EFFECT OF THIAMINE DEFICIENCY ON COLLAGEN METABOLISM

C. Upendra Prasad and S. M. Bose

Biochemistry Laboratory, Central Leather Research Institute, Madras-20, India
(Received February 5, 1974)

Young weanling albino rats were pair fed with control and thiamine deficient diets for five weeks. The analyses of skins and granulomas showed that, compared with the control, the thiamine deficient group had a decrease in the neutral salt soluble, insoluble, and total collagen contents. The incorporation of glycine-1-14C into the skin collagen and the free glycine content of skins were also decreased. There was no change in the RNA and DNA contents of skins and granulomas. The urinary excretion of hydroxyproline or plasma hydroxyproline was not affected. The results suggest that there is a reduction of collagen synthesis in thiamine deficiency.

In several skin diseases the metabolism of thiamine is disturbed (1–4). Thiamine was shown to accelerate wound healing (5). Gould (6) suggested a potential role for B-complex vitamins in collagen metabolism. Natarajan and Bose (7) reported a decrease of total hydroxyproline content of skins in thiamine deficient rats. In the present investigation the effect of thiamine deficiency on the metabolism of collagen has been studied.

MATERIALS AND METHODS

Twenty-one-day-old weanling albino rats were pair fed with a basal diet for five days and then divided into two groups of thirty-six each. The first group was fed a thiamine deficient diet (Nutritional Biochemicals, Cleavland, Ohio), while the second group was fed with the same diet, but with a supplement of thiamine hydrochloride (0.5 mg/100 g diet) and also the other required vitamins. The first group was given all the required vitamins except thiamine. The control animals were pair fed with thiamine deficient rats. Water was supplied ad lib. Daily records for food consumption were kept and the body weights were taken weekly. On the last day of the experimental period of five weeks, the urine from each rat was collected under toluene for a period of 24 hr. Glycine-1-14C was then injected into each rat intraperitoneally in 0.9% NaCl, the dose being
10 μg/100 g body weight. Five hours after injecting the labelled glycine, the rats were killed by decapitation, the blood was collected and the skin was removed from each rat immediately. For the convenience of analysis, skins, urine or blood from six animals in each group were pooled together, thus making six samples in each group.

Analysis of skin

Total collagen content. The skins were shaved with a razor blade and fleshed. An aliquot of the skins from each pooled sample was analysed for total hydroxyproline content by the method of Prockop and Udenfriend (8) and the total collagen content was calculated by multiplying the hydroxyproline content by the factor 7.46 (9).

Fractionation of collagen. Another aliquot of the skins from each pooled sample was extracted with 1 M NaCl by the method of Levene and Gross (10) for neutral salt soluble collagen. The residue left after NaCl extraction was thrice extracted further with 0.5 M citrate buffer (pH 3.6) for acid soluble collagen. The pooled extract obtained after 1 M NaCl or 0.5 M citrate buffer extractions was centrifuged and the collagen content of the supernatant was calculated by estimating the hydroxyproline content. The insoluble collagen content was calculated by subtracting the sum of soluble collagens from the total collagen. The results obtained are presented in Table 1.

Table 1. Effect of thiamine deficiency on neutral salt soluble, acid soluble and insoluble collagen content of skins.*

| Group            | Neutral salt soluble collagen b | Acid soluble collagen b | Insoluble collagen b | Total collagen b |
|------------------|---------------------------------|------------------------|---------------------|------------------|
| Control          | 3.97 ± 0.34                     | 1.62 ± 0.22           | 75.49 ± 4.13        | 81.08 ± 4.36     |
| Thiamine deficient | 1.83 ± 0.21                    | 1.44 ± 0.18           | 64.89 ± 3.75        | 68.16 ± 3.92     |

*Expressed as g/100 g dry defatted skin.

Values are mean ± standard error, six samples, six skins in each sample.

Radioactivity of gelatin. Collagen from another aliquot of each pooled sample was prepared as purified gelatin by the method of Jackson (11). The gelatin was hydrolysed and converted to DNP amino acids by the method of Sanger (12). After removing the dinitrophenols by the cold finger sublimation technique of Mills (13), DNP-glycine was isolated as described by Neuberger et al. (14) and its radioactivity was measured by the procedure of Henriques et al. (15). The values expressed as specific activity are presented in Table 2.

Free glycine content of skins. To check whether the decreased incorporation of labelled glycine is due to an increase in the glycine precursor pool and to the greater dilution of the injected labelled glycine, the free glycine content of an aliquot of the skins from each pooled sample was determined by the method of
EFFECT OF THIAMINE DEFICIENCY ON COLLAGEN METABOLISM

Table 2. Effect of thiamine deficiency on the specific activity of gelatin and free glycine content of skins.\textsuperscript{a}

| Group               | Specific activity\textsuperscript{b} | Free glycine content\textsuperscript{c} |
|---------------------|--------------------------------------|--------------------------------------|
| Control             | 122.3±6.2                            | 149.6±7.5                            |
| Thiamine deficient  | 87.7±4.2                             | 124.8±6.9                            |

\textsuperscript{a} Values are mean ± standard error, six samples, six skins in each sample.
\textsuperscript{b} Expressed as counts per minute/mg of gelatin.
\textsuperscript{c} Expressed as mg/100 g dry defatted skin.

Table 3. Effect of thiamine deficiency on nucleic acid contents of skins.\textsuperscript{a}

| Group               | RNA\textsuperscript{b} | DNA\textsuperscript{b} |
|---------------------|------------------------|------------------------|
| Control             | 498±19                 | 163±7                  |
| Thiamine deficient  | 484±21                 | 159±9                  |

\textsuperscript{a} Values are mean ± standard error, six samples, six skins in each sample.
\textsuperscript{b} Expressed as mg/100 g dry defatted skin.

SMITH and ALLISON (16). The results obtained are presented in Table 2.

RNA and DNA contents of skin. The RNA and DNA contents of the skins were calculated by estimating the ribose and deoxyribose contents of an aliquot of the skins from each pooled sample according to the method described by NATARAJAN and BOSE (7). The results are presented in Table 3.

Analysis of urine and plasma. The total and free hydroxyproline contents of urine or plasma were determined for hydrolysed and unhydrolysed samples, respectively, by the method of PROCKOP and UDENFRIEND (8) and the bound hydroxyproline was calculated. In the case of plasma, the hydroxyproline content was determined after deproteinization with trichloro-acetic acid. The results are presented in Table 4.

Analysis of granulomas. For this study, two groups of thirty-six rats each were raised according to the same plan as described in the previous section. Five

Table 4. Effect of thiamine deficiency on urinary hydroxyproline excretion and plasma hydroxyproline content.\textsuperscript{a}

| Group               | Urinary hydroxyproline\textsuperscript{b} | Plasma hydroxyproline\textsuperscript{c} |
|---------------------|--------------------------------------------|----------------------------------------|
|                     | Free                                       | Bound                                  | Free                                     | Bound                                  |
| Control             | 74.3±6.7                                   | 372.1±11.9                             | 13.7±.8                                  | 20.8±1.6                               |
| Thiamine deficient  | 72.8±6.4                                   | 367.8±12.2                             | 13.2±1.1                                  | 19.3±1.5                               |

\textsuperscript{a} Values are mean ± standard error, six samples, each sample containing urine or plasma from six animals.
\textsuperscript{b} Expressed as µg/24 hr/rat.
\textsuperscript{c} Expressed as µg/ml.
days before the termination of the experimental period, two granulomas were induced in each rat by bilateral implantation of sterilised cotton sponges subcutaneously in the dorsum by the method adopted by Sekharavarma and Bachhawat (17). On the fifth day after the implantation, the rats were killed and the granulomas were removed by careful dissection of the connective tissue. The granulomas from each group were pooled together to make one sample for analysis, as the collagen content in granulomas is very small.

Fractionation of collagen. Neutral salt soluble, acid soluble and insoluble collagen contents of granulomas were determined by the same methods as adopted in the case of the skins. The results are presented in Table 5.

RNA and DNA contents of granulomas. The RNA and DNA contents of granulomas were determined as in the case of skins. The results are presented in Table 6.

Table 5. Effect of thiamine deficiency on neutral salt soluble, acid soluble and insoluble collagen contents of granulomas.

| Group              | Neutral salt soluble collagen^a | Acid soluble collagen^a | Insoluble collagen^a | Total collagen^a |
|--------------------|---------------------------------|-------------------------|----------------------|------------------|
| Control            | 0.511                           | 0.187                   | 7.684                | 8.382            |
| Thiamine deficient | 0.247                           | 0.172                   | 6.448                | 6.867            |

^a Expressed as g/100 g dry defatted granulomas.

Table 6. Effect of thiamine deficiency on nucleic acid content of granulomas.

| Group              | RNA^a | DNA^a |
|--------------------|-------|-------|
| Control            | 1.7   | 2.8   |
| Thiamine deficient | 1.7   | 2.7   |

^a Expressed as g/100 g dry defatted granulomas.

RESULTS AND DISCUSSION

Table 1 shows that, compared with the control, the neutral salt soluble, acid soluble, insoluble, and total collagen contents of skins of the thiamine deficient group decreased by 53.9%, 11.1%, 14.0%, and 15.9%, respectively. In the case of thiamine deficient granulomas also, the neutral salt soluble, acid soluble, insoluble, and total collagen contents were found to decrease by 51.6%, 8.0%, 16.0%, and 18.0%, respectively (Table 5). The neutral salt soluble collagen is known to be the most recently synthesised collagen molecules (18). A close relationship between the level of neutral salt soluble collagen and the rate of collagen synthesis in healing wounds has also been reported (19). The appreciable decrease of neutral salt soluble collagen may, therefore, infer a reduction in the collagen synthesis (18, 20, 21). The role of acid soluble collagen in collagen
metabolism is not clear, but it is now considered that it may be a degradation intermediate of insoluble collagen, rather than its precursor (21-23). Jackson (11) and Woessner (24, 25) proposed solubilization of collagen to precede the catabolism.

Table 2 shows decreased incorporation of glycine-1-14C in the thiamine deficient group as the specific activity of gelatin from this group was reduced by 28.3%, compared with the control. The free glycine content of skins of thiamine deficient animals shows a decrease of 16.5%. These results suggest a decreased synthesis of collagen.

Table 3 shows a negligible change in the RNA and DNA contents of the skins in thiamine deficient animals compared with those of the controls. Similar results are obtained in the case of granulomas (Table 6).

Table 4 shows a negligible reduction in the urinary excretion of hydroxyproline or plasma hydroxyproline, thus suggesting that catabolism is probably not affected. Though urinary hydroxyproline excretion usually serves as a parameter of collagen catabolism (26-28), it may not always be the case, since it may be altered by changes in kidney functions and resorption (25).

The mechanism by which thiamine deficiency affects the collagen metabolism is not known, but since no change has been observed in RNA and DNA contents of skins and granulomas in thiamine deficiency, the mechanism may be different from that of the inhibition of general protein synthesis. Thiamine deficiency is known to decrease the tissue ascorbic acid levels (29, 30) and a role for thiamine in the biosynthesis of ascorbic acid has been suggested (30, 31). In ascorbic acid deficiency the hydroxylation of proline to hydroxyproline is impaired (32-39). Ascorbate is also shown to be a necessary cofactor for the enzymes (proline hydroxylase and lysine hydroxylase), hydroxylating proline and lysine (40, 41). It may be possible that the observed changes in thiamine deficiency are due to an interference in the hydroxylation of proline and lysine.

Our thanks are due to Dr. M. Santappa, Director, Central Leather Research Institute, Madras, for his keen interest in this work and for permission to publish the results. The financial assistance to one of us (C. U. P.) by the Council of Scientific and Industrial Research, New Delhi, is gratefully acknowledged.

REFERENCES

1) Takenouchi, K., Aso, K., and Yamamoto, S., Vitamin (Kyoto), 18, 211 (1959).
2) Yamamoto, S., Vitamin (Kyoto), 18, 231 (1959).
3) Aso, K., Japan J. Dermatol. Urol., 69, 1732 (1959).
4) Tatarenkov, L. M., Vestn. Dermatol. Venerol., 38, 23 (1964).
5) Yabuki, T., Hiroshima Igaku, 8, 2611 (1964).
6) Gould, B. S., Treatise on Collagen, 2, Part B, 323 (1968).
7) Natarajan, M. and Bose, S. M., Leath. Sci., 12, 111 (1965).
8) Prockop, D. J. and Udenfriend, S., Anal. Biochem., 1, 228 (1965).
9) Neuman, R. E. and Logan, M. A., *J. Biol. Chem.*, 186, 349 (1950).
10) Levine, C. I. and Gross, J., *J. Exp. Med.*, 110, 771 (1959).
11) Jackson, D. S., *Biochem. J.*, 65, 277 (1957).
12) Sanger, F., *Biochem. J.*, 39, 507 (1945).
13) Mills, G. L., *Biochem. J.*, 50, 707 (1952).
14) Neuberger, A., Perrone, J. C., and Slack, H. G. B., *Biochem. J.*, 49, 199 (1951).
15) Henriques, O. B., Henriques, S. D., and Neuberger, A., *Biochem. J.*, 60, 409 (1955).
16) Smith, Q. T. and Allison, D. J., *Endocrinology*, 77, 785 (1965).
17) Sekharavarma, T. N. and Bacchawat, B. K., *Biochim. Biophys. Acta*, 69, 464 (1964).
18) Jackson, D. S. and Bentley, J. P., *Biophys. Biochem. Cytol.*, 7, 37 (1960).
19) Peacock, E. E., *Surg. Cynecol. Obstet.*, 113, 329 (1961).
20) Harkness, R. D., Marko, A. M., Muir, H. M., and Neuberger, A., *Biochem. J.*, 56, 558 (1959).
21) Kuhn, K., Iwangoff, P., Hammerstein, F., Stecher, K., Durruti, M., Holzmann, H., and Korting, G. W., *Z. Physiol. Chem.*, 33, 4 (1964).
22) Tsurufuji, S. and Ogata, Y., *Biochim. Biophys. Acta*, 104, 193 (1965).
23) Hurych, J. and Chvapil, M., *Soveren. Biokhim. Morfol. Probe. Soldin. Tkani 1968* (Pub. 1971), Ed. A.D. Soboreva, p. 93.
24) Woessner, J. F., *Biochem. J.*, 83, 304 (1962).
25) Woessner, J. F., in B. S. Gould (Editor), *Treatise on Collagen*, Academic Press, New York, London, Vol. 2, 253 (1968).
26) Dull, T. A. and Heneman, P. H., *New Engl. J. Med.*, 268, 132 (1963).
27) Klein, L., *Metabolism*, 13, 386 (1964).
28) Johnston, C. C. and Deiss, W. F., *Metabolism*, 14, 523 (1965).
29) Kennaway, E. L. and Daff, M. E., *Brit. J. Exp. Pathol.*, 27, 63 (1946).
30) Roy, S. C., Roy, S. K., and Guha, B. C., *Nature*, 158, 238 (1946).
31) Little, P. L. and Edgar, S. A., *Poultry Sci.*, 50, 26 (1971).
32) Robertson, W. V. B. and Hiwett, J., *Biochim. Biophys. Acta*, 49, 404 (1961).
33) Robertson, W. V. B. and Sanborn, E. C., *Endocrinology*, 63, 250 (1958).
34) Gould, B. S., *Vitam. and Horm.*, 17, 89 (1960).
35) Gould, B. S., *Ann. N.Y. Acad. Sci.*, 92, 168 (1961).
36) Gross, J., *J. Expil. Med.*, 107, 265 (1959).
37) Hurych, J. and Chvapil, M., *Naturwissenschaften*, 49, 17 (1962).
38) Ross, R. and Benditt, E. P., *Fed. Proc.*, 21, 173 (1962).
39) Ross, R. and Benditt, E. P., *Fed. Proc.*, 23, 441 (1964).
40) Kivirikko, K. I. and Prockop, D. J., *Arch. Biochem. Biophys.*, 118, 611 (1967).
41) Bhatnagar, R. S., Liu, T. Z., and Rapaka, S. R., *Fed. Proc.*, 31, 3528 (1972).