# THE ISOLATION OF PURE HYPOTAURINE FROM THE URINE OF RATS FED CYSTINE*

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A ninhydrin-reacting, sulfur-containing unknown substance was shown to be present, by paper chromatography, in the urine of rats fed a diet supplemented with cystine (1). From the evidence then available the compound was tentatively identified as an incomplete oxidative product of cystamine: either a sulfoxide or a sulfinic derivative. From the pooled urines of a number of rats fed cystine, a small amount of an impure sample of the natural compound was extracted and was shown to contain cystamine disulfoxide by a number of properties (2, 3). From these results the new spot was attributed to the presence of cystamine disulfoxide in the urine of rats. A similar spot, found in the chromatograms of the liver of rats injected with cysteine, was attributed to the same compound (4).

Hypotaurine (2-aminoethanesulfinic acid) synthesized independently by different methods by Awapara (5) and by Cavallini, De Marco, and Mondovì (6) was shown by the latter authors to give, by paper chromatography, the same picture as cystamine disulfoxide, a fact attributable to a dismutation and partial oxidation of cystamine disulfoxide to hypotaurine in the chromatographic run. The suspicion then arose that cystamine disulfoxide contributed only in part to the new spot found in chromatograms from the urine and liver of rats and that hypotaurine could have been present. This was shown to be the case, as far as it concerns the urine, by using ion exchange chromatography. The place of synthetic hypotaurine in the chromatographic pattern of the amino acids in the urine of rats was checked by the method of Stein (7). It was also found that cystamine disulfoxide, under the mild conditions of the ion exchange chromatography, is retained in the column and does not contribute to the hypotaurine peak. By this procedure, a substance having the position and possessing the properties of hypotaurine was shown to be present and was quantitatively estimated in the urine of rats fed cystine (8).

Meanwhile Awapara (5) and Awapara and Wingo (9) found that the injection of cysteine in the rat, in lieu of increasing the amount of alanine, as first reported by Awapara (10), gives rise in the paper chromatogram to a new spot having the same migration characteristics as the spot found by Cavallini *et al.* (1, 4), which they identified as hypotaurine. The identi-

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fication was made by comparing the chromatographic characteristics of the natural with the synthetic compound. In view of the inconclusive evidence given by the experiments of Cavallini *et al.* and Awapara, the isolation of hypotaurine in pure form from the urine of rats fed cystine and its characterization by orthodox chemical and physical methods were deemed necessary. Until now, hypotaurine has been extracted and analyzed only from a liver extract incubated with cysteinesulfinic acid by Bergeret and Chatagner (11).

## EXPERIMENTAL

*Diet and Collection of Urine*—Ten male albino rats were kept in pairs in metabolic cages on the diet previously described (1), containing 6 per cent L-cystine. The urines were collected daily over 2 ml. of 2 N HCl, pooled, and kept in the refrigerator until a total amount of 1000 ml. was obtained. At the end of the collection period the liquid was divided into five portions, decolorized with charcoal, and filtered. The filtrates were placed separately on the anion resin, but the eluates were pooled and treated as one sample in the subsequent steps.

*Anion Resin*—A bed of 170 ml. of Dowex 2 (80 to 100 mesh) in a column 2.5 cm. in diameter was used as the anion resin column. The regenerative cycles were as follows: 5 N HCl, 300 ml.; $CO_2$-free water until the effluent was neutral; $CO_2$-free 1 N NaOH, for 200 ml. after the emergence of the alkaline front; and $CO_2$-free water until the effluent was neutral.

*Cation Resin, Na Form*—A bed of 200 ml. of Dowex 50 (80 to 100 mesh) in a column 2.5 cm. in diameter, saturated with NaOH and equilibrated with 0.1 M citric acid, was used as the cation resin, Na form. This column reproduced on a preparative scale the analytical column used by Stein (7) for determination of urinary amino acids. The resolving power, at least for the amino acids eluted with citric acid, is as good as that of the analytical column. With citric acid as eluent, taurine, urea, and hypotaurine are resolved and eluted separately, while the other amino acids are retained on the column (8). The preparative and regenerative cycles were as follows: 5 N HCl, 1000 ml.; water until the effluent was neutral; 0.2 N NaOH, for 200 ml. after emergence of the alkaline front; 0.1 M citric acid, until the effluent was strongly acidic.

*Cation Resin, H Form*—An amount of Dowex 50 equal to that used for the preparation of the Na form of resin bed was prepared in the H form by the following cycles: 2 N NaOH, 1000 ml.; water until the effluent was neutral; 5 N HCl, 1000 ml.; water, to neutrality of the effluent.

The eluates were collected by a time-regulated fraction collector, the columns being operated at room temperature. Hypotaurine was localized by spotting with a glass rod from every second or fourth tube on a sheet of

Whatman No. 4 paper, drying with warm air, running in phenol, and developing with ninhydrin.

*Isolation of Hypotaurine*

After the removal of charcoal each of the five portions of urine was passed separately through the anion resin column. The column was washed with 1000 ml. of $CO_2$-free water, eluted with 1 N acetic acid, and the effluent collected in about 10 ml. portions. Hypotaurine, together with taurine, aspartic acid, glutamic acid, and other unidentified compounds, was eluted in the first 150 ml. after the appearance of the acidic front. The five eluates containing hypotaurine were concentrated to 20 to 30 ml. *in vacuo* with slight warming. The concentrate was transferred to the column of Dowex 50 in the Na form and resolved with 0.1 M citric acid. The effluent was collected in about 4 ml. portions at a rate of 20 ml. per hour. The first 100 ml. of effluent contained taurine and urea, and hypotaurine appeared uncontaminated in the next 200 ml. Citric acid was removed in the next step. The liquid containing hypotaurine was made 1 N with 50 per cent $H_2SO_4$ and passed through the Dowex 50 column in the H form. The bed was washed with 200 ml. of 1 N $H_2SO_4$, then with water until the effluent was neutral. The elution was carried out with 1 N ammonia. The bulk of hypotaurine was contained in the first 50 ml. after the ammonia front. Ammonia was removed in a vacuum desiccator over concentrated $H_2SO_4$ and the solution brought to dryness *in vacuo* with slight warming. The fractionation by Dowex 50 in the Na form and the removal of citric acid were repeated. At the end, a perfectly clear slightly hygroscopic mass of pure hypotaurine was obtained, approximately 150 mg.

*Identification of Hypotaurine*

The natural hypotaurine has a melting point of 175–177° (micro, uncorrected); a pure sample of synthetic hypotaurine, prepared as described previously (6), melted at 175–177°; previously reported 186–188°;[1] mixed m.p. 174–175°. By paper chromatography the natural compound was indistinguishable from the synthetic one when all the solvents were used. Both the compounds tested on paper gave only one positive spot with the following reagents: ninhydrin, KI + HCl (20 per cent KI in 2 N HCl), $FeCl_3$ (10 per cent in water), and iodoplatinate (12). Microanalyses of the natural compound gave the following:

| | | |
|---|---|---|
| $C_2H_7NO_2S$. | Calculated. | N 12.83, S 29.37 |
| 109.138 | Found. | " 12.69, " 29.63 |

[1] The difference of the present value from that previously reported (6) may be attributable to the use of different apparatus. The reliability of the melting point reported here has been checked by calibrating the micro melting point apparatus (Kofler) with standard compounds.

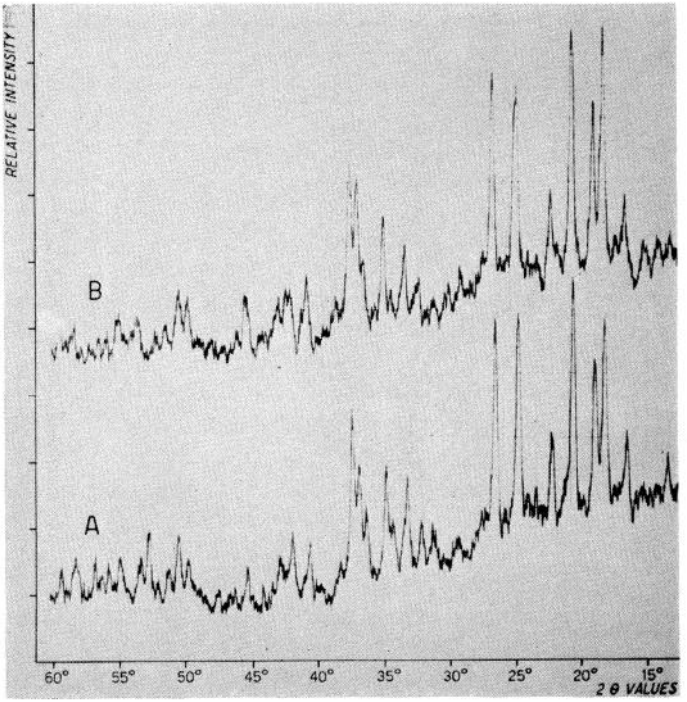


FIG. 1. X-ray diffraction recording of hypotaurine. *A*, synthetic compound; *B*, extracted from the urine of rats fed cystine.

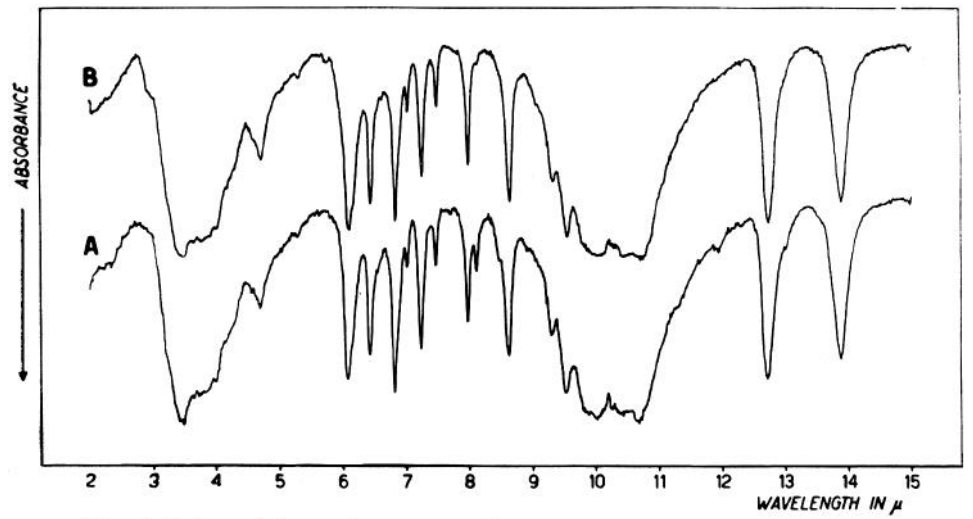


FIG. 2. Infra-red absorption spectra of hypotaurine. *A*, synthetic compound; *B*, extracted from the urine of rats fed cystine. (Suspended in paraffin oil; oil peaks at 3.43, 6.81, and 7.22 $\mu$.)

As further criteria of identity, the x-ray diffraction patterns and infra-red spectra of both the natural and the synthetic compound have been compared. The x-ray powder spectra were obtained by the Brentano parafocusing technique (13) with a General Electric Company automatic x-ray spectrometer. A Perkin-Elmer automatic infra-red spectrophotometer was employed for the infra-red spectra. As shown in Figs. 1 and 2, the Bragg's angles of the diffraction peaks and the wave-lengths of the absorption maxima in the infra-red region are the same for the natural and the synthetic compound.

## DISCUSSION

The data presented above are conclusive for the identification of hypotaurine in the urine of rats fed cystine, in agreement with our previous finding (8). Since hypotaurine has been shown to be converted in part to taurine by the living rat (14), its occurrence in the urine corroborates the hypothesis that it may play a rôle in the metabolic reactions leading from cystine to taurine.

Previously hypotaurine has been found to arise from the following compounds: (*a*) cystine *in vivo* (8), (*b*) cysteine *in vivo* (5, 9), (*c*) cysteinesulfinic acid *in vitro* (11) and *in vivo* (15), (*d*) cystamine *in vitro* (16) and *in vivo* (17), (*e*) autoxidation of cystamine and cystamine disulfoxide in alkaline solution (6), (*f*) dismutation of cystamine disulfoxide (6). It is not yet clear what is the route of conversion of cystine to hypotaurine, or whether some one of the above compounds, apart from cystine and cysteine, plays a rôle as an intermediate in this metabolic process. Our first findings on the presence of cystamine disulfoxide in the urine of rats fed cystine are suggestive in pointing to cystamine disulfoxide as one of the intermediates. Nevertheless, the great amount of hypotaurine in the same urine casts some doubt that the disulfoxide we extracted could be an artifact due to the condition of extraction. It is known that a sulfinic acid derivative may be converted to a disulfoxide by heating the acidic solution (18), a procedure which was largely employed in the course of our first extraction of cystamine disulfoxide. Further work is necessary to elucidate this point. Though cystamine is converted to hypotaurine, it is highly unlikely that this compound contributes more than traces to the hypotaurine excreted after feeding cystine. When fed or injected in less than toxic amounts, cystamine is not changed to hypotaurine in the urine of rats (4).

Cysteinesulfinic acid is generally accepted as the most probable precursor of hypotaurine, and it has been unequivocally shown to be decarboxylated to hypotaurine *in vitro* (11, 19). Nevertheless, when fed or injected into rats, cysteinesulfinic acid is converted only in part to hypotaurine, and hypotaurine, though detectable in small amount in the liver (15, 4), does

not appear in the urine (4, 20), as is the case when cystine and cysteine are fed or injected in comparative amounts. This fact necessitates caution in concluding that cysteinesulfinic acid is the main intermediate between cystine and hypotaurine.

SUMMARY

Hypotaurine has been extracted in pure form from the urine of rats fed a diet supplemented with cystine. The natural compound is identical with synthetic hypotaurine, as shown by comparison of the infra-red absorption spectra, x-ray diffraction patterns, and other properties.

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