S^{35} Taurine Metabolism in Normal and Mongoloid Individuals.* (30740)

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Although urinary taurine excretion varies widely in the general population, some mongoloids excrete significantly less taurine than any subjects free of disease studied in our laboratories or elsewhere(1,2). Sufficient mongoloids and normals have been studied to permit tentative separation of discrete classes of taurine excretors in either population. For the present study, "low" excretors are provisionally defined as those excreting in their first morning urine less than 20 mg of taurine per gram creatinine. Such values have been found in only 10% of normal subjects but comprised 57% of mongoloids studied(3). "Intermediate" excretors are defined as those excreting 20-70 mg taurine per gram creatinine and include the lowest excretors (about 59%) among the normal population, 37% of mongoloids studied and 14 of 18 non-mongoloid institutionalized retardates. "High" excretors are defined as those excreting more than 70 mg taurine per gram creatinine. This class includes about 30% of normal individuals, 6% of mongoloids and 3 of the 18 institutionalized non-mongoloid retardates. Support for designation of a class of low excretors among mongoloids is provided by the fact that urinary taurine excretion does not increase significantly in the low excretor groups after ingestion of large amounts of protein rich in methionine and cystine, whereas the other mongoloid groups and control subjects show a several-fold increase after eating such taurine precursors. Similar results are obtained after the administration of taurine itself(2). No group differences have been found in plasma taurine levels.

The purpose of the present work was to determine the fate of S^{35} taurine administered to mongoloid and normal individuals. The results indicate that the aforementioned differences reflect differences in rate or degree, rather than kind, of taurine metabolism.

Materials and methods. S^{35} taurine, given in doses of 0.5 microcuries/kg, was purchased from Volk Radiochemical Co. Taurine derived from natural sources was purchased from Nutritional Biochemicals Corp. Radioactive samples were counted in a gel suspension medium(4) using a Nuclear Chicago liquid scintillation counter. Paper strip chromatograms were assayed in a RSC 365 scanner from Atomic Accessories.

Of the 18 mongoloid subjects, most were chosen from the previously studied institutionalized group of 47 whose taurine excretion was known, but several were outpatients. The 12 normal individuals were in 8 cases parents of mongoloids. In early experiments S^{35} taurine was given at convenient times during the day in doses of from 20-50 μc per individual, given intravenously (carrier free) or orally with carrier taurine, depending on the purpose of the experiment. In later experiments 2 protocols were followed. In the first protocol, overnight-fasted individuals were injected with a tracer dose of S^{35} taurine before breakfast and fasting continued for one hour. Urine and blood samples were collected and a meal consisting of 2 eggs, bacon, toast and milk was eaten. Urine was collected at the third and fourth hours following injection and at the fourth hour, taurine (4 mg/kg body weight) was taken orally by the subjects. Urine collection continued for the next 3 hours. In the second protocol, fasting individuals were given a tracer dose of S^{35} taurine with carrier taurine (4 mg/kg body weight) orally. Urine was collected hourly for 4 hours and blood was collected 30 minutes and 60 minutes after administration of taurine.

Urine samples were assayed for radioactivity by counting 0.5 ml aliquots in 10 ml

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of gel suspension as described above. Plasma samples were counted by adding to the gel either 0.2 ml untreated plasma or 0.5 ml of a protein-free supernatant of plasma prepared by adding 3 ml of 1% sulfosalicylic acid to 1 ml of plasma. Internal standards of S^{35} taurine were used for quench correction.

For paper chromatograms, urine was treated as follows: 5 ml was passed through a 5 × 1 cm Dowex 50 W × 4 (H^+) column. The column was washed with 5 ml of water and the combined effluents were rotary-evaporated to dryness at 80°, 5 ml of water added and redried. The residue was dissolved in 1 ml of water and aliquots spotted on strips of Whatman No. 1 paper. The strips were ascended for 18 hours in *n*-butanol, acetic acid and water (12:3:5), then radioassayed.

Percentage radioactivity present in urine as inorganic sulfate was determined by adding a known amount of carrier sulfate to the urine and then precipitating sulfates as the barium salt. The radioactivity of the $BaSO_4$ was measured as previously described(5) and, after appropriate corrections, compared to the total activity of the urine.

Aliquots of untreated urine were analyzed for taurine in a Technicon Amino Acid Autoanalyzer, using pH 2.875 citrate buffer for eluant. It had been shown that all the radioactivity emerged at or before taurine. Twenty-five 2.5-minute fractions of the column effluent were collected after having passed through the analytical system and 0.50 ml aliquots counted. Plasma samples were deproteinized (1 ml plasma plus 3 ml 3% sulfosalicylic acid) before adding the equivalent of 0.75 ml plasma to the column.

Results. Fig. 1 shows representative radiochromatograms obtained on mongoloids, both low excretors and those excreting more than 20 mg taurine/g creatinine, 2 hours after intravenous tracer doses of S^{35} taurine. Virtually no radioactive taurine is demonstrable in urine of low excretors but at least 2 nontaurine peaks are present, whereas the paper chromatograms of normal excretors always exhibit a taurine peak and, depending on the time since the last meal, may show nontaurine peaks of varying magnitude and with

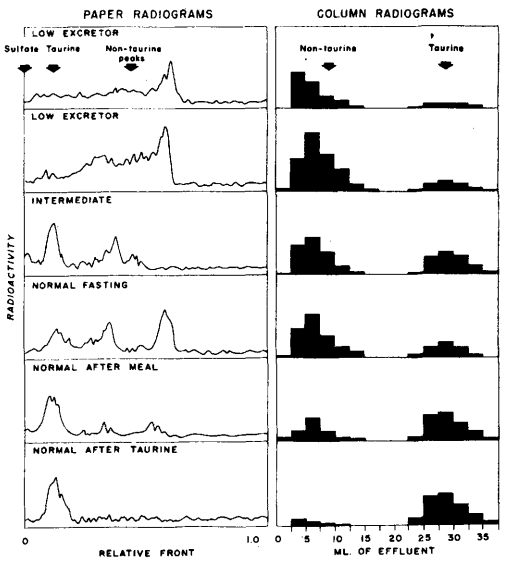

FIG. 1. Paper and column radiograms of urine from normal and mongoloid individuals. Both methods demonstrate the difference between the patterns obtained from normal individuals and the low excretors. The positions of taurine and nontaurine peaks are indicated. In the column radiograms all nontaurine radioactivity emerged immediately after one void volume. In the paper radiograms, more than one nontaurine peak is often seen.

the same chromatographic behavior on column and paper.

In normal individuals the nontaurine peaks decrease as a source of taurine (either dietary protein or taurine itself) is supplied. The decrease in the nontaurine peaks is not simply relative since the total amount of taurine excreted after a meal may not significantly increase. The urinary taurine does increase greatly in normal individuals after ingestion of 4 mg taurine/kg and in these cases the shift in radioactivity is so nearly complete that it is difficult to ascertain whether the decrease in the nontaurine radioactivity is relative or absolute.

On the other hand S^{35} taurine could be detected in significant quantities in the urine of a low excretor only when large amounts of carrier taurine were given; for example, in one case in which 20 mg taurine/kg body weight was given to a very low excretor (usually less than 5 mg urinary taurine/g creatinine), little nontaurine radioactivity was found. Patterns from the intermediate and

TABLE I. Urinary Taurine and Nontaurine Radioactivity Following Injection of S^{35} Taurine.*

Subject	Taurine excretion mg/g creatinine	Fraction of radioactivity as taurine	Taurine + nontaurine radioactivity mg/g creatinine
P.W.—Mongoloid	9.5	.139	68
T.M.—Mongoloid	3.8	.143	26
P.H.—Mongoloid	4.2	.145	29
A.M.—Mongoloid	73.7	.416	177
A.L.—Mongoloid	27.1	.150	181
A.W.—Non-mongoloid	57.1	.530	115
F.W.—Non-mongoloid (fasting)	58.7	.495	120
F.W.—Non-mongoloid (post meal)	46.6	.625	75
P.B.—Non-mongoloid (fasting)	42.6	.233	182
P.B.—Non-mongoloid (3 hr post meal)	51.2	.696	73
P.B.—Non-mongoloid (4 hr post taurine)	216.0	.866	251

* Values are from fasting (pre-breakfast) urines unless otherwise indicated. The taurine values were determined by amino acid analysis. The fraction of radioactivity as taurine was determined from aliquots of column fractions and the taurine plus nontaurine values in the last column were calculated assuming that the specific activity of the nontaurine radioactivity was the same as the taurine activity.

high excretors among mongols were similar to those obtained from non-mongoloid individuals; *i.e.*, the higher excretors tend to excrete more taurine than nontaurine radioactivity.

The paper and column radiochromatograms represent two independent methods of determining the fate of the S^{35} taurine administered. Both methods show that low excretors have virtually no urinary taurine and nontaurine peaks are ninhydrin negative. The data from the column are more precise and quantitative, since the urine is untreated and total activity is recovered, but all nontaurine radioactivity emerges unresolved with the column front (which suggests that the metabolites are either neutral or strongly acid). Paper chromatography demonstrates the presence of more than one radioactive nontaurine peak.

Since in the above experiments no carrier taurine was given, the data for total radioactivity excreted do not necessarily reflect the amount of either changed or unchanged taurine excreted. If it is assumed that the specific activity of taurine products is equal to that of taurine, one can estimate the total amount of taurine plus taurine intermediates excreted. Such data are shown in Table I. It appears that the low excretors indeed excrete less taurine plus metabolites than do the intermediates and high excretors.

The identity of the nontaurine radioactivity is as yet unknown. The per cent of total activity appearing as inorganic $S^{35}O_4$ ranges from about 10-30% of the total activity of the urine with no apparent pattern of difference between the various types of taurine excretors.

No useful information was obtained from a comparison of the radioactivity of plasma and urine in the above cases. This was mainly the result of low activities in the plasma which precluded determination of activity after amino acid analysis in most cases. Also, the plasma radioactivity is in a dynamic state, capable of continuous change, whereas the urinary activity is static. However, some useful information on taurine absorption was obtained from the radioassays of plasma described below.

Data obtained from individuals given oral doses of S^{35} taurine plus carrier taurine are shown in Table II. It can be seen that the intermediate and high groups excrete much more taurine than the low excretors. The difference in the groups is not ascribable to intestinal absorption since there is little difference in plasma taurine (which is markedly elevated, normal levels being 88.6 $\pm$ 30.8

TABLE II. Taurine Excretion Following Oral Dose of S^{35} Taurine Plus Carrier Taurine.*

Subject	Excretor status	% radio-activity excreted in 4 hrs	% urinary activity as taurine	Urinary taurine at 2 hrs mg/g creatinine	Plasma taurine		
					Min after dose	μmole/l	CPM/ml plasma
W.M.	Low	4.7	50	45	30	189	652
					60	160	645
G.S.	Low	7.2	75	117			
A.R.	Intermediate	10.6	75.5	166	30	133	553
					60	146	498
D.W.	Intermediate	15.8	84	184	30	130	604
					60	130	494
A.L.	Intermediate	18.2	88	364	30	150	651
					60	145	710
H.G.	Non-mongoloid, high	20.0	95	200	60	160	

* All subjects with the exception of G.S. were given 4 mg of taurine/kg body weight. G.S. was given 16 mg/kg body weight.

(st. dev.) μmoles/l) or in plasma radioactivity. Less than 2% of the total radioactivity was recovered in the feces collected for 38 hours after administration of S^{35} taurine.

Discussion. Several factors are known to influence the urinary excretion of taurine. Thus, after surgery or after administration of certain drugs urinary taurine may increase greatly(6,7). Also, numerous amino acids are known to hinder renal tubular reabsorption of taurine in mice and therefore cause increased excretion(8). Recently high excretion levels of taurine have been noted in certain types of mental disorders(9). Decreased taurine excretion has been found thus far only during fasts and in cases of pyridoxine deficiency(10,11). Pyridoxine is required for the synthesis of taurine from cysteine. Some preliminary experiments reported elsewhere (2) indicate that in some cases the administration of pyridoxine to low excretors results in greatly increased taurine excretion.

The chromatograms of low excretors resemble those of higher excretors when the latter are fasting. Also, the low excretors excrete less taurine plus taurine metabolites than do higher excretors under both normal conditions and when given a load of taurine. The low excretors therefore act as though depleted of body taurine. The fact that blood levels remain in the normal range has analogy to the experiments in pyridoxine deficiency in which urinary taurine greatly decreased but brain taurine remained high(11). There may be some mechanism of maintaining blood taurine at the expense of urinary excretion and also taurine in the tissues.

Superficially, a direct approach to the problem would seem to be the study of taurine synthesis from S^{35} cystine. However, the problem is one of logistics. 1) Only about 1% of cystine is excreted as taurine in normal individuals(12) and thus at least 100 times the radioactivity would have to be given (this was confirmed experimentally); 2) also, the metabolic products of cystine are more varied than those of taurine and some of these products (which behave chromatographically similar to taurine) may interfere with the measurement of taurine; 3) finally, to compare the synthesis of taurine from cystine in low and high excretors requires measurement of specific activities. Since the urine of the low excretors may have so little taurine, the only reliable measurements would be obtained from plasma. This requires even greater levels of radioactivity as described above. At the present time the use of such high doses of radioactivity (*e.g.*, about 1-5 millicuries per individual) is being avoided, and would be impracticably expensive.

Experiments on the effects of pyridoxine on taurine excretion under normal conditions and when given loads of cysteine are now being performed and should help elucidate the reasons for the low excretion.

The nontaurine nonsulfate radioactivity

after administration of S^{35} taurine probably represents normal metabolites of taurine such as taurocyamine and carbamyl taurine(13, 14). The identity of these products is of interest but, since they are found in the urine of fasting individuals in relatively large amounts, they probably reflect the low level of body taurine rather than the synthesis of unique metabolites. The reason for the increased excretion of taurine metabolites in fasting states remains unknown.

Summary. The metabolism of S^{35} taurine has been studied in normal individuals and mongols. The latter contains a group that excretes very low amounts of urinary taurine and urine from these individuals yields radiograms similar to those obtained from fasting normal individuals. These radiograms are characterized by the appearance of taurine metabolites in relatively greater amounts than taurine itself. In contrast, normal individuals after a meal produce patterns characterized by large taurine peaks compared to taurine metabolites. When administered loads of taurine, the low excretors among the mongols excrete much less of the load than do higher excretors. The low excretors behave as if deficient in body taurine.

1. Goodman, H. O., King, J. S., Jr., Thomas, J. J., Nature, 1964, v204, 650.

2. King, J. S., Goodman, H. O., Thomas, J. J., Acta Genet. Stat. Med., accepted for publication.

3. King, J. S., Wainer, A., Goodman, H. O., Thomas, J. J., Proc. of 1965 Technicon Symposium, Mediad, to be published.

4. Gordon, C. F., Wolfe, A. L., Anal. Chem., 1960, v32, 574.

5. Wainer, A., Biochem. Biophys. Res. Commun., 1964, v16, 141.

6. Turner, F. P., Brum, V. C., J. Surg. Res., 1960, v4, 423.

7. Pentz, E. I., Moss, W. T., Denko, C. W., J. Clin. Endocrin. and Metab., 1959, v19, 1126.

8. Gilbert, J. B., Ku, Y., Rogers, L. L., Williams, R. J., J. Biol. Chem., 1960, v235, 1055.

9. Agur, A., Pinsky, A., Israel J. Chem., 1964, v2, 318.

10. Chatagner, F., Tabechian, H., Bergeret, B., Biochem. and Biophys. Acta, 1954, v13, 313.

11. Hope, D. D., J. Neurochem., 1957, v1, 364.

12. Swan, P., Wentworth, J., Linkswiler, H., J. Nutrition, 1964, v84, 220.

13. Thoai, N.-V., Roche, J., Olomuchi, A., Biochim. Biophys. Acta, 1954, v14, 448.

14. Schram, E., Crokaert, R., Biochem. J., 1957, v66, 20.

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Experimental Glomerulonephritis. IX. Factors Influencing the Development of Kidney in Adjuvant Nephritis in Rats.*† (30741)

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The production of a nephritis in rats by immunization with homologous kidney in adjuvant was first reported in 1959(1). After 6-8 intraperitoneal injections of homologous kidney in complete Freund's adjuvant, rats developed a disease very similar to the human nephrotic syndrome with hyperlipemia, reversal of albumin-globulin ratios and massive proteinuria. In spite of considerable study (2-6), the etiologic and pathogenetic mechanisms operative in this disease have still not been defined; and therefore, the name "kidney in adjuvant nephritis" rather than "autoimmune nephritis" with its pathogenetic implications has been used here.

The experiments to be reported were undertaken in an attempt to define more clearly some of the factors important in the induction of this disease. *First,* the immunization procedures were altered by increasing the frequency of injections and by altering the components of the adjuvant. *Second,* the role of the host animal was assessed by immuniz-

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