cytes, were those of competitive inhibition. This is contrasted with the non-competitive kinetics of sulfonamide inhibition of a number of carbonic anhydrase-mediated hydration reactions. K_I for acetazolamide was 8.6×10^{-8} M while that for sulfanilamide was 1.8×10^{-5} M; these values are similar to those reported for inhibition of CO_2 hydration in the presence of bovine erythrocyte enzyme.

Note added in proof. From other types of evidence, J. E. Coleman (personal communication) has written a reaction scheme similar to that presented here.

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Received January 26, 1967. P.S.E.B.M., 1967, v125.

^{35}S 2-Hydroxyethanesulfonic Acid (Isethionic Acid) in Urine of Human Subjects Given ^{35}S Taurine.* (32026)

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Koechlin(1) was the first to report the occurrence of isethionic acid in a biological system. He found it to be the major anion in the axone of the squid, *Loligo pealii.* Later Braun and Fromageot(2) found that the mold *Aspergillus niger* could grow in a medium containing taurine as the sole source of sulfur and that isethionic acid accumulated as a presumed intermediate in the degradation of taurine to sulfate. Welty, Read and Shaw (3) demonstrated isethionic acid in the dog heart.

* This work was supported by Grants HD-2206, AM-07233, AM-08050 and MH-12319 from Nat. Inst. Health.

† Recipients of Career Development Award, Nat. Inst. Health.

In studying the metabolism of ^{35}S taurine in Down's syndrome (mongolism, trisomy 21) and in normal subjects, Wainer, King, Goodman and Thomas(4) observed radioactivity in urine not attributable to taurine or sulfate. The present paper reports on studies indicating that isethionic acid is present in the urine of normal and mongoloid subjects as a catabolite of taurine.

Experimental procedures. Normal subjects were the present investigators and mongoloid subjects were institutionalized young adults whose clinical diagnoses of mongolism were confirmed by chromosomal analyses. For most studies, ^{35}S taurine obtained from Volk Radiochemical Co. was administered orally in doses of 50 μc to fasting subjects. Urine

specimens were collected at 2, 4, 6 and 8 hours following administration of ^{35}S taurine. For crystallization of isethionic acid, doses of 250 μc were administered to one normal and one mongoloid subject. Radioactive urine samples were counted in a gel suspension medium(5) using a Nuclear Chicago liquid scintillation counter.

For paper chromatography, urine samples were treated as previously described(4) using 1 × 5 cm columns packed with Dowex 50W-X8 (H^+), 200-400 mesh. Solvent systems used included n-butanol, acetic acid, water (12:3:5); n-butanol, pyridine, water (1:1:1); tertiary butanol, pyridine, water (1:1:1); and isobutanol, pyridine, water (1:1:1). These will be referred to below as n-BuA, n-BuP, t-BuP, and IsobuP, respectively.

Ascending chromatograms of urine were run without equilibration at 25°C in 5 × 35 cm glass cylinders for 10-14 hours. Whatman No. 1 paper strips were used for all chromatograms and strips were scanned with an RSC 365 scanner (Atomic Accessories). Bromphenol blue (50 mg in 100 ml ethanol) was used as an indicator for acids and ninhydrin (200 mg in 100 ml acetone) for taurine. When necessary, taurine content of urine specimens was measured with the Technicon Amino Acid Autoanalyzer as previously described(4), using cysteic acid as internal standard(6). Isolation and paper chromatography of the taurine peak revealed no other ninhydrin-positive component.

For crystallization of isethionic acid, 125 mg of crystalline sodium isethionate (Eastman #8541) was added to 20 ml of urine and the urine was cleared of sulfate with 7.5% $BaCl_2$. The cleared urine was applied to a 2.5 × 45 cm column containing Dowex 50W-X8 (H^+), 200-400 mesh, and eluted with water at a flow rate of 2 ml per minute, using a Buchler micropump. Preliminary study with sodium isethionate showed that isethionic acid emerged immediately following void volume. Thirty ml of eluant following void was rotary-evaporated to near dryness at 50°C. Five ml of water was added and the solution re-evaporated. This step was repeated 4 times to eliminate HCl and other acids volatile under these conditions. Prior to final evaporation, the redissolved residue was brought to pH 7 with 0.1 N NaOH. The final residue was dissolved in 0.4 ml of water and sodium isethionate crystallized from cold absolute ethanol as previously described(3). Crystals were washed with three 2 ml aliquots of cold absolute ethanol and dried to constant weight at 100°. Yields of crystals from urine to which sodium isethionate had been added were 40-50% of the weight of added sodium isethionate. These yields were comparable to those obtained when crystallizing added sodium isethionate from water. An aliquot was counted and the remainder crystallized a second time. The low yield precluded recrystallization to constant radioactivity. Though yields could have been increased by adding larger amounts of carrier isethionate, the concomitant reduction in specific activity of recovered crystals would have made detection of radioactivity on paper chromatograms tenuous. Samples (1-2 mg) of the crystalline material were dissolved in 1 ml water and run through 1 × 4 cm cation exchange columns (Dowex 50W-X8) to convert the salt to acid. The eluent (2 ml following void) was evaporated to about 0.2 ml and 300 μg of the acid spotted on paper strips. The strips were developed in the solvent systems described above, concurrent with strips containing known isethionic acid, then dried, scanned, and sprayed with bromphenol blue.

An anion exchange resin (AG1-X8, (Cl^-) -325 mesh, spherical particles) kindly provided by Bio-Rad Laboratories was used for independent confirmation of the identity of isethionic acid in urine. The resin was converted batchwise to the monochloroacetate form and then placed in a 70 × 0.6 cm column. Aliquots (0.5 ml) of urine were pumped through the column using 1 M sodium monochloroacetate as eluent(7). The first radioactivity emerged at 16 minutes and the last at 50 minutes when the column flow rate was adjusted to 0.5 ml per min. The effluent was run through a continuous-flow scintillation counting cell (Nuclear Chicago) and radioactivity was recorded using a chart recorder and a digital counter-printer

simultaneously. ^{35}S Isethionic acid prepared from ^{35}S taurine according to Schmidt and Clark(8) was added to urine and run through the column to identify the isethionic acid peak. Similarly, ^{35}S taurine was combined with urine samples to identify the taurine peak detected in untreated urine. A sample of the material crystallized from urine was also run through this system. The peak attributed to sulfate was identified by its chromatographic behavior and by its disappearance when sulfate was cleared from urine as its barium salt. One ml fractions of the effluent from untreated urine placed on the anion exchange column were also chromatographed on paper to confirm that the presumed isethionic acid peak moved as expected in the four solvent systems.

Results. Paper chromatographic analyses of urine run through a short cation exchange column revealed several radioactive peaks in all four solvent systems coincident either with acid spots produced with bromphenol blue or taurine spots produced with ninhydrin. The R_F of the fastest moving component in all four systems corresponded to that of known isethionic acid, which was .50, .58, .75, and .51 in BuA, nBuP, t-BuP and IsobuP, respectively.

Chromatograms of crystals obtained from a 4-hour normal urine specimen to which carrier sodium isethionate had been added revealed single acid spots coincident with radioactive peaks having R_F values corresponding to concurrently run known isethionic acid. Fig. 1 illustrates a typical run with 300 μg of crystalline material in t-BuP. Comparable results were obtained in the other three systems and with crystals obtained in the same way from urine of a mongoloid subject. The presence of the isethionic acid spot was, of course, expected because isethionate was added to the urine. However, the fact that the single peaks detected in radiograms of the same strips coincided with the acid spot in all 4 solvent systems supports the identification of ^{35}S isethionic acid as the catabolite responsible.

Fig. 2 presents tracings of the radioactive peaks detected in the effluent from the anion exchange column for 0.5 ml of urine from

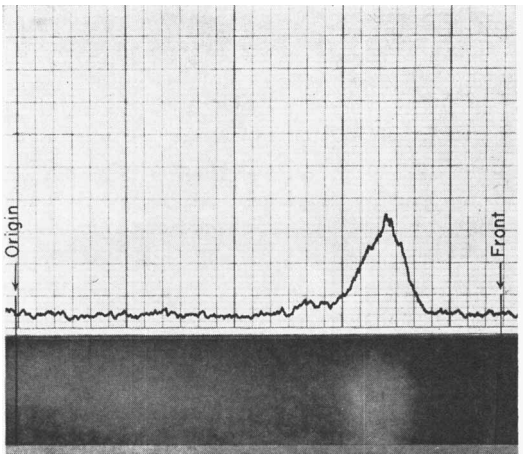

FIG. 1. Photograph of paper chromatogram and corresponding radiogram of 300 μg of crystalline ^{35}S sodium isethionate isolated from human urine to which 125 mg of carrier sodium isethionate was added. Chromatogram developed in t-BuP, dried and sprayed with bromphenol blue. Scanner operated with time constant, 100 sec; scale, 300 cpm; slit, 0.45 cm; chart rate, 0.5″ per min.

a normal and a mongoloid subject. The location of known peaks (taurine, isethionic acid, and sulfate) is indicated along with the percentage of total radioactivity accounted for by each component. The relative proportions of taurine radioactivity in the mongoloid compared to the normal subject correspond to differences in absolute taurine values obtained when the same urine samples were examined by column chromatography, 8.9 mg per 2 hour period and 64 mg per 2 hour period for the mongoloid and normal subject, respectively. The relatively smaller amount of radioactive taurine in some mongoloid urines is reflected in larger relative amounts of other radioactive metabolites derived from taurine, as seen previously(4). Previous findings(4) indicate that total radioactivity excreted during the first few hours following a dose of ^{35}S taurine is less among mongoloid low taurine excretors than is found for normal subjects.

The nature of the first emerging peak and the two smaller unidentified peaks is unknown to us. Taurine is known to be converted to carbamyl taurine by the dog(10) and rat(11) and to taurocyamine by the rat(12). To determine whether the first unknown component might be either taurocyamine or carbamyl taurine, the elution time

for both authentic compounds‡ and the unknown first peak were determined on the cation exchange column under routine conditions previously described(9). One-half ml fractions of the effluent were collected and aliquots of these were spotted on paper. Carbamyl taurine was detected using Ehrlich's reagent(13) and taurocyamine using a modified Sakaguchi spray(14). The unknown compound was detected by its radioactivity. Carbamyl taurine emerges just prior to taurine; taurocyamine emerges coincident with urea; the unknown first peak emerges almost coincident with ammonia. Hence, the first-emerging peak is neither carbamyl taurine nor taurocyamine; the two smaller peaks could, of course, be carbamyl taurine and taurocyamine but present data do not provide information on this point.

The small proportion of total radioactivity attributable to the two smaller unidentified components suggests that they may represent ^{35}S sulfate incorporated into metabolites by intestinal flora and subsequently reabsorbed. However, the possibility that they represent intermediate metabolites in the degradation of taurine to sulfate cannot be controverted by present data.

A dose of 50 μc of ^{14}C taurine was given to a mongoloid subject to determine whether the isethionic acid was a direct derivative of taurine or was being produced from ^{35}S sulfate. Only taurine and isethionic acid were detected, indicating that isethionic acid is derived directly from taurine rather than being resynthesized from sulfate.

Discussion. To our knowledge, the present data are the first indicating that isethionic acid is a normal metabolite of taurine in normal and mongoloid humans. It is possible that administered taurine is being converted by intestinal flora to isethionic acid, a capacity already demonstrated for certain microflora(2). However, the relatively large amount of isethionic acid in the urine of some normal and mongoloid subjects, the reported presence of isethionic acid in squid axone(1), honey bee brain(15) and dog heart(3) and its synthesis from taurine by dog heart slices (16) all support the inference that this acid may be an intermediate of taurine in man

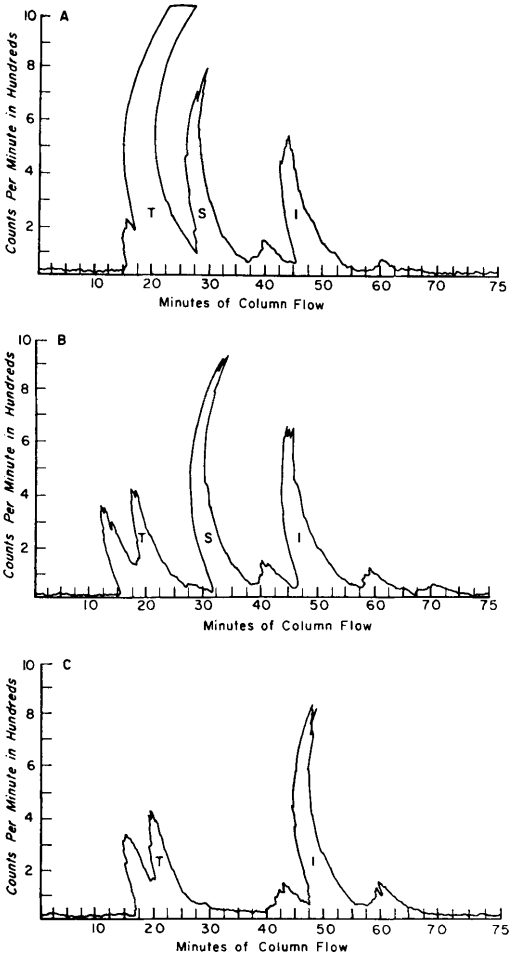

FIG. 2. Tracings of radiograms of monitored effluent from anion exchange column packed with AG1-X8, -325 mesh spherical particles, monochloroacetate form. Aliquots of 0.5 ml of urine, collected 4 hr after ^{35}S taurine dose, placed on columns and eluted with 1 M sodium monochloroacetate at flow rate of 0.5 ml per min. Scanner operated with time constant, 10 sec; scale, 1000 cpm; flow cell, 1 ml; chart rate, 6" per hour. Urine in (A) from normal subject; taurine (T) peak 76%, sulfate (S) 10%, and isethionic acid (I), 9% of total radioactivity corrected for background. Urine in (B) from mongoloid low excretor; (T), 14%; (S), 29%; and (I), 28% of total radioactivity. Urine in (C) same as that in (B) but cleared of sulfate. The first-emerging unidentified peak and the smaller two unidentified peaks in order of emergence accounted for 4, 2, and 1% of total radioactivity in urine from normal subject; 13, 6 and 5% in mongoloid urine.

‡ Taurocyamine was kindly provided by Dr. N. van Thoai, Collège de France, Paris, and carbamyl taurine by Dr. R. Crokaert, Université Libre de Bruxelles.

as well. The fact that this acid is the major anion of the squid axone(1) and has been suggested as a regulator of irritability in the dog heart(15) prompts study of isethionic acid levels in nervous and other human tissues.

Summary. The derivatives of orally administered ^{35}S taurine have been studied in the urine of normal and mongoloid subjects. Using paper and ion exchange column chromatography, 2-hydroxyethanesulfonic acid (isethionic acid) and sulfate were found to be the major derivatives. One major and two minor radioactive constituents remain unidentified. ^{14}C taurine administration indicated that the isethionic acid is derived directly from taurine rather than from reincorporation of sulfate. The present study also demonstrates the usefulness of anion exchange column chromatography for the separation of ^{35}S taurine derivatives.

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Received January 3, 1967. P.S.E.B.M., 1967, v125.

Plasma and Adrenal Corticosterone Levels Following Exposure of the Two-Day-Old Rat to Various Stressors.* (32027)

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Previous literature on the response of the neonatal rat to stress indicate an inability of stressful stimuli to activate the pituitary adrenal axis(1-9). Jailer(2) suggested that a post-natal period was necessary for maturation of the pituitary-adrenal system and Schapiro(8) has concluded that the rat goes through a stress-non-responsive period during the first 8 days of life, which he describes as "that stage in an animal's early life when pituitary-adrenal activation is not evoked by stressor agents." Leeman(9) as well as Baca and Chiodi(10) have reported somewhat similar data. But, contrary to the above, we(11,12) and Levine(13,14) have recently demonstrated that such stress situations as heat or electric shock will induce the release of adrenal corticoids in the 2- to 3-day-old rat.

The discrepancy between our results and those of Schapiro and others has been resolved by the finding that the type of stressor employed, the time interval between stimulation and sacrifice, and the endpoint used are all critical parameters(12).

The current study is an extension of our previous findings and investigates qualitatively different stressors to determine which are capable of inducing an increased release

* Aided in part by Grant HD-02068 from Nat. Inst. Health.