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A chemical study of cystine from kidney stones.*

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In 1923, Dr. C. E. Tennant[1] reported a surgical case in which 15 stones having a total weight of 73 grams were removed from a kidney. He noted that these stones were composed chiefly of cystine, which, upon purification, crystallized in hexagonal plates. Inasmuch as this material offered an unusual opportunity of again investigating the old question—is stone cystine identical in chemical composition with protein cystine—we secured, through the kindness of Dr. Tennant, a number of the kidney stones, and have analyzed them and prepared certain organic derivatives of the "stone" cystine. Our data, in summary, are:

1. 5.20 grams of the cystine stones yielded 4.84 grams, or 93 per cent of pure cystine crystallizing in typical hexagonal plates. Qualitative tests on the filtrate from the cystine crystallization indicated that small amounts of calcium and phosphate were present. Neuberg and Mayer[2] state that "protein" cystine crystallizes in hexagonal plates but "stone" cystine crytallizes in needles. We have found "protein" cystine to crystallize in the typical hexagonal plates, whereas our "isomeric"[3] cystine, prepared from "protein" cystine by long boiling with 20 per cent HCl crytallizes in microscopic needles.

2. The cystine crystals analyzed for 11.63 per cent nitrogen (theory 11.65 per cent) and 26.55 per cent sulfur (theory 26.67 per cent), and a 1 per cent solution in approximately 0.1 N HCl had a specific optical rotation of $[\alpha_D^{20}] = -242.6°$. Neuberg and Mayer[2] report —224° for the optical rotation of "protein" cystine and —206° for "stone" cystine. The value usually ac-

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[1] Tennant, E. C., *J. Am. Med. Assn.*, 1923, lxxx, 305-7.

[2] Neuberg, C., and Mayer, P., *Z. physiol. Chem.*, 1905, xliv, 472-510.

[3] Hoffman, W. F., and Gortner, R. A., *J. Am. Chem. Soc.*, 1922, xliv, 341-360.

cepted[4] for "protein" cystine is $[\alpha_D] = -223°$ in HCl solution. Andrews[5] finds that the optical rotation is somewhat dependent upon the pH value. His values for a 1 per cent concentration of cystine range from $[\alpha_D^{29}] = -206.7°$ in 2.5 N HCl solution to $[\alpha_D^{29}] = -231°$ in 0.05 N HCl. Our value of $[\alpha_D^{20}] = -242.6°$ is decidedly higher than any value recorded in the literature for a solution of corresponding concentration. It would appear as though the usual methods for isolating cystine from protein material cause a slight racemization. This view agrees with our earlier findings.[3]

3. A microscopical examination of the di-hydrochloride showed the long needle crystals typical of the di-hydrochloride of "protein" cystine.

4. The di-benzoyl derivative (N found = 6.10 per cent, theory 6.25 per cent; S found = 14.11 per cent, theory 14.28 per cent) melted at 158° to 160° (uncor.) and crystallized in *diamond shaped plates.* The di-benzoyl derivative of "protein" cystine melts at 181° and crystallizes in long silky needles,[6, 7] while that of the "isomeric"[3] cystine melts at 110°[8] and crystallizes in diamond shaped plates. Neuberg and Mayer[2] report the melting point of the di-benzoyl derivatives of "stone" cystine as 157° to 159° (cor.) whereas that from "protein" cystine melts at 182° to 184° (cor.). Goldmann and Baumann[9] report a melting point for di-benzoyl cystine as 156° to 158°, but it is uncertain whether they were working with protein cystine or "stone" cystine. Apparently they were dealing with cystine derived from a case of cystinurea.

5. The phenylisocyanate crystallized in flat plates, M. P. 132° to 133° (uncor.) (N found = 11.64 per cent, theory 11.72 per cent; S found = 13.27 per cent, theory 13.39 per cent). Neuberg and Mayer[2] report "stone" cystine phenylisocyanate as melting at 170° to 172° (cor.) and "protein" cystine phenylisocyanate as melting at 160° (cor.). Shiple and Sherwin[10] also

[4] Abderhalden, E., "Biochemisches Handlexikon," Vol. 4, p. 657.

[5] Andrews, J. C., *J. Biol. Chem.*, 1925, lxv, 147-159.

[6] Brenzinger, K., *Z. physiol. Chem.*, 1892, xvi, 552-588.

[7] Gortner, R. A., and Hoffman, W. F., *J. Am. Chem. Soc.*, 1921, xliii, 2199-2202.

[8] Unpublished data. Data reported before the Organic Division of the American Chemical Society at the New York Meeting, September, 1921.

[9] Goldmann, E., and Baumann, E., *Z. physiol. Chem.*, 1888, xii, 244-261.

[10] Shiple, G. J., and Sherwin, C. P., *J. Biol. Chem.*, 1923, lv, 671-686.

report 160° (uncor.) for protein cystine. In our own work[8] we have found the following melting points for the pure phenylisocyanates: "protein" cystine M. P. = 148° to 149° (uncor.), "isomeric" cystine M. P. = 181° (uncor.). Both our "protein" cystine and "isomeric" cystine phenylisocyanates crystallized in long silky needles.

6. The phenyl hydantoin of the stone cystine was easily prepared from the phenylisocyanate derivative. It crystallized in needles, M. P. 112° (uncor.) (N found = 12.71 per cent, theory 12.67 per cent). Neuberg and Mayer[2] report that they were unable to prepare the phenyl hydantoin of "stone" cystine, whereas the corresponding derivative of "protein" cystine was easily prepared and melted at 110° (cor.). Shiple and Sherwin[10] and Patten[11] both report the melting point of "protein" cystine phenyl hydantoin at 117° (uncor.). We have found[8] "protein" cystine hydantoin to crystallize in fine needles and melt at 122° to 123° (uncor.), whereas the "isomeric" cystine derivative crytallizes in needles which melt at 166° (uncor.).

Conclusions. In the present instance some of the properties of the "stone" cystine are essentially identical with those which have been reported for "stone" cystine, in others with those reported for "protein" cystine, and in still others are apparently distinct from both. The only conclusion which can be drawn from the above conflicting observations, considered in the light of the cited literature, appears to be that cystine is an extremely labile compound and possibly occurs in more than one form, so that persons working with cystine are probably working with a mixture of substances and that this mixture varies in composition depending at least upon (1) the source of the biological material from which the cystine is prepared, and (2) the method of preparation which is used for the isolation and purification of this amino acid.

[11] Patten, A. J., *Z. physiol. Chem.*, 1903, xxxix, 351-355.