# GLUTATHIONE METABOLISM IN NORMAL AND INDUCED STATES OF GROWTH

by

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Results of studies on the metabolism of liver glutathione with the aid of $^{15}$N-glycine[1], $^{15}$N-ammonia[2], and $^{15}$N-glutamic acid[2] indicate a relatively high metabolic activity of liver glutathione nitrogen in comparison with that of total liver protein nitrogen. On this basis WAELSCH AND RITTENBERG[1] suggested that the tripeptide might be involved in the transfer of amino acids into protein or in the regulation of the process. Results of similar studies with the aid of $^{35}$S-cystine[3] confirm the findings with $^{15}$N labeled compounds. Aside from the demonstration of the participation of glutathione in transpeptidation reactions[4,5], however, there appears to be little additional evidence supporting the attractive hypothesis that glutathione acts as an intermediate in the biosynthesis of other peptides and proteins.

If the tripeptide is involved, as the hypothesis suggests, however, the relative metabolic activity of liver glutathione and total protein nitrogen in rapidly growing immature normal rats, which are actively engaged in the synthesis and accumulation of body protein, might be expected to differ considerably from that in normal adult rats. We have thus studied this aspect of glutathione metabolism following administration of $^{15}$N-glycine. For comparative purposes similar studies were also conducted on normal adult rats in which nitrogen retention and gain in body weight were induced with anterior pituitary growth hormone and on groups of immature rats in which growth was arrested by hypophysectomy and then induced with growth hormone. Conveniently included were liver glutathione determinations made in parallel with the measurements of $^{15}$N uptake.

## EXPERIMENTAL

### *Animals*

Female rats of the Sprague-Dawley strain, both normal and hypophysectomized, were used in all experiments. The hypophysectomized rats were about 30 days old at the time of operation, and were held for a period of sufficient duration to establish the fact that the growth process had been arrested as indicated by a plateau in body weight.

### *Diet*

The animals were maintained in separate cages, and fed *ad libidum*. The diet was that described by BENNETT, LI AND LAUNDRIE[6] with the following modifications: Corn oil was substituted for Crisco, and Haliver Oil for Sardilene. This diet was supplemented twice weekly with fresh lettuce.

### *Methods*

Body weight and food intake were determined daily for each rat until it was ascertained that

*References p. 350.*

the animal was either growing rapidly or had reached a plateau in body weight. The animals were then grouped into rapidly growing immature normal, normal adult, and hypophysectomized immature rats and observations continued for a period of 10 days. During the latter period, nitrogen storage and gain in body weight were induced in a group of the hypophysectomized immature rats and in a group of the normal adult rats by small daily subcutaneous injections of anterior pituitary growth hormone. Immediately following the administration of the final dose of growth hormone to these rats, food was withdrawn and $^{15}N$ glycine, containing 32–33 atom % excess $^{15}N$, administered intraperitoneally in doses of 2.5 mg per 100 g body weight. Two hours later the animals were sacrificed by stunning and decapitation. Unstimulated control groups of rapidly growing immature normal rats, hypophysectomized immature rats, and normal adult rats were similarly treated and sacrificed at the same time as the stimulated animals.

Following sacrifice of each rat, the liver was quickly removed, weighed, and a sample removed for glutathione concentration determination. The remainder of the liver was immediately ground in a mortar previously chilled with dry ice. Livers from the rats in each group were combined and processed as described by WAELSCH AND RITTENBERG[1] for the isolation of glutathione as a copper mercaptide. Trichloroacetic acid residues remaining after extraction of glutathione were handled in the manner described by PETERSON AND GREENBERG[7]. Some residues were also subjected to performic acid treatment to remove glutathione which might be bound to the protein through disulfide linkages.

The different nitrogenous fractions were processed in the manner described by SPRINSON AND RITTENBERG[8] and enrichment of the nitrogen of each fraction determined in a Model 21-401 CEC Mass Spectrometer. Comparison of the $^{15}N$ content of the nitrogen obtained from the performic acid treated trichloroacetic acid residues with that from the untreated residues showed no significant difference.

Liver glutathione concentrations were determined on each sample of liver tissue by the modified nitroprusside procedure described by GRUNERT AND PHILLIPS[9].

## RESULTS

Since the publication of a preliminary report on growth and the metabolism of glutathione[10], we have found that the termination procedure used in these studies, in which hypophysectomized rats were fasted 5 hours prior to administration of $^{15}N$ glycine and for a subsequent 2 hour period before sacrifice, produced marked alterations in liver glutathione concentrations in such animals and covered effects of growth hormone on $^{15}N$ incorporation into liver glutathione nitrogen. In subsequent experiments, the finding that liver glutathione concentrations were not significantly altered in such animals fasted 2 hours before sacrifice, led to the adoption of this procedure in the termination of the experiments reported in this paper. The results presented in Table I thus differ from, and are in contrast to, those previously reported[10].

The significance of differences between concentrations of glutathione in the livers of rapidly growing immature normal rats, normal adult rats, and a group of the latter stimulated with growth hormone were statistically analyzed by the methods of FISHER[11], as was also the significance of the difference between liver glutathione concentrations in hypophysectomized immature rats and in similar animals stimulated with growth hormone. Differences were considered highly significant when $p$ values less than 0.01 were obtained.

Liver glutathione concentrations, expressed in terms of mg glutathione per 100 g wet tissue and in terms of mg per 100 g body weight, were found to be significantly lower in normal adult rats than in rapidly growing immature normal animals, and stimulation of the growth process in the normal adult rat with growth hormone resulted in a significant elevation in the concentration of liver glutathione expressed on either basis. Stimulation of the growth process in the hypophysectomized rat with growth hormone also resulted in a significant elevation in the concentration of liver glutathione, expressed on the basis of mg per 100 g wet tissue. Data from the same

*References p. 350.*

## TABLE I

GLUTATHIONE METABOLISM IN NORMAL AND INDUCED STATES OF GROWTH*

| Group | No. in group | Initial body weight g | Change in body weight g | Daily food intake g | Terminal liver weight g | Liver glutathione concentration | | Liver fractions | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | mg/100 g wet wt | mg/100 g body wt | Glutathione N | Protein N |
| | | | | | | | | Atom % excess $^{15}N$ | |
| Normal immature | 6 | 82 | +62.3 | 12.0 | 6.98 | 201 (± 8.7) | 9.7 (±0.8) | 0.295 (0.9 %) | 0.013 (0.04 %) |
| Normal adult | 6 | 271 | + 3.1 | 9.6 | 7.29 | 170 (±14.3) | 4.5 (±0.4) | 0.524 (1.6 %) | 0.017 (0.052%) |
| Normal adult stimulated with growth hormone** | 6 | 264 | +30.8 | 13.6 | 8.98 | 198 (±17.2) | 5.7 (±0.5) | 0.430 (1.32%) | 0.016 (0.049%) |
| Hypophysectomized immature | 6 | 88 | — 3.4 | 4.8 | 2.69 | 204 (±19.6) | 6.8 (±1.1) | 0.392 (1.17%) | 0.014 (0.043%) |
| Hypophysectomized immature stimulated with growth hormone*** | 6 | 95.2 | +10.9 | 6.3 | 3.98 | 242 (±14.5) | 8.6 (±0.8) | 0.316 (0.97%) | 0.015 (0.046%) |

* The ± values in parentheses are standard deviations calculated by the methods of FISHER[11]. The percentage values in parentheses indicate the percentage of the nitrogen of the administered $^{15}N$ glycine incorporated into the various fractions. In making this calculation, it was assumed that the nitrogen of the glycine administered was 100% labeled with $^{15}N$.

** Stimulated with 500 micrograms Armour's Somar Growth Hormone Preparation, M-10810, per day for 10 days.

*** Stimulated with 50 micrograms Armour's Somar Growth Hormone Preparation, M-10810, per day for 5 days followed by 100 microgram per day for a subsequent 5 day period.

group of rats, statistically analyzed in terms of liver glutathione concentrations per 100 g body weight, yielded a $p$ value between 0.02 and 0.01.

In all rats, both normal and hypophysectomized, the percentage of the nitrogen of administered $^{15}N$ glycine incorporated into liver glutathione during the 2 hour period of assimilation was much greater than that incorporated into liver protein. Nitrogen obtained from glutathione isolated from livers of normal adult rats was approximately 78% higher in $^{15}N$ content than that from rapidly growing immature normal rats. Stimulation of normal adult rats with growth hormone reduced the $^{15}N$ enrichment of liver glutathione nitrogen in such animals by about 18% and had no effect on the enrichment of the nitrogen of total liver proteins. The $^{15}N$ enrichment of liver glutathione nitrogen of the hypophysectomized immature rat was reduced 19.5% by stimulation of this animal with growth hormone.

## DISCUSSION

In considering the results of the present studies and their relationship to the growth process, several observations seem of interest. In both normal and hypophysectomized rats there appears to be a direct relationship between liver glutathione concentration and the growth process. Thus liver glutathione concentrations are relatively higher in rapidly growing immature normal rats than in normal adult rats, and relatively higher in normal adult rats stimulated with growth hormone than in untreated adult animals. Similarly liver glutathione concentrations are relatively higher in hypophysectomized immature rats in which the growth process has been stimulated with growth hormone than in untreated hypophysectomized immature rats.

While the maintenance of relatively higher concentrations of glutathione in livers of rats in either normal or induced states of growth, in comparison with that in rats in which the growth process has been arrested by attainment of the adult state or by hypophysectomy, might be considered evidence for an enhanced biosynthesis of the tripeptide during growth, such an interpretation must be tempered by the lack of information on liver glutathione turnover.

The percentage of administered $^{15}N$ incorporated into liver glutathione nitrogen, however, is strikingly different in the three groups of normal rats and in the two groups of hypophysectomized rats. In this respect it seems of interest to note that in normal adult rats and in hypophysectomized immature rats, the alterations produced in $^{15}N$ liver glutathione enrichment are of the same order of magnitude as those produced in liver glutathione concentration. Thus, for example, stimulation of the normal adult rat with growth hormone produced a 16.5% increase in liver glutathione concentration, expressed on the basis of wet tissue weight, and decreased the $^{15}N$ enrichment of liver glutathione nitrogen by 17.9%. Similarly in hypophysectomized rats stimulated with growth hormone, the concentration of liver glutathione was increased 18.5%, while the $^{15}N$ enrichment of liver glutathione nitrogen decreased 19.4%. In comparing similar data for rapidly growing immature normal rats with that of normal adult rats, however, we find that the nitrogen of liver glutathione isolated from the immature animals contains 43.7% less $^{15}N$ than that isolated from the normal adult rats. This finding can be partially accounted for by the dilution effect of an 18.2% increase in the concentration of liver glutathione in the immature rat. In this connection studies on the contribution of certain other

*References p. 350.*

factors, such as the size of the glycine pool and the rate of turnover of both the glycine and glutathione pools, to the $^{15}N$ enrichment of liver glutathione would be of special interest.

Under the experimental conditions, the $^{15}N$ enrichment of the nitrogen of total liver proteins obtained from normal adult rats or from hypophysectomized immature rats was not significantly different from that obtained from similar groups of rats in which the growth process had been stimulated with growth hormone. Similarly the $^{15}N$ enrichment of the nitrogen of total liver proteins obtained from rapidly growing immature normal rats does not appear to be significantly different from that obtained from normal adult rats. Whether or not this is due to unfavorable conditions for detecting such differences must still be determined. Certainly the 2 hour period of assimilation of $^{15}N$ glycine, which was selected as an experimental condition because of the rapid turnover rate of liver glutathione (half-life time of about 3 hours), is much more favorable for the detection of differences in the metabolic activity of liver glutathione nitrogen than it is for the detection of differences in the metabolic activity of total liver proteins (half-life time of 5–6 days). Results of experiments now in progress in our laboratories on the $^{15}N$ enrichment of liver glutathione and total protein nitrogen at varying time intervals following administration of $^{15}N$ glycine may be particularly helpful in establishing this experimental condition. Data from preliminary experiments on the incorporation of the nitrogen of $^{15}N$-alanine into total liver protein suggest that even under more favorable conditions of incorporation (*i.e.* administration of highly labeled $^{15}N$-alanine on the third, fourth, and fifth days of a 5 day experimental period) only slight differences in the $^{15}N$ enrichment of total liver protein nitrogen may be observed between hypophysectomized immature rats and hypophysectomized immature rats stimulated with growth hormone[12].

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## SUMMARY

1. Liver glutathione concentrations were found to be significantly higher in rapidly growing immature normal rats than in normal adult rats. Stimulation of the latter with growth hormone resulted in a significant elevation in liver glutathione concentrations.

2. Stimulation of the growth process in the hypophysectomized immature rat with growth hormone resulted in a significant elevation in the concentration of liver glutathione.

3. The percentage of administered $^{15}N$ found in the liver glutathione nitrogen obtained from normal adult rats and from hypophysectomized immature rats is markedly reduced as a result of stimulation of these animals with anterior pituitary growth hormone. This appears to be largely due to alterations in the size of the metabolic pool of liver glutathione. The relatively low percentage of administered $^{15}N$ found in the nitrogen of liver glutathione obtained from rapidly growing immature normal rats compared with that obtained from normal adult rats, on the other hand,

*References p. 350.*

can be accounted for only partially in terms of the dilution effect of a larger metabolic pool of liver glutathione.

4. The $^{15}N$ enrichment of the nitrogen of total liver proteins of rats in normal or induced states of growth, during a 2 hour period of assimilation of $^{15}N$ glycine, does not appear to be significantly different from that observed in rats in which the growth process is arrested.

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