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The Resolution of Inactive Cystine.

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Ever since the earlier investigators found that in the preparation of cystine continued hydrolysis with acid decreased the rotation of the cystine and resulted in the disappearance of the typical hexagonal crystals of cystine and the appearance of needle-like crystals, the question as to the identity of this product has been the subject of repeated investigation. Morner[1] heated horn with hydrochloric acid for 2 weeks and obtained cystine partly in needle form which was inactive. Rothera[2] made similar observations on the length of hydrolysis. Neuberg and Mayer,[3] studying the inactive material suggested the possibility that it was either meso cystine or a racemic mixture. They grew *Aspergillus niger* on some of the inactive material and obtained a dextro-rotatory product having a rotation of +93.78° while a 1% solution of l-cystine in 1 *N* HCl acid gives a rotation of about —210 at 26°. Van Slyke[4] and Plimmer[5] both found that on boiling l-cystine with 20% HCl acid it was converted into a more soluble form yielding a more soluble phosphotungstate. Recently Gortner and Hoffman[6, 7] investigated the inactive cystine and synthesized for comparison many derivatives of the l-cystine and the inactive cystine. In all cases the derivatives had the same empirical formulas but in no instance did they find them to have the same melting point and in but few instances to be identical in crystal form. They attempted to obtain dextro cystine from the inactive material by growing *Aspergillus niger* on the inactive material but no optically active material was obtained. They tried to resolve the inactive benzoyl-cystine into its optical isomers but upon hydrolysis of the various fractions the cystine obtained was completely inactive. They suggested that the inactive cystine might be the internally compensated meso form. Andrews[8] concluded that the inactive cystine

[1] Morner, *Z. Physiol. Chem.*, 1899, **28**, 595.
[2] Rothera, *J. Physiol.*, 1905, **32**, 175.
[3] Neuberg and Mayer, *Z. Physiol. Chem.*, 1905, **44**, 472, 498.
[4] Van Slyke, *J. Biol. Chem.*, 1911, **10**, 15.
[5] Plimmer and Lowndes, *Biochem. J.*, 1927, **21**, 247.
[6] Hoffman and Gortner, *J. Am. Chem. Soc.*, 1922, **44**, 341.
[7] Gortner and Hoffman, *J. Biol. Chem.*, 1927, **62**, 433.
[8] Andrews and de Beer, *J. Phys. Chem.*, 1928, **32**, 1031.

was a mixture of the meso and racemic forms and predicted that d-cystine is 4 times as soluble as l-cystine and that the d-cystine would have a lower numerical rotation than the l-cystine.

In spite of the negative results previously obtained in the attempt to resolve inactive cystine, the question was reinvestigated by using other derivatives for the resolution. Furthermore the preparation of d-cystine was highly desirable not only in order to study its chemical and physical behavior but also its physiological behavior.

The resolution of inactive cystine was accomplished by means of the brucine salt of acetyl-cystine. Since cystine is decomposed by acetic anhydride and pyridine[9] the acetylation was attempted through the action of acetic anhydride on an alkaline solution of cystine.* The brucine salt was then prepared from the acetyl cystine. The dried brucine salt of acetyl-l-cystine melted indistinctly at 158-160° and had a rotation of $(\alpha)_{D}^{27°}$—66° in water, the concentration being one per cent. From the brucine salt l-cystine was obtained having a rotation of $(\alpha)_{D}^{25°} = -205°$.

The inactive cystine was prepared by the method of Hoffman and Gortner[6] and the brucine salt prepared as in the case of l-cystine. Upon recrystallization from water the negative rotation decreased, finally becoming positive. After 12 recrystallizations the rotation appeared to remain constant at a value of +17.5° for a one per cent solution. Upon hydrolysis of the salt d-cystine was obtained in hexagonal platelets having a rotation of $(\alpha)_{D}^{25°} = +200°$ in 1% solution in 1*N* HCl. Analyses agreed with that expected of cystine. Recrystallizations using methyl alcohol have given even better results than with water. Other salts and solvents are being tried to find the optimum conditions for effecting the separation.

The resolution of the inactive cystine with the isolation of dextro cystine has not of course ruled out the possibility of there being present a small amount of meso-cystine. It is hoped that a thorough investigation of the various fractions may throw some light on this possibility. The isolation of dextro cystine affords an opportunity for the study of the utilization of this isomer by the animal body, an investigation of which is planned in this laboratory.

[9] Dakin and West, *J. Biol. Chem.*, 1928, **78**, 91.

* This method was also tried independently by C. S. Marvel and A. White with successful results. Private communication.