble liver protein with time*

The specific radioactivity of plasma protein (counts per minute per milligram) in normal (▲) and prednisolone-treated (△) animals after the administration of radioactive leucine is compared with the radioactivity curves of liver protein. The symbols for liver proteins are: ○, hormone-treated animals given L-leucine-^{14}C; ●, control animals given L-leucine-^{14}C; ×, control animals given L-arginine-(guanido-^{14}C).

difference in the half-life of alanine aminotransferase in normal and glucocorticoid-treated animals. The values obtained are closely comparable to those determined previously (3.5 days) from the decline of enzyme activity from the high-hormone to the normal level (1). Nor did the hormone affect the average turnover of the soluble liver protein pool. The half-life value of 4.2–4.3 days for this fraction is within the range of those (3–6.5 days) previously reported (14, 16–18). However, most previous reports have neglected the early, rapid decay phase, which appears to represent secretion rather than degradation of protein.

Very little is known regarding the rate-limiting factors in the disappearance of proteins from tissues. Among the possibilities to be considered are proteolytic susceptibility, the intrinsic physical-chemical lability in the intracellular environment, and the secretion rate; all these mechanisms are consistent with a first-order decay process.

Whatever the rate-limiting step may be, it has been established that the rate of disappearance is controllable in at least two cases (4, 9), so that this mechanism, in addition to other, more familiar ones, appears to be one of the devices utilized in metabolic regulation, at least in cells of higher organisms. However, no reports have appeared as yet regarding the hormonal control of this process.

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