TIME-COURSE OF CHANGES OF LIVER TRYPTOPHAN PYRROLASE (TRYPTOPHAN OXYGENASE) AND LIVER AND KIDNEY GLUCOSE-6-PHOSPHATASE IN RATS SHIFTED FROM HIGH-TO ZERO-PROTEIN DIETS

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(Received January 16, 1980)

Summary Time-courses of changes in the activities of liver and kidney glucose-6-phosphatase [EC 3.1. 3.9] and hepatic tryptophan pyrrolase [EC 1.13. 1.12; TPO] in rats pre-fed high-protein diets for 5 days and then shifted to zero-protein diets were studied. Liver glucose-6-phosphatase activity decreased 1 day after the dietary shift but then increased and remained significantly higher than the 0 day value for the next 2 days. Changes in liver glycogen were found to be intimately and inversely related to liver glucose-6-phosphatase activity. Changes in kidney glucose-6-phosphatase activity paralleled the pattern of changes observed in liver activity. An initial decrease in TPO activity was followed by increased enzyme activity up to the 3rd day of the dietary shift. Later there was a rapid fall in tryptophan pyrrolase activity. Changes observed in these specific enzyme proteins differed from those observed in total tissue proteins. Alterations in the activities of these enzymes and changes in other parameters are compared with those observed earlier with the reverse type of dietary shift.

Keywords tryptophan pyrrolase, glucose-6-phosphatase, protein, glycogen, liver, kidney, enzyme regulation

In earlier investigations by this laboratory (1, 2) we studied the alterations in activity of liver and kidney glucose-6-phosphatase [EC 3.1.3.9; G-6-Pase] and hepatic tryptophan pyrrolase (tryptophan oxygenase, EC 1.13.1.12; TPO) in rats adapted to swimming for short periods, and in rats ingesting different levels of dietary protein with and without the administration of prednisolone. Alteration in the activities of these enzymes produced by adaptation, protein and hormones and their interactions, may be caused by changes in the synthesis and degradation of enzyme protein or changes in the rate of conversion of inactive enzyme precursors to enzyme proteins. One or more of these reactions may be modified by the above-
mentioned parameters. Among other procedures, the time-course of changes in the enzyme activities under a given set of conditions can be indicative of the rates of degradation of the enzyme protein. The rationale and basis of such calculations have been explained by Segal and Kim (3), Freedland (4) and Schimke and Doyle (5). Many workers (3–7) have utilised these time-courses of enzyme activities for calculation of the half-life of enzyme proteins.

Similar studies from this laboratory (8) on liver and kidney G-6-Pase and hepatic TPO in rats pre-fed zero-protein diets for 4 days and then shifted to high protein have revealed differences in the nature and magnitude of the responses of these enzymes. In the present investigation we have studied the time-course of changes in the activities of the above enzymes in rats pre-fed high-protein diets for 5 days and then shifted to zero-protein diets for 7 days. The alterations in activities of these enzymes and changes in other parameters are compared with those reported earlier (8) in the reverse dietary shift.

MATERIALS AND METHODS

Adult male albino rats used in this investigation were purchased from the Postgraduate Institute of Medical Education and Research, Chandigarh. Twenty-four animals, with an average weight of 131 g, were divided into 5 groups of 4 animals each. All the animals in different groups were housed in separate cages with raised screen bottoms and were allowed water and food ad libitum. All the animals in different groups were fed a diet containing 750 g casein/kg diet for 5 days and then were shifted to protein-free diets. The diet was composed of the following ingredients (g/kg of the diet): til oil, 40; shark liver oil, 10; yeast, 20; salt IV (9), 40; and sucrose as the source of carbohydrate and casein as the source of protein. High-protein diets were made at the expense of sucrose. The animals in different groups were then sacrificed on the 0, 1st, 2nd, 3rd, 5th, and 7th day of the dietary shift.

At the end of each respective experimental period the animals in various groups were sacrificed by decapitation. All the animals were killed between 8 AM and 9 AM to eliminate the effects of circadium rhythm. The liver and kidneys were removed, washed and weighed, and immediately processed for enzyme assay and analysis. Liver tryptophan pyrrolase activity (10), liver and kidney glucose-6-phosphatase activity (11), liver glycogen (12), and liver and kidney protein (13) were estimated.

Enzyme activities have been expressed as specific activities (nmol kynurenine liberated/min/g tissue for TPO and μmol of inorganic phosphorus liberated/min/g tissue for G-6-Pase), and as total activities (activity/100 g body weight). The values of other constituents are mg per 100 g body weight of the animals. The reason for expressing the values in this way and details of other methods have been explained previously (1).
The food consumption averaged 9.9 g/rat/day during the period of high-protein intake for 5 days and this value is taken for the 0 day shift. The food consumption increased when the animals were shifted to zero-protein diets and averaged 12.5, 12.0, 14.0, 12.5, and 12.0 g per rat per day in animals on protein-free diets for 1, 2, 3, 5, and 7 days, respectively. Relative liver size (RLS) averaged 3.92 g in rats pre-fed a high-protein diet for 5 days (Table 1) and this value increased significantly (10% level) 24 hr after dietary shift to a protein-free diet, but subsequent changes were small and insignificant. The relative kidney size (RKS) showed minor and insignificant changes with time after the dietary shift.

A marked and highly significant increase in liver glycogen concentration was observed 1 day after the dietary shift, and this enhanced value was maintained for the next 24 hr. This was followed by a significant and sharp reduction in the liver glycogen value 3 days after the dietary shift. Small but insignificant changes were observed thereafter.

The dietary shift from high protein to zero protein in these animals was followed by a progressive decrease in liver protein and at the end of 7 days the content was reduced to less than 50% of the value observed on the day of the dietary shift (Fig. 1). A similar decrease in the kidney protein up to 5 days of the dietary shift was also observed in these animals. However, this decrease was followed by an increase in kidney protein 7 days after dietary shift. Further, the decrease in kidney protein was of a lesser magnitude than that observed in liver.

The shift from high-protein to protein-free diets caused changes in liver and kidney G-6-Pase activities and in hepatic TPO activity; however, the time-course of changes in these enzymes had some similarities as well as contrasts. A decline in liver G-6-Pase activity observed during the first 24 hr of feeding the zero-protein diet was followed by a rapid and marked increase for the next 2 days and then showed minor and insignificant variations.

Changes in kidney glucose-6-phosphatase activity paralleled the pattern of changes observed in liver G-6-Pase activity but the magnitude of changes differed in two tissues. Further, the activity of the kidney enzyme at different intervals after dietary shift was always lower than that observed at the beginning of dietary shift. After 7 days of feeding a zero-protein diet total activity of kidney G-6-Pase was practically equal to that observed on day 0.

An initial decrease in hepatic tryptophan pyrrolase (TPO) activity observed 1 day after dietary shift was followed by an increase in activity for the next 2 days and a very rapid decline thereafter. The TPO activity after 7 days of feeding zero-protein diet was far below the value observed in animals maintained on a high-protein diet for 5 days.

An idea about the relative magnitude of these changes in various parameters can be had if the values are plotted as percentages of the 0 day value taken to be 100 (Fig. 1). For comparison these figures also include the time-course of changes
Table 1. Time-course of changes in liver glycogen, liver and kidney glucose-6-phosphatase, and liver tryptophan pyrrolase in rats pre-fed high-protein diets for 5 days and then shifted to protein-free diets.

| Parameter measured | Number of days on protein-free diet |
|--------------------|------------------------------------|
|                    | 0       | 1       | 2       | 3       | 5       | 7       |
| RLS                |         |         |         |         |         |         |
| Liver weight × 100 | 3.92    | 4.67    | 4.67    | 4.68    | 5.34    | 4.85    |
| Body weight        | ±0.144<sup>a</sup> | ±0.21  | ±0.27   | ±0.21   | ±0.38   | ±0.09   |
| RKS                |         |         |         |         |         |         |
| Kidney weight × 100| 1.02    | 0.92    | 0.92    | 1.00    | 0.96    | 0.87    |
| Body weight        | ±0.08   | ±0.06   | ±0.04   | ±0.12   | ±0.08   | ±0.02   |
| Liver glycogen     | 99.67   | 289.32  | 286.60  | 130.68  | —       | 120.25  |
| (mg/100g body weight) | ±21.47  | ±13.64  | ±28.60  | ±39.18  | ±6.44   |         |
| Liver tryptophan pyrrolase: | | | | | | |
| Specific activity<sup>a</sup> | 25.00   | 16.33   | 18.50   | 38.83   | 14.00   | 10.00   |
|                       | ±0.66   | ±2.50   | ±3.00   | ±5.16   | ±0.83   | ±2.50   |
| Total activity<sup>b</sup> | 97.83   | 76.00   | 85.16   | 146.00  | 75.33   | 47.83   |
|                       | ±4.50   | ±9.16   | ±12.16  | ±29.50  | ±8.83   | ±11.66  |
| Liver G-6-Pase:     |         |         |         |         |         |         |
| Specific activity<sup>c</sup> | 2.69    | 1.16    | 2.83    | 4.95    | 3.43    | 3.84    |
|                       | ±0.70   | ±0.26   | ±0.36   | ±0.34   | ±0.48   | ±0.27   |
| Total activity<sup>b</sup> | 10.69   | 5.26    | 13.39   | 21.68   | 17.77   | 18.62   |
|                       | ±2.99   | ±0.98   | ±2.09   | ±2.47   | ±1.54   | ±1.34   |
| Kidney G-6-Pase:    |         |         |         |         |         |         |
| Specific activity<sup>c</sup> | 3.50    | 1.24    | 2.39    | 3.41    | 3.36    | 4.12    |
|                       | ±0.40   | ±0.06   | ±0.14   | ±0.05   | ±0.34   | ±0.32   |
| Total activity<sup>b</sup> | 3.71    | 1.12    | 2.18    | 3.41    | 3.12    | 3.59    |
|                       | ±0.67   | ±0.06   | ±0.10   | ±0.40   | ±0.12   | ±0.31   |

<sup>a</sup> nmol of kynurenine liberated/g tissue/min.  
<sup>b</sup> Activity/100g body weight.  
<sup>c</sup> μmol of inorganic phosphorus liberated/g tissue/min.  
<sup>d</sup> Mean ± SEM for 4 observations.
Fig. 1. Relative changes in liver glycogen (a), liver protein (b), liver glucose-6-phosphatase (c), and liver tryptophan-pyrrolase activity (d) of rats shifted from zero-protein diets to "75% casein" diet (●) and those shifted from high-protein to zero-protein diets (○). The values on the 0 day of the dietary shift are taken as 100 and the other values are represented as percentages of this value.

Liver glycogen increased nearly 3-fold within 24 hr after dietary shift from high-protein to protein-free diets and this value was maintained during the next 24 hr. A dietary shift from zero-protein to high-protein diets on the other hand caused a small decline in liver glycogen up to 5 days after dietary shift and then showed a fairly rapid rise in the next 2 days (Fig. 1a). One day after the dietary shift liver G-6-Pase activity showed an opposite response in the two types of dietary
shifts (Fig. 1c), but later changes in G-6-Pase activity were more or less parallel and were always higher than the 0 day value in both types of dietary shifts. Changes in hepatic TPO in rats pre-fed high-protein diets and then shifted to protein-free diets followed a time-course which is the reverse of that observed in rats pre-fed protein-free diets and then shifted to high-protein diets (Fig. 1d) throughout the 7-day experimental period.

**DISCUSSION**

The abrupt increase in liver glycogen one day after dietary shift to zero-protein diets is explicable on the basis of the sudden influx of glucose from the diet. Similarly, the decrease in liver glycogen follows the withdrawal of carbohydrate in the reverse type of experiments (8). Subsequent changes in the liver glycogen may be caused by adaptation to high carbohydrate and rearrangement of metabolising machinery (14). The rapid decline in liver G-6-Pase activity one day after dietary shift is one such change which may bring about rapid accumulation of liver glycogen. The subsequent rapid increase in liver G-6-Pase 2 days after dietary shift initially prevents further accumulation of liver glycogen and the value thus stayed constant. Later changes in liver glycogen tended to follow the changes in liver G-6-Pase activity. It appears that changes in liver glycogen are inversely related to liver G-6-Pase activity, a fact also observed in our earlier studies involving reverse dietary shift (8). The changes in kidney G-6-Pase activity are similar to those in liver G-6-Pase, but of smaller magnitude, and in both types of dietary shifts tend to reach the 0 day value 7 days after dietary shift. As these changes occur the kidney G-6-Pase may also be playing an important role in adaptive response of the animal under various stress conditions (8, 15). Either type of dietary shift leads to increased G-6-Pase activity; however, this increase is immediate in rats shifted from zero-to high-protein diets but has a lag period of 1 day in rats shifted from high-to zero-protein diets. The activity continues to increase only for 2 days and then levels off in both cases, indicating approach to a steady-state level of the enzyme. An interesting observation is the increase in concentration of G-6-Pase even though other liver proteins show a progressive decline with time on protein-free diets. This indicates a preferential retention/synthesis of G-6-Pase protein at the expense of other liver proteins. Maintenance of high activity of G-6-Pase under the condition of zero-protein intake is indicative of the importance of this enzyme in the metabolic economy of the cell and organism as a whole, and further confirms the previous observations (8, 16) that different proteins undergo alterations to different extents during adaptive responses. Freedland and Harper (17) reported increased activity of liver G-6-Pase 24-48 hr after dietary carbohydrates were replaced by protein or fat and then a gradual return to normal. A similar type of change is seen in kidney G-6-Pase within 7 days, but liver G-6-Pase does not return to a normal value within 7 days. Physiological interpretation of changes in G-6-Pase activity is, to some extent, complicated by the fact that the same enzyme also hydrolyses...
pyrophosphate and acts as phosphotransferase (18).

In contrast, the changes in TPO (a tryptophan metabolising enzyme whose synthesis is very much under the control of the exogenous supply of tryptophan) follows a distinctly different pattern when the rats are shifted to zero-protein diets. The observations suggest that up to 3 days after dietary shift, tryptophan from body stores helps to maintain TPO activity; later, TPO activity falls rapidly. The changes are exactly the reverse of those observed during a zero-protein to high-protein shift (8). The results indicate that the 3-day period of adjustment of metabolic machinery is followed by a true effect of high protein, or withdrawal of protein, on the TPO activity in rats. The effects of dietary protein on the liver tryptophan pyrrolase activity are, presumably, mediated through its tryptophan content and availability. However, the changes in activity of this enzyme, even after the period of adjustment, are very much slower than those reported to be produced by administration of L-tryptophan to rats. A 2–3-fold increase in the liver TPO activity within 4–5 hr of administration of a single dose of L-tryptophan has been reported (19–22). A higher-fold increase in TPO activity was observed when the dose of tryptophan was increased (21) or when TPO activity was assayed after 12–16 hr in rats administered L-tryptophan every 4 hr (20, 22). The slower effects in our investigation may have been caused by the slow release/absorption of tryptophan from the gut and consequently slow increase in the blood and liver levels of free tryptophan. This is supported by the observations that the inducing effect of tryptophan disappears 10 hr after tryptophan administration (23); the effect produced by L-tryptophan on TPO activity depends upon the dose of L-tryptophan administered (21); also, that the increased induction of liver TPO by tryptophan is related to an increased level of tryptophan in liver (23). The tryptophan circulating in blood and in other tissues at the time of shift to a zero-protein diet might be responsible for the slower decrease in TPO activity with time. The increased TPO activity observed 3 days after shifting to zero protein may have been caused by an increase in the level of tryptophan in blood and liver. Increased plasma levels of some amino acids after 3 days of carbohydrate feeding or starvation have been reported (24, 25) and this is a situation analogous to that in our investigation.

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