ORAL TYROSINE TOLERANCE TEST IN RHEUMATOID ARTHRITIS

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On the basis of their observations that the patients with collagen diseases, but not their controls, excrete 2·5 dihydroxy phenylpyruvic acid in the urine, and that the urinary and plasma tyrosine levels of these patients are increased, Nishimura and his associates consider that the collagen diseases are disorders of the tyrosine metabolism (Nishimura, Yasui, Okamoto, Kanazawa, Kotake, and Shibata, 1958; Nishimura, Maeda, Yasui, Okamoto, Matsunaka, and Toshina, 1961; Nishimura, 1963).

Rivlin, Melmon, and Sjoerdsma (1965) have introduced an oral tyrosine tolerance test, using a fluorometric method for estimation of tyrosine in the plasma. Adopting this test, we have endeavoured to study the metabolism of tyrosine in rheumatoid arthritis because the question is still open for discussion.

Material and Methods

Our series consists of fifteen patients with rheumatoid arthritis (4 men, 11 women; average age 48·6 years), and sixteen control subjects with various internal diseases (4 men, 12 women; average age 53·7 years). Some clinical characteristics of the patients with rheumatoid arthritis are shown in Table I and the diagnoses of the controls in Table II. All the patients of the two groups were treated in hospital during the study. No drugs were given on the day before the test or on the test day.

The tyrosine tolerance test was performed as suggested by Rivlin and others (1965). We used 1-tyrosine puriss. (Fluka AG Buchs SG, Switzerland) and the dose given was 50 mg./kg. orally in orange juice. The drug was administered in the morning, 10 ml. of blood first being drawn for estimation of the 0-value of the blood tyrosine level. The same amount of blood was drawn at $\frac{1}{2}$, 1 $\frac{1}{2}$, 3, 4 $\frac{1}{2}$, and 6 hours after ingestion of the test dose of tyrosine. Heparin was added as an anticoagulant. Duplicate plasma samples were run through the entire procedure. The Aminco-Bowman spectrophotofluorometer was used for fluorescence measurement, and the fluorometric method presented by Waalkes and Udenfriend (1957) for estimation of tyrosine in plasma.

Results

These are given in Tables I and II (overleaf).

The plasma tyrosine level is significantly ($t = 3.02$; $P < 0.01$) lower in the rheumatoid arthritis group than in the control group. The differences in the plasma tyrosine level in the two groups after oral ingestion of tyrosine are statistically insignificant. In the rheumatoid arthritis group there were six patients with a plasma tyrosine level $< 11.0 \mu\text{g./ml.}$ The mean increase in the plasma tyrosine level after the test dose did not deviate significantly in these cases as compared with those with an initial tyrosine level $\geq 11.0 \mu\text{g./ml.}$ If the changes are expressed as a percentage of the control value, the difference between the two groups is still insignificant.

The patients with rheumatoid arthritis noticed no changes in their joint symptoms during and after the tyrosine tolerance test.

Discussion

In our patients with rheumatoid arthritis the plasma level was significantly lower than in the controls. This observation is not new. Waalkes and Udenfriend (1957) reported seven patients with rheumatoid arthritis whose plasma tyrosine level seemed to be lower than that of the healthy adults included in their series. Nettelblatt and Sandell (1963), using paper chromatography, studied the content of seventeen amino acids in the serum of patients with rheumatoid arthritis, and recorded low values for arginine, glutamine, tyrosine, and histidine, while the concentration of glutamic acid was higher in patients with rheumatoid arthritis than in healthy subjects. Kulonen and Kulonen (1960) studied the blood and urinary amino acids in rheumatoid arthritis also by paper chromatography. No difference specific to rheumatoid arthritis was observed in the blood, but the urinary elimination of glycine, glutamine, and alanine was increased, and the basic amino acids (cysteine, glutamic acid, and threonine)

TABLE I
RESULTS OF TYROSINE LOADING TEST IN 15 PATIENTS WITH RHEUMATOID ARTHRITIS

Case No.	Sex	Age (yrs)	Duration of Disease (yrs)	Stage of Disease	Waller-Rose Titre	Latex Test	E.S.R. (mm./hr)	Hb (g./100 ml.)	Tyrosine Loading (μ g./ml.) (hrs)					
									0	$\frac{1}{2}$	1 $\frac{1}{2}$	3	4 $\frac{1}{2}$	6
1	Female	53	1	I	128	3+	136	10.5	12.0	21.5	29.5	33.0	24.5	22.5
2	Female	31	1	I	0	0	60	9.8	10.5	13.3	20.5	18.8	14.1	11.1
3	Male	32	5	III	0	0	60	12.1	9.8	17.0	29.6	28.5	23.0	26.5
4	Male	26	$\frac{1}{2}$	I	0	0	22	11.4	9.7	21.0	27.5	30.3	21.8	15.5
5	Female	58	5	III	lacking	2+	25	13.0	13.8	16.3	28.8	22.8	25.8	20.5
6	Female	62	40	III	32	3+	65	11.8	12.0	18.2	17.8	24.5	29.8	27.5
7	Female	51	10	III	8	2+	53	12.7	15.3	18.7	27.8	28.0	28.0	21.5
8	Female	37	2	III	16	3+	83	9.9	9.7	23.1	23.8	23.8	23.7	16.5
9	Female	52	2	II	8	2+	75	12.5	12.8	17.3	17.3	21.5	25.8	19.5
10	Female	65	2	IV	lacking	lacking	115	10.0	12.5	15.3	16.8	19.3	18.1	19.5
11	Female	53	15	III	32	3+	113	9.6	15.3	45.3	48.5	35.0	24.5	18.5
12	Female	47	10	III	256	3+	73	10.6	8.5	21.8	22.5	21.8	lacking	14.5
13	Female	65	20	III	lacking	lacking	56	13.8	14.5	24.0	24.2	33.3	29.8	25.5
14	Male	47	1/12	I	0	2+	37	10.2	12.8	15.0	20.5	17.6	17.3	12.5
15	Male	51	1 $\frac{1}{2}$	II	8	2+	71	13.6	10.0	15.0	21.5	20.5	19.0	20.5
Mean Increase of Blood Tyrosine Content from Control Value										8.2	13.1	13.3	10.1	7.5
Mean Plasma Tyrosine Content									11.9	20.2	25.1	25.2	23.2	19.5
SEM									0.60	2.00	2.02	1.50	1.28	1.33
SD									2.32	7.65	7.85	5.82	4.80	4.44

TABLE II
RESULTS OF TYROSINE LOADING TEST IN 16 CONTROL SUBJECTS

Case No.	Sex	Age (yrs)	Diagnosis	Tyrosine Loading (μ g./ml.) (hrs)					
				0	$\frac{1}{2}$	1 $\frac{1}{2}$	3	4 $\frac{1}{2}$	6
1	Female	44	Observation	12.0	22.3	23.3	25.8	27.8	19.0
2	Male	62	Cardiac infarction	16.3	16.8	28.0	23.3	22.8	18.0
3	Female	48	Abdom. pains	13.8	19.4	19.5	19.8	21.3	23.0
4	Male	58	Hemiparesis	11.8	16.3	24.5	25.5	24.0	23.0
5	Female	59	Hypertensio art.	11.8	19.3	22.3	20.5	lacking	lacking
6	Female	40	Migraena	10.8	18.0	27.8	31.8	21.5	15.3
7	Female	43	Peptic ulcer	15.0	25.5	31.3	38.8	30.0	19.5
8	Female	61	Hypertensio art.	13.0	16.3	17.5	19.3	lacking	lacking
9	Female	55	Little stroke	17.6	31.8	42.0	31.0	27.3	21.5
10	Female	51	Hypertensio art.	16.3	33.3	50.3	38.3	32.8	25.8
11	Male	39	Haematuria	13.2	25.6	34.0	19.0	17.6	16.8
12	Female	67	Little stroke	16.0	24.8	37.5	27.0	25.8	25.8
13	Female	70	Angina pectoris	16.0	22.8	22.8	30.0	23.0	19.0
14	Female	50	Cardiac infarction	14.8	27.8	23.8	35.5	28.8	25.8
15	Female	72	Little stroke	19.8	24.0	31.8	41.8	27.9	lacking
16	Male	40	Observation	13.3	20.0	20.5	24.0	23.0	22.3
Mean Increase of Blood Tyrosine Content from Control Value					8.3	14.1	13.7	10.6	6.5
Mean Plasma Tyrosine Content				14.5	22.8	28.5	28.2	25.3	21.1
SEM				0.62	1.29	2.25	1.86	1.05	0.94
SD				2.48	5.15	9.00	7.45	3.94	3.39

decreased in patients with rheumatoid arthritis compared with cases of infection and with healthy controls. The elimination of tyrosine, phenylalanine, and leucines was increased in both rheumatoid arthritis and in cases of infection.

Rivlin and others (1965) observed very high plasma tyrosine levels after oral tyrosine loading in hyperthyroidism and low values in hypothyroidism. It has been suggested that thyroidectomy may aggravate rheumatoid arthritis or that this condition may develop after such an operation (Laine, Vainio, and Holopainen, 1954; Kalliomäki, 1954; Kalliola, Kalliomäki, and Rintala, 1957).

The results here presented show that, although the plasma tyrosine level is initially low in rheumatoid arthritis, its increase and decrease after oral tyrosine loading is normal. From earlier studies we know that the urinary elimination of tyrosine is increased in rheumatoid arthritis. This discrepancy—low basal level in plasma, normal "clearance" from plasma, and increased urinary elimination—is difficult to explain. The tyrosine content of the tissues and the renal handling of tyrosine during this kind of loading test must be evaluated more closely. The increased extracellular fluid phase observed in rheumatoid arthritis (Kalliomäki, Kirpilä, Koskinen,

and Laine, 1958) must also be taken into consideration in this connexion.

Summary

(1) An oral tyrosine tolerance test—50 mg./kg. l-tyrosine being given orally in the morning and the plasma tyrosine level followed thereafter by a fluorometric method for 6 hours—was performed on fifteen patients with rheumatoid arthritis and on sixteen patients with various internal disorders.

(2) The initial plasma tyrosine level was significantly ($P < 0.01$) lower in the rheumatoid patients than in the controls.

(3) The change in plasma tyrosine content after oral tyrosine loading was, within the limits of statistical error, the same in both groups of patients.

REFERENCES

- Kalliola, H., Kalliomäki, J. L., and Rintala, A. (1957). *Ann. med. intern. Fenn.*, **46**, 97.
 Kalliomäki, J. L. (1954). *Ann. rheum. Dis.*, **13**, 341.
 —, Kirpilä, J., Koskinen, H.-M., and Laine, V. A. I. (1958). *Acta rheum. scand.*, **4**, 79.
 Kulonen, M., and Kulonen, E. (1960). *Scand. J. clin. Lab. Invest.*, **12**, 84.
 Laine, V. A. I., Vainio, K. J., and Holopainen, T. E. (1954). *Ann. rheum. Dis.*, **13**, 250.
 Nettelbladt, E., and Sandell, B. M. (1963). *Ibid.*, **22**, 269.
 Nishimura, N. (1963). *J. chron. Dis.*, **16**, 343.
 —, Maeda, K., Yasui, M., Okamoto, H., Matsunaka, M., and Toshina, H. (1961). *A.M.A. Arch. Derm.*, **83**, 644.
 —, Yasui, M., Okamoto, H., Kanazawa, M., Kotake, Y., and Shibata, Y. (1958). *Ibid.*, **77**, 255.

Rivlin, R. S., Melmon, K. L., and Sjoerdsma, A. (1965). *New Engl. J. Med.*, **272**, 1143.

Waalkes, T. P., and Udenfriend, S. (1957). *J. Lab. clin. Med.*, **50**, 733.

Le test de tolérance à la tyrosine par voie buccale dans l'arthrite rhumatismale

RÉSUMÉ

(1) Le test de tolérance à la tyrosine par voie buccale—50 mg./kg. de l-tyrosine le matin, suivi pendant 6 heures de détermination fluorométrique du taux plasmatique de la tyrosine—fut effectué chez 15 malades atteints d'arthrite rhumatismale et 16 malades atteints de diverses affections internes.

(2) Le taux plasmatique initial de la tyrosine fut significativement ($P < 0.01$) plus bas chez les malades rhumatisants que chez les témoins.

(3) L'élévation du contenu du plasma en tyrosine après la surcharge par voie buccale fut, dans les limites de l'erreur statistique, le même dans les deux groupes de malades.

El test de tolerancia a la tirosina por vía oral en la artritis reumatoide

SUMARIO

(1) Se efectuó un test de tolerancia a la tirosina por vía oral—50 mg./kg. de l-tirosina por la mañana, seguidos de la determinación fluorométrica por seis horas de las cifras plasmáticas de tirosina—en 15 enfermos con artritis reumatoide y 16 enfermos con varios trastornos internos.

(2) Las cifras plasmáticas iniciales de tirosina fueron significativamente ($P < 0.01$) más bajas en los enfermos reumáticos que en los demás.

(3) El cambio en la concentración plasmática de la tirosina después de su sobrecarga por vía oral fué, en los límites del error estadístico, igual en ambos grupos de enfermos.