ALTERATIVE METABOLIC PATHWAY OF RATS SUFFERED FROM THIAMINE DEFICIENCY

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It has been shown that during the ensuing anorexia, the thiamine-deficient rats mobilized their fat reserves, but the reserves mobilized were utilized inefficiently (1). In a previous paper (2) it has been observed that there was a rise and subsequent fall of the concentration of glyoxylate (GA) in the blood and in the urine of rats suffered from thiamine deficiency. There was also a similar change in the concentration of glycine in the blood and urine due to excessive tissue breakdown. This study is to show how the normal and thiamine-deficient rats handle this amino acid.

From the present knowledge of glycine metabolism two pathways may be envisaged from complete oxidation of glycine. One pathway involves the intermediary formation of serine and subsequent oxidation of this amino acid by way of alanine, pyruvate (Pyr) and TCA-cycle (3–5). This pathway has been proposed more recently by a glycine cleavage system where glycine undergoes serine, NH₃ and CO₂ (6–9). Both pathways seemed to occur in the rats in this present study; the relative importance of these two pathways and that they are being modified by the nutritional state of the animals were revealed by the following study:

A single dose of glycine-²¹⁴C (1.0 μCi/ml/100 g body weight) was injected intraperitoneally into a rat which was then placed immediately into a metabolic jar which was connected with a series of absorption tubes; the first absorption tube contained 20 ml of 0.5% 2,4-dinitrophenylhydrazine reagent in 2 N HCl to trap any volatile carbonyl compound expired. The other two tubes each contained 20 ml 1 N NaOH for the absorption of the expired CO₂ (Fig. 1). A gentle stream of air was allowed to pass through the whole system by means of a suction pump. The experiment was carried out at a temperature of 18°C for 3 hr. The rat was then taken out of the metabolic jar and it was immediately sacrificed. Blood from the neck was collected in the heparinized centrifuge tube. Urine inside the watch glass at the bottom of the metabolic jar and that from the urinary bladder was collected and measured.
A portion of the blood was centrifuged to separate the plasma which was then hydrolyzed with 1 N HCl. After neutralizing and desalting, the hydrolyzate was applied to a sheet of Whatman’s No. 1 filter paper and carried out the two dimensional ascending chromatograph for 20 hr at room temperature. After drying in the air and spraying with ninhydrin reagent and heating to reveal the amino acid spots, the chromatogram was autoradiographed on a sheet of X-ray photographic film. The radioactive spots from the chromatogram were cut out and they were eluted. Their radioactivity in counts per minute was recorded and converted to the percentage of the radioactivity in counts of the glycine injected.

The remaining sample of blood was treated with 2,4-dinitrophenylhydrazine reagent as described in a previous paper (10) to identify the spots of GA and other oxo-acids. Their radioactivity in counts was similarly calculated.

It was noted that when radioactive glycine was injected into the normal rats, only a fraction (15.70–17.12%) was eliminated in the expired CO₂ within 3 hr, and a fraction was converted into serine (2.70–3.80%), Pyr and α-oxo-glutarate (OG) in the plasma (Table 1).

When radioactive glycine was injected into rats when the thiamine deficiency first appeared, some radioactivity was found in GA, Pyr and OG; rats suffered from prolonged thiamine deficiency have more than half of the amount of radioactivity derived from glycine within 3 hr in the expired CO₂, and GA contained more radioactivity. Apart from the part escaped into the urine, the part of glycine remained in the body was under constant metabolic changes in many ways, it was probable that the peak of this conversion was missed. Therefore there was probably a higher rate of GA formation from glycine in the thiamine deficient rats.

The second fact obtained is the different ways in which the injected glycine is utilized. In the thiamine-deficient animals, glycine is used for the energy as judged by the oxidation to higher percentage of CO₂; while in normal rats, only a small portion was used for energy purpose, and a large portion was supposed to be used for the anabolic purpose.

The third fact obtained from this study illustrates the modifying effect on metabolism of the nutritional state of the animals. In the course of developing thiamine deficiency, the

### Table 1. Conversion of peritoneally injected glycine-2₁⁴C into normal and thiamine-deficient rats into radioactive expired CO₂, radioactive blood constituents: serine, other amino acids (α–α), glyoxylate (GA), pyruvate (Pyr), α-oxo-glutarate (OG) and radioactive urinary constituents: GA, Pyr, and OG at ambient temperature of 18°C in 3 hr.

| Condition of rats | Radioactivity of expired CO₂ (%) | Radioactivity of blood constituents (%) | Radioactivity of urinary constituents (%) |
|-------------------|---------------------------------|---------------------------------------|-----------------------------------------|
| Sex               | Body weight (g) | Days on thiamine-deficient diet | Radioactivity of expired CO₂ (%) | Serine | a–α | GA | Pyr | OG | GA | Pyr | OG |
| F                 | 173              | 0                                  | 15.70                                 | 3.80   | 0.13 | 0  | 0.60 | 0.60 | 0  | 0.80 | 0.20 |
| M                 | 252              | 0                                  | 16.20                                 | 2.70   | 0.14 | 0  | 0.50 | 0.50 | 0  | 0.60 | 0.28 |
| M                 | 263              | 0                                  | 15.80                                 | 3.15   | 0.17 | 0  | 0.30 | 0.30 | 0  | 0.20 | 0.25 |
| M                 | 284              | 10                                 | 17.12                                 | 2.84   | 0.19 | 0  | 0.90 | 1.10 | 0  | 1.12 | 0.32 |
| M                 | 181              | 18                                 | 18.70                                 | 2.68   | 0.15 | 0.18 | 2.80 | 2.80 | 0.16 | 2.86 | 1.72 |
| F                 | 152              | 18                                 | 24.80                                 | 2.23   | 0.13 | 0.98 | 3.20 | 3.53 | 0.42 | 2.60 | 1.48 |
| M                 | 176              | 32                                 | 32.66                                 | 0.89   | 0.11 | 1.57 | 2.13 | 1.86 | 8.42 | 1.56 | 1.68 |
| M                 | 138              | 35                                 | 31.23                                 | 0.75   | 0.11 | 2.89 | 2.76 | 2.22 | 15.17 | 1.87 | 1.23 |
| F                 | 121              | 40                                 | 62.00                                 | 0.37   | 0.08 | 3.21 | 1.84 | 1.54 | 2.86 | 1.33 | 1.12 |
| M                 | 125              | 43                                 | 45.30                                 | 0.25   | 0.05 | 3.85 | 0.97 | 0.98 | 0.54 | 0.24 | 0.18 |
| M                 | 118              | 47                                 | 38.95                                 | 0.15   | 0   | 5.98 | 0.53 | 0.32 | 0.43 | 0.18 | 0.09 |
| F                 | 98               | 51                                 | 55.50                                 | 0      | 0   | 6.12 | 0   | 0    | 0.12 | 0.08 | 0   |
Table 2. Average metabolic CO₂ (ml) liberated from incubating 5 ml liver homogenate from 5 normal rats, 10 thiamine-deficient rats (35 days on thiamine-deficient diet) and 10 paired-fed rats (35 days on restricted diet) without and with added thiamine pyrophosphate (TPP) (1.0 mg) respectively. The CO₂ obtained after aerated for 30 min with various substrates added to the liver homogenate at 37°C.

| Substrate added | Quantity (μmole) | CO₂ produced |          |          |          |          |          |
|-----------------|-----------------|--------------|----------|----------|----------|----------|----------|
|                 |                 | No TPP added | With TPP added |          |          |          |          |
|                 |                 | Total | Net | % of recovery | Total | Net | % of recovery |          |          |          |          |

**Normal rats**

| Substrate added | Quantity (μmole) | CO₂ produced |          |          |          |          |          |
|-----------------|-----------------|--------------|----------|----------|----------|----------|----------|
| Nil (endogenous) | 0.775           | 0.807        |          |          |          |          |          |
| Glucose         | 2.555           | 2.883        | 0.700    | 2.076    | 92.65    |          |          |
| Glycine         | 1.066           | 1.149        | 0.000    | 0.342    | 15.27    |          |          |
| Serine          | 2.215           | 2.497        | 0.200    | 1.723    | 76.83    |          |          |
| Na-formate      | 1.019           | 1.073        | 0.266    | 11.87    |          |          |          |
| Na-glyoxylate*  | 0.767           | 0.837        | 0.008    | 0.300    | 13.39    |          |          |
| Na-glyoxylate*  | 0.785           | 0.819        | 0.010    | 0.012    | 26.76    |          |          |

**Thiamine deficient rats**

| Substrate added | Quantity (μmole) | CO₂ produced |          |          |          |          |          |
|-----------------|-----------------|--------------|----------|----------|----------|----------|----------|
| Nil (endogenous) | 0.483           | 0.700        |          |          |          |          |          |
| Glucose         | 0.904           | 1.325        | 0.625    | 27.90    |          |          |          |
| Glycine         | 2.025           | 2.830        | 2.130    | 94.70    |          |          |          |
| Serine          | 0.884           | 1.278        | 0.578    | 25.80    |          |          |          |
| Na-formate      | 1.008           | 1.226        | 0.526    | 23.50    |          |          |          |
| Na-glyoxylate*  | 0.385           | 0.706        | 0.006    | 26.78    |          |          |          |
| Na-glyoxylate*  | 0.499           | 0.724        | 0.024    | 53.60    |          |          |          |

**Pair-fed rats**

| Substrate added | Quantity (μmole) | CO₂ produced |          |          |          |          |          |
|-----------------|-----------------|--------------|----------|----------|----------|----------|----------|
| Nil (endogenous) | 0.703           | 0.788        |          |          |          |          |          |
| Glucose         | 2.438           | 2.763        | 1.975    | 88.15    |          |          |          |
| Glycine         | 1.028           | 1.280        | 0.492    | 21.96    |          |          |          |
| Serine          | 2.127           | 2.552        | 1.764    | 78.35    |          |          |          |
| Na-formate      | 0.951           | 1.065        | 0.277    | 12.36    |          |          |          |
| Na-glyoxylate*  | 0.698           | 0.801        | -0.013   | 12.36    |          |          |          |
| Na-glyoxylate*  | 0.715           | 0.804        | 0.016    | 35.73    |          |          |          |

*a* The net CO₂ production was the total CO₂ production minus the endogenous CO₂ production.

*b* The % of recovery was the net CO₂ production divided by the expected CO₂ produced from the amount of substrate added.

*c* Since higher glyoxylate concentrations were found to inhibit metabolism in this experiment, the concentration of Na-glyoxylate was kept low to minimize the inhibitory effect which would mask the actual oxidation of this substrate to CO₂.

**oxidation of glycine was shifted from the serine to the GA route. This shift of the metabolic pathway will be further demonstrated in the experiment below:**

The diversified aspect of energy provision can further be noted by incubating liver homogenate (Fig. 2) from normal rats, thiamine-deficient rats, and paired-fed rats (1, 10) reared on a restricted diet. Different degrees of utilization of various added substrates on liver homogenate were observed (Table 2).

In the normal rats glucose and serine were metabolized more efficiently than other substrates to provide energy; but in the thiamine-deficient rats, the increased rate of glycine metabolism revealed that glycine was being metabolized for energy more completely and faster than glucose and serine. It was quite
possible there was a diversion to GA, formate and CO₂ pathway. In the presence of thiamine pyrophosphate (TPP), the metabolism of all substrates was increased; however, it increased more in the thiamine-deficient rats.

The present study revealed an alteration of the metabolic pathway in thiamine deficiency as shown in the accelerated rate and the more complete metabolism of glycine compared with other common substrates. Although GA can form malate (11, 12) which could enter the TCA-cycle in the normal animal tissue metabolism. Thiamine deficiency produced blocks in the TCA-cycle, preventing the completed oxidation of the oxo-acids which could not produce enough energy and would endanger the life of the animal.

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