CCXXXIII. STUDIES IN THE SULPHUR METABOLISM OF THE DOG.

XII. THE PREPARATION AND METABOLISM OF *d*-ACETYLCYSTEINE.

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THE racemising action of acetic anhydride on amino-acids has attracted a considerable amount of attention recently. For this reason and also because du Vigneaud and Sealock [1932] have suggested that the acetylcysteine made by one of us [Pirie, 1931, 1] was partly racemic it seemed advisable to study this substance further. The metabolic work which we carried out with this doubtful material [Hele and Pirie, 1931] had also to be repeated.

Using the method of preparation already described, very little racemisation does in fact occur; the preparation has, however, been simplified by the substitution of lead acetate for cuprous oxide as a precipitant for the acetylcysteine. The use of ketene as an acetylating agent was suggested to us by the work of Bergmann and Stern [1930]. The reaction proceeds very smoothly in alkaline solution and gives, as is shown later, an unracemised product in nearly quantitative yield.

The fact that the cysteine in mercapturic acid is acetylated gives a special interest to metabolic studies on acetylcysteine. The results of previous papers on the metabolism of related compounds [Hele and Pirie, 1931; Pirie, 1932] may be summarised as follows:

Substance	% excreted as sulphate in 2 days	% excreted as neutral sulphur in 2 days
Cysteine or cystine	70	4
Glutathione	72	10
Glycylcysteine	56·5	19·5
Methionine	66	16

The present work on acetylcysteine gives the following mean figures. After oral administration 48 % of the dose is excreted as sulphate and 32 % as neutral sulphur; after subcutaneous administration the figures are 28 and 42 %. Our previous results with orally administered acetylcysteine were similar but on the single occasion when it was given subcutaneously a larger percentage was oxidised. It is clear that acetylation has reduced considerably the oxidisability of the molecule.

Preparation of acetylcysteine. A brisk current of ketene, prepared by the method of Ott *et al.* [1931], is passed into a cold suspension of 20 g. of cystine in 130 cc. of 0·6 *N* NaOH. The mixture is kept cool with tap water and after

half an hour 30 cc. of 2·5 N NaOH are added. All the cystine soon goes into solution and after about an hour the action is complete, *i.e.* a sample has no formaldehyde titration. The solution is now slightly acid, 20 cc. of glacial acetic acid and some zinc are added and the mixture warmed to 50–60° and allowed to cool. It is stirred occasionally and filtered when cold.

The filtrate is made up to about 400 cc., and normal lead acetate solution is added till the supernatant fluid after centrifuging a sample gives no further precipitate with more lead acetate. The precipitate is centrifuged and washed two or three times on the centrifuge with 1 % lead acetate solution; it is then suspended in water and decomposed with hydrogen sulphide. There should be no avoidable delay during the operations described in this paragraph or a brown colour will develop, and the yield will be small.

The lead sulphide is removed by filtration and the filtrate distilled to small bulk *in vacuo*. Acetylcysteine crystallises readily in thick prisms and occasionally in well-formed octahedra; yield 21·5 g.

The product made in this way is purer than that obtained by the old method; its M.P. is 111° instead of 107–109°, and a 2·7 % solution in water has $[\alpha]_{546}^{21^\circ} = +6{\cdot}3^\circ$.

1·28 g. of this *d*-acetylcysteine were hydrolysed by boiling for 2 hours with 20 cc. of 6 N HCl and evaporated to dryness. The residue was taken up in water, neutralised and aerated after adding a trace of iron. 0·95 g. of cystine was obtained; this is 94 % of the theoretical amount. A 0·4 % solution in $N/9$ HCl showed $[\alpha]_{546}^{20^\circ} = -274^\circ$. This value is that to be expected for a dilute solution of cystine in weak acid [Pirie, 1931, 2]; it is clear therefore that, when prepared by the ketene method, acetylcysteine undergoes little or no racemisation.

If a ketene lamp is not available an almost equally unracemised product may be made, though rather less conveniently, with acetic anhydride as in Nicolet's [1930], Hollander and du Vigneaud's [1931] and Pirie's [1931, 1] methods if the reaction mixture is kept cold. The reduction and precipitation with lead are carried out exactly as in the method described in this paper. A sample of acetylcysteine prepared in this way was hydrolysed with 6 N HCl: the cystine obtained from it had $[\alpha]_{546}^{20^\circ} = -265^\circ$. Under these conditions therefore racemisation, as might be expected from the work of Bergmann and Zervas [1928], does not occur very readily.

Diacetylcystine is easily prepared from acetylcysteine by oxidation with hydrogen peroxide. Experiments carried out in the same manner as those already published by one of us [Pirie, 1931, 2; 1933] show that this oxidation is catalysed by iron and thiocarbamide in much the same way as the oxidation of glutathione, but that copper has only a slight catalytic power. The oxidation is therefore carried out in the same way as that of glutathione [Pirie, 1931, 1] but using a trace of iron as catalyst. Diacetylcystine prepared in this way, like that prepared by Hollander and du Vigneaud [1931], is a non-crystalline, slightly gummy solid, exceedingly soluble in water.

Metabolic experiments.

Two bitches, Patsy (8·5 kg.) and Christina (8·7 kg.), were used. The diets and analytical methods were the same as in our earlier work [Hele and Pirie, 1931]. When fed in the amounts used in this work *d*-acetylcysteine showed no signs of being poisonous and did not affect the dog's nitrogen excretion appreciably.

The results of nine experiments are as follow.

	Dog	Dose g.	Route	% of dose excreted as sulphate in 2 days	% excreted as neutral sulphur	% of dose accounted for
1.	Patsy	1·63	Orally	34	32	66
2.	,,	,,	,,	59	35	94
3.	,,	,,	,,	56	31	87
4.	Christina	,,	,,	46	39	85
5.	,,	,,	,,	40	31	71
6.	,,	,,	,,	47	30	77
7.	,,	,,	Subcutaneously	29	39	68
8.	,,	0·815	Orally	52	27	79
9.	,,	1·63	Subcutaneously	27	46	73

In Exps. 1, 3, 5 and 9 *d*-acetylcysteine was prepared by the old method and in the others by the ketene method.

The rise in neutral sulphur observed after feeding acetylcysteine is due, in part at least, to the excretion of that substance unaltered in the urine. From a mixed batch of urines passed on the day following the dose an amount of acetylcysteine corresponding to one-fifth of the rise in neutral sulphur was isolated. The method of isolation (precipitation with acid mercuric sulphate followed by the removal of sulphuric acid from the decomposed mercury precipitate and precipitation with lead acetate) could not, however, be expected to give even an approximately quantitative recovery.

A sample of each day's urine was reduced with zinc and sulphuric acid and titrated with $N/100$ iodine. The titration figure agreed closely with the rise in neutral sulphur on each dose day, but we feel that the actual value has little quantitative significance.

Summary.

d-Acetylcysteine can be conveniently prepared by the action of ketene on cystine in alkaline solution.

When *d*-acetylcysteine is fed to a dog 48 % is excreted as sulphate and 32 % as neutral sulphur. It is less readily oxidised when given subcutaneously.

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