Tryptophan Metabolite Excretion in Connective Tissue Diseases Demonstrating a Difference Between Rheumatoid Spondylitis and Rheumatoid Arthritis.* (29689)

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Previous studies have shown that patients with certain chronic diseases excrete elevated amounts of tryptophan metabolites in their urine. McMillan reported abnormal levels of the tryptophan metabolite, 3-hydroxyanthranilic acid (3HAA), in the urine of patients with rheumatoid arthritis(1). This finding has been subsequently confirmed by Bett(2) and Spiera(3). Another tryptophan metabolite, kynurenine, has also been found to be excreted in increased amounts by patients with rheumatoid arthritis before and after a loading dose of L-tryptophan(2,4). However, these findings are not specific for rheumatoid arthritis since abnormal urinary excretion of tryptophan metabolites following ingestion of L-tryptophan has been observed in other conditions including bladder cancer, porphyria, scleroderma, systemic lupus erythematosus, and pregnancy(4).

It is not clearly established whether or not rheumatoid spondylitis is a variant of rheumatoid arthritis or a distinct clinical entity. Therefore, it was deemed worthwhile to determine the urinary excretion of kynurenine in patients with rheumatoid spondylitis and rheumatoid arthritis to see if there is a significant difference in kynurenine excretion in these two diseases. Furthermore, Spiera has shown that patients with rheumatoid arthritis excrete elevated levels of 3HAA when compared to patients with various other "collagen" diseases and hospital controls(3). However, other studies have demonstrated a significant increase in kynurenine excretion in patients with scleroderma and systemic lupus erythematosus after ingesting a load of L-tryptophan(5,6). In view of these somewhat inconsistent findings in the "collagen" or connective tissue disease group other than rheumatoid arthritis, it was also considered worthwhile to determine the amount of urine kynurenine excreted in a group of patients with a variety of other connective tissue diseases including systemic lupus erythematosus, scleroderma, and polymyositis. The results are reported here.

Materials and methods. All the patients on this study were admitted to the Clinical Research Unit at the University Hospital in Birmingham. Studies were obtained on 40 patients with various connective tissue diseases. The connective tissue disease group consisted of 14 patients with rheumatoid arthritis, 12 patients with rheumatoid spondylitis, 7 patients with systemic scleroderma, 5 patients with systemic lupus erythematosus and 2 patients with polymyositis. All patients with rheumatoid arthritis fulfilled the American Rheumatism Assn. criteria for definite or classical rheumatoid arthritis(7). All the patients with rheumatoid spondylitis had limitation of spinal motion, decreased chest expansion, and radiographic evidence of bilateral sacroiliac joint involvement and as a group represented rather severe disease. The control group consisted of 3 normal females and 9 normal males.

All of the subjects were maintained on a constant normal diet. The daily tryptophan intake ranged from 0.7 to 1.9 g with an average intake of 1.064 g. The average normal diet contains approximately 1 g of tryptophan per day. After 2 days of equilibration, 5 consecutive 24-hour urine collections were obtained for analysis from each subject. The kynurenine content of the urine samples was determined according to the method of Tompsett(8) using a reagent grade kynurenine as a standard. Creatinine values were determined on each 24-hour urine collection and were within normal limits. Statistical analysis of the data was by means of an ap-

* This study was supported by grants from Nat. Inst. Health (USPH AM 03555, USPH TI AM 5000 & USPH 2M01 FR-32-04).

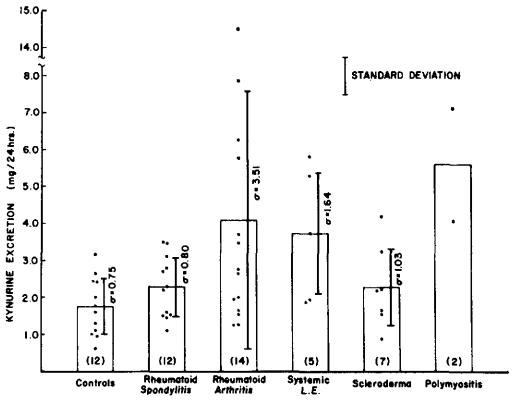

FIG. 1. Mean 24-hour kynurenine values excreted in urine of various disease groups and normal controls. Number enclosed by parenthesis on each bar is number of individuals in each group. Height of the bar represents mean kynurenine value for each group. Line which extends above and below height of each bar represents one standard deviation. The mean 24-hour kynurenine value for each individual studied was calculated from 5 separate 24-hour urine collections obtained from each subject, shown on this Figure by a single dot.

proximate "T" test or by analysis of variance where appropriate.

Results. The mean 24-hour urine kynurenine values for patients with rheumatoid arthritis and systemic lupus erythematosus of 4.08 mg and 3.73 mg respectively were significantly elevated ($P>.05$) when compared to the mean value of 1.75 mg for the control group. However, no significant difference was found between the scleroderma group (2.28 mg) or the rheumatoid spondylitis group (2.27 mg) and the control group (Fig. 1). The kynurenine values for the control and rheumatoid spondylitis groups and to a lesser extent the scleroderma group lie relatively close together as reflected by standard deviations of 0.75, 0.80, and 1.03 respectively. However, there is a marked spread of kynurenine values obtained from patients with rheumatoid arthritis, and the standard deviation (3.51) is especially large in this group. Kynurenine values for patients with systemic lupus erythematosus also very considerably as reflected by a standard deviation of 1.64, but this spread is less marked than in the group with rheumatoid arthritis. Also of particular interest is the finding that the mean kynurenine value for 2 patients with polymyositis is about 3 times that of the control patients.

Some of the kynurenine values obtained from patients with rheumatoid arthritis lie well within the range of values found in the normal control group; whereas other kynurenine values in the rheumatoid group are markedly elevated. These findings are in agreement with other studies that also found a wide range of values for the tryptophan metabolites 3HAA and kynurenine in the urine of patients with rheumatoid arthritis (2,3,4). In the rheumatoid group there is no apparent correlation between the levels of kynurenine excreted and other clinical or laboratory parameters such as duration of the disease, subjective discomfort, titer of rheumatoid factor, or radiological changes. These findings also agree with previous studies(2,3).

The kynurenine content was determined on 5 different 24-hour urine collections obtained from each subject. The level of kynurenine in each of these 24-hour urine collections was found to be reasonably constant for each individual.

Discussion. There has been considerable controversy as to whether rheumatoid spondylitis is a variant of rheumatoid arthritis or a distinct clinical entity. There are some well established clinical differences between these conditions(9). For example, rheumatoid arthritis is somewhat more common in the female; whereas rheumatoid spondylitis is much more common in males. Rheumatoid arthritis is frequently associated with the presence of rheumatoid factor in the blood, but it is most unusual to find rheumatoid factor in the blood of patients with rheumatoid spondylitis. Rheumatoid nodules are relatively common in rheumatoid arthritis; however, they rarely if ever occur in rheumatoid spondylitis. Rheumatoid spondylitis is often associated with calcification of spinal ligaments which is apparently not seen in rheumatoid arthritis. Also rheumatoid spondylitis has a marked tendency to involve both sacroiliac joints which is unusual for patients with rheumatoid arthritis. The demonstrated difference in kynurenine excretion between patients with rheumatoid spondylitis and

those with rheumatoid arthritis in this study provides additional evidence that the two conditions are distinct clinical entities. The main argument in favor of these diseases being similar is that about 20% of patients with rheumatoid spondylitis develop peripheral joint involvement which clinically and pathologically closely resembles that of rheumatoid arthritis(10).

In contrast to previous studies, no significant difference was found between the 7 patients with scleroderma and the control group. Both Brown(5) and Price *et al*(6) found elevated excretion of kynurenine in the urine after a loading dose of tryptophan in patients with systemic scleroderma. The subjects in this study were on a normal diet, and it is possible that if they had received a loading dose of tryptophan, abnormal levels of kynurenine excretion might have been demonstrated in the patients with scleroderma.

The finding of elevated urinary excretion of kynurenine in the systemic lupus erythematosus group agrees with the observations of Brown(5) who demonstrated elevated levels of kynurenine in patients with systemic lupus erythematosus following a loading dose of tryptophan. It is not surprising to find evidence of abnormal tryptophan metabolism in both rheumatoid arthritis and systemic lupus erythematosus since it is widely recognized that there are many similarities and overlapping features in these 2 diseases. It was of particular interest to find unusually high levels of kynurenine in the 2 patients with polymyositis since, to our knowledge, this has not been previously reported.

The cause and significance of the increased excretion of kynurenine in certain chronic diseases such as rheumatoid arthritis has not been established. It is known that both kynurenine and 3HAA are intermediates in the metabolism of tryptophan by the kynurenine pathway. In this pathway, the indole ring of tryptophan is split by the enzyme tryptophan pyrrolase, and after a series of steps kynurenine and subsequently 3HAA are obtained. 3HAA is oxidized to an aldehyde intermediate which eventually is metabolized to become chinolinic, picolinic, and nicotinic acid.

The increased amounts of tryptophan metabolites in the urine of some patients with an active disease process may reflect the catabolic effect of a systemic disease, but the demonstration by Bett(11) and Flinn *et al*(4) that administration of pyridoxine (Vitamin B_6) can restore tryptophan metabolism towards normal in patients with rheumatoid arthritis suggests that there may be a specific abnormality of tryptophan metabolism. An active catabolic disease process in itself need not necessarily result in an abnormality of tryptophan metabolism since patients with a variety of chronic diseases such as diabetes mellitus, gout, cirrhosis, and rheumatoid spondylitis excrete normal amounts of various tryptophan metabolites in the urine(3).

Summary. Urinary excretion of kynurenine was measured in a group of patients with various connective tissue diseases and compared to normal controls. Elevated kynurenine excretion was found in patients with rheumatoid arthritis, systemic lupus erythematosus, and in 2 patients with polymyositis; whereas excretion of kynurenine in patients with scleroderma and rheumatoid spondylitis was not significantly different from normal controls. The demonstrated difference in kynurenine excretion between patients with rheumatoid spondylitis and those with rheumatoid arthritis in this study provides additional evidence that the two conditions are distinct clinical entities.

The valuable technical assistance of Miss Geneva Blackburn is gratefully acknowledged. The authors also wish to thank Miss Carol Brewster for supervision of the diet used in this study.

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Received September 2, 1964. P.S.E.B.M., 1964, v117.

Effect of Protein Reserves on Liver Regeneration After Partial Hepatectomy. (29690)

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It is well known that animals can maintain nitrogen balance on widely varying intakes of nitrogen(1). If an animal on a high protein diet is transferred to one low in protein content, nitrogen excretion gradually diminishes until, if the intake is not below a certain minimum, equilibrium is again reached. On the other hand, if an animal being maintained on a low or poor quality protein regime is transferred to a diet containing a higher level or better quality protein, initially a period of nitrogen retention occurs which is followed by rising excretion until nitrogen equilibrium is again established. This retained or easily lost nitrogen, is generally considered to represent the more labile protein in the body and is referred to collectively as "protein reserves."

The importance of protein reserves has been discussed elsewhere(2). One of the attributes of a reserve supply of any material is to furnish the substance in question in time of need. One such time is the need for amino acids by the regenerating liver after partial hepatectomy. The experiments to be reported here were designed to learn whether the state of the animals' protein reserves prior to removal of ⅔ of the liver would affect the subsequent liver regeneration taking place during the feeding of a protein free diet.

Methods. Two experiments were carried out. In the first, adult Charles River C-D male rats, weighing approximately 300 g, were placed on a protein-free diet for 2 weeks and then divided into 4 groups of 12 rats each which were fed respectively diets of (1) wheat gluten, (2) gluten + lysine monohydrochloride, (3) gluten + lysine monohydrochloride + threonine or (4) egg white for 1 week. (The nitrogenous part of each diet was added at the expense of corn starch to give 1.60% nitrogen in the diet.) The composition of these diets is shown in Table I. After this 7-day repletion period, one-half of the rats receiving each diet were subjected to partial hepatectomy by the method of Higgins and Anderson(3) and all groups were again placed on the protein-free diet. Seven days later, both operated and unoperated rats were anesthetized with nembutal and blood samples taken by heart puncture. The animals were then killed and the livers removed and weighed.

A second experiment was carried out in the same general manner, except that slightly smaller rats were used (average wt. 225 g), the group size was increased from 12 to 20

TABLE I. Composition of Diets (g/100 g Diet).

	1	2	3	4
Salt mixture USP XIV	4.0	4.0	4.0	4.0
Cellu-flour	5.0	5.0	5.0	5.0
Corn oil	10.0	10.0	10.0	10.0
Vitamin mix*	1.0	1.0	1.0	1.0
Wheat gluten†	11.1	11.1	11.1	—
Dried egg white‡	—	—	—	11.7
L-Lysine monohydrochloride	—	.35	.35	—
L-Threonine	—	—	.10	—
Corn starch	68.9	68.5	68.4	68.3

* Furnishing the following micronutrients per 100 g of food: In mg—thiamine, 1.0; riboflavin, 2.0; pyridoxine, 1.0; calcium pantothenate, 10.0; niacinamide, 10.0; inositol, 5.0; choline, 100.0; p-aminobenzoic acid, 30.0; biotin, 0.05; folic acid, 0.2; α-tocopherol, 14.2; menadione, 14.2; B_{12} triturate (0.1% trituration with mannitol), 10.0. In units—calciferol, 300; vitamin A palmitate, 1600.

† General Mills Pro-80 Wheat Gluten.

‡ Obtained from Henningsen Bros., N. Y.