CXXV. STUDIES IN THE SULPHUR METABOLISM OF THE DOG.

VIII. THE METABOLISM OF GLUTATHIONE COMPARED WITH THAT OF OTHER CYSTINE DERIVATIVES.

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THE remarkable activity of glutathione *in vitro* and its wide distribution in different animal tissues have, in recent years, focussed attention on its metabolic function. So far however no wholly satisfactory explanation of the high concentration of glutathione in such diverse tissues as liver, lens, and testis has been put forward. The possibility therefore suggests itself that it is a metabolic end-product and that its occurrence is due to an animal's inability to decompose it. A comparison of the behaviour of glutathione and cystine when administered to a dog should throw light on this question since the latter substance is only present in traces, if at all, in such fresh, non-pathological, tissues as have been studied. Some difference in the degree of oxidation was to be expected since Abderhalden and Samuely [1905] have found that less than half of the sulphur given to a dog as dialanylcystine or dileucylcystine is excreted as sulphate. We find however that there is no difference between the behaviour of glutathione and that of cystine in the dog. Of all the cystine-containing peptides which have been studied glutathione is the most completely oxidised¹.

EXPERIMENTAL.

The methods employed in this work were identical with those already described in the earlier papers of this series [Hele, 1924]. Three bitches were used and they received the following diets, half being given in the morning and half in the afternoon. They were catheterised at the same hour each morning and fed immediately afterwards.

	Lean meat	Sugar	Margarine	Milk	Water
	g.	g.	g.	cc.	cc.
Perky (10.5 kg.)	80	70	20	160	100
Violet (10.5 kg.)	80	70	20	160	100
Patsy (8.2 kg.)	80	50	20	160	100

When given orally the substances studied were put in small gelatin capsules which were wrapped up in a thin slice of meat (part of the diet) and when injected they were dissolved in 5–10 cc. of water containing, in the case of acids, the amount of sodium hydroxide necessary to bring the p_H to about 7.

¹ It has been shown in some unpublished work by Hele, Hopkins, Lawrie, Leese, Meldrum and Pirie that a cystinuric could oxidise 3 g. of glutathione, given by mouth, as readily as cystine.

On two of the dogs we tested the experimental technique by injecting subcutaneously 3.22 g. of sodium sulphate (decahydrate) dissolved in 10 cc. of water. The "extra" sulphate in the urine on the following day was 98 % of the dose in each case.

Cysteine hydrochloride and *l*-cystine were prepared from human hair in the usual way and *r*-cystine was prepared from the latter by the method of Hoffman and Gortner [1922]. *r*-Cysteine hydrochloride was made by reducing the *r*-cystine with tin and hydrochloric acid. The methods of preparation of acetylcysteine, chloroacetylcysteine, dichloroacetylcysteine, glycylcysteine and oxidised glutathione have already been described [Pirie, 1931] as has the method of preparation of reduced glutathione [Pirie, 1930].

These substances are not wholly without ill effect on the dogs. Unless the dose is fed in two portions some hours apart the acidic compounds occasionally cause vomiting and on the occasion when oxidised glutathione was injected the dog had a mild attack of diarrhoea. Experiments involving any alimentary disturbance have been excluded from Table I except in the case of oxidised glutathione given subcutaneously.

Fig. 1. Perky: 0.16 g. of glycylcysteine anhydride given subcutaneously on day 3.
0.5 g. of glycylglycine anhydride given subcutaneously on day 10.

Once the feeding of 1.2 g. of cystine on a low protein diet (*i.e.* meat 10 g., sugar 60 g., margarine 40 g., milk 50 cc., water 100 cc.) caused haematuria, but this did not happen on four other occasions although there was always a great diminution in the volume of urine passed on the day after the dose.

We attempted to study the metabolism of glycylcysteine anhydride prepared by the method of Hopkins [1929]. 1.2 g. was fed to Patsy on December 9th, she had a marked diuresis on the 11th, 12th, 13th and 14th; the daily volume of urine increased from about 210 cc. to 420 cc. and, since her fluid intake remained constant, she lost 600 g. weight. Her nitrogen and sulphur excretions were very erratic. In an experiment on Perky (Fig. 1) 0.16 g. of glycylcysteine anhydride was given subcutaneously on the 3rd day. The diuretic action and general metabolic upset are clearly shown in the graph. In the urine there were blood cells but no casts and the delayed excretion of

Fig. 2. Patsy: day 3. 1/100 g. mol. cysteine hydrochloride *sub cutem.*
 7. 1/100 glycylcysteine *sub cutem.*
 12. 1/100 acetylcysteine *sub cutem.*
 17. 1/200 oxidised glutathione *sub cutem* (see note, Table I).
 20. 1/200 " " *per os.*
 23. 1/100 *r*-cysteine hydrochloride *per os.*
 27. 1/100 glycyl cysteine *per os.*
 33. 1/200 cystine *per os.*

nitrogen and sulphur shows that there was some alteration in the permeability of the kidney. On the 10th day she received 0.5 g. of glycylglycine anhydride subcutaneously without any such effect. The oral administration of 0.3 g. of diglycylcystine dianhydride had a similar, but smaller, diuretic action while 1.2 g. of glycylglycine anhydride had none. Four rats, which had each received 6 mg. of glycylcystine anhydride, all showed a similar diuresis, but even 0.1 g. was without effect on each of three rabbits whether they were fed on a diet of cabbage or on one of bread and milk. Lewis and Lewis [1927] found that dialanylcystine dianhydride was absorbed by a rat and Brand and Sandberg [1926] injected 70 mg. into a rabbit. In neither paper is any ill effect mentioned. Abderhalden and Wacker [1908] fed 10 g. of glycylglycine anhydride

Fig. 3. Violet: day 3. 1/200 gm. mol. dichloroacetylcystine *per os*.
 8. 1/100 acetylcystine *per os*.
 13. 1/100 γ -cystine hydrochloride *sub cutem*.
 3. 1/100 cystine *per os*.
 8. 1/100 γ -cystine *per os*.
 14. 1/100 reduced glutathione *per os*.
 18. 1/100 " " *sub cutem*.
 3. 1/100 dichloroacetylcystine *sub cutem*.
 9. 2/100 cysteine hydrochloride *sub cutem*.

to a rabbit and mention a diuretic effect; they also found that most of it was absorbed. It is clear however that the diuresis caused by glycylcysteine anhydride is of an entirely different order from that due to glycylglycine anhydride. In spite of its fairly high solubility only a small part of the glycylcysteine anhydride which we fed to a dog seemed to be absorbed, and only 140 mg. (12 % of the dose) could be recovered from Patsy's urine although such a compound is relatively easy to isolate. This amount corresponded to the rise in neutral sulphur.

Only one experiment was carried out with *p*-iodophenylcysteine [Baumann and Schmitz, 1895] since 1.6 g. given orally made the dog vomit, and iodine estimations on the urine, vomit and faeces showed that less than 5 % of the dose had been absorbed.

Analytical data for some of the experiments are shown graphically in Figs. 2 and 3. In all cases there were two or three more blank days at the beginning and end of each set of experiments than are shown. The course of the experiments of which the results only are given was similar.

Table I.

Substance	Dog	Mg. S given	How given	% excreted as sulphate			% excreted as neutral sulphur			% accounted for
				1st day	2nd day	Total	1st day	2nd day	Total	
<i>l</i> -Cystine	P.	320	Orally	66	7	73	0	0	0	73
"	P.	160	"	74	0	74	0	0	0	74
"	V.	320	"	70	0	70	7	0	7	77
"	V.	320	"	61	14	75	0	0	0	75
" *	P.	320	"	31	31	62	4	7	11	73
" *	P.	320	"	69	6	75	6	0	6	81
" *	V.	320	"	41	22	63	—	—	—	—
" *	V.	640	"	48	24	72	—	—	—	—
<i>r</i> -Cystine	V.	320	"	67	13	80	9	0	9	89
<i>d</i> -Cysteine-HCl	V.	640	Subcutaneously	66	4	70	10	0	10	80
"	P.	320	"	58	3	61	0	0	0	61
" †	V.	320	Orally	69	3	72	0	0	0	72
<i>r</i> -Cysteine-HCl	V.	320	Subcutaneously	54	0	54	31	4	35	89
"	P.	320	Orally	72	0	72	10	0	10	82
"	Pk.	320	"	56	4	60	19	0	19	79
" †	V.	320	"	57	7	64	8	0	0	72
Acetylcysteine	P.	320	Subcutaneously	40	0	40	46	5	51	91
"	V.	320	Orally	34	4	38	33	10	43	81
"	Pk.	320	"	50	0	50	28	0	28	78
Dichloroacetylcystine	V.	320	"	14	26	40	42	30	72	112
"	V.	640	Subcutaneously	6	3	9	89	3	92	101
Chloroacetylcysteine†	Pk.	240	Orally	17	0	17	75	5	80	97
Glycylcysteine	V.	288	Subcutaneously	43	4	47	9	3	12	59
"	P.	320	"	56	0	56	18	0	18	74
"	P.	320	Orally	66	0	66	6	5	11	77
"	V.	320	Subcutaneously	57	0	57	25	12	37	94
Reduced glutathione	V.	320	Orally	72	6	78	0	0	0	78
"	V.	320	Subcutaneously	66	0	66	7	6	13	79
Oxidised glutathione†	P.	320	"	56	0	56	24	0	24	80
"	P.	320	Orally	77	11	88	0	5	5	93

* With "low protein" diet.

† Given in two doses.

‡ Diarrhoea, urine contaminated.

DISCUSSION.

The work of Abderhalden and Samuely [1905] on the sulphur metabolism of dogs may be summarised as follows.

Substance	Amount given per day (g.)	Number of days	% in urine as sulphate	% in urine as neutral sulphur
Cystine (orally)	1.0	10	65	19
Dialanylcystine (O)	1.6	8	58	14
„ (subcutaneously)	1.6	5	38	24
Dileucylcystine (C)	1.9	2	33	29

Stearns and Lewis [1930] found that rabbits oxidised 92.8 % of a dose of cystine ethyl ester hydrochloride to sulphate in 2 days. We are aware of no other quantitative work on the metabolism of sulphur compounds of this type except the very thorough investigations by Lewis and co-workers and Sherwin and co-workers on derivatives of cystine containing an aromatic group. For our present purpose, on account of the aromatic group, that work is not strictly relevant.

It is clear from Table I that the extent to which a substance (except dichloroacetylcystine) is oxidised is very variable but is much the same whether it is given in the sulphhydryl or disulphide form and whether it is given subcutaneously or orally. Our results may therefore be summarised as follows:

Substance	% excreted as sulphate in 2 days	% excreted as neutral sulphur in 2 days
Cystine and cysteine	69.7	3.7
Racemic cystine and cysteine	66.0	16.2
Acetylcysteine	42.7	40.7
Chloroacetylcysteine and dichloroacetylcysteine	22.0	81.3
Glycylcysteine	56.5	19.5
Glutathione	72.0	10.5

The similarity between the figures for acetylcysteine, dialanylcystine, dileucylcystine and, to a lesser extent, glycylcysteine is interesting when compared with the relatively much greater extent to which cystine and its ester and glutathione are oxidised. It has now been shown definitely by Nicolet [1930] and by Kendall and co-workers [1930] that glutathione, as was thought, though on somewhat inadequate evidence [Pirie and Pinhey, 1929], in this laboratory, is glutamylcysteinylglycine. A study of the oxidation of the related peptides glutamylcysteine and cysteinylglycine would be interesting.

It is clear from the figures given that a dog can oxidise *r*-cystine to much the same extent as *l*-cystine; it appears that a greater proportion of the racemic compound is excreted as neutral sulphur. In no case has the nature of this neutral sulphur been investigated but we intend to do this at a later date. The percentage of the sulphur administered which is accounted for is greater in the case of inactive cystine or cysteine than in that of the normal

isomeride. This suggests that, as would be expected, the unnatural isomeride is not used to replace tissue breakdown.

The marked difference between the extent to which dichloroacetylcystine is oxidised when given orally and when given subcutaneously suggests that the substance is only oxidised after hydrolysis. In addition to oral administration chloroacetylcystine was given subcutaneously after neutralisation. There was no rise in sulphate excretion but 84 % of the sulphur in the dose appeared as neutral sulphur. 2 mols. of sodium hydroxide are necessary to raise the p_H of a solution containing 1 mol. of chloroacetylcystine to 7. The neutral solution contains chlorine ions, no amino-group is present and the sulphydryl group is unaffected. Presumably either hydroxyacetylcystine or its anhydride is formed but this has not yet been demonstrated conclusively.

SUMMARY.

The capacity of the dog to oxidise cystine and allied compounds, given orally or subcutaneously, has been studied.

The dog can oxidise to the same degree, *l*- and *r*-cystine and *l*- and *r*-cysteine and glutathione.

Acetylcystine and glycylcystine are not so readily oxidised.

Chloroacetylcystine and dichloroacetylcystine are very resistant.

The nature of the neutral sulphur excreted as result of administering certain of the compounds has not yet been investigated.

The administration of glycylcystine anhydride causes diuresis in dogs and rats but not in rabbits.

p-Iodophenylcystine upsets the dog and is not well absorbed.

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[Note added 15. viii. 31.] As the neutral sulphur figure (24 %) in the experiment with injected oxidised glutathione appeared high, we repeated this experiment and certain others on the same dog, Patsy. Stocks being low, we used smaller doses, containing in each case 190 mg. sulphur. The results were as follows: oxidised glutathione given orally; excreted as sulphate 58 % and as neutral sulphur 6 %; given subcutaneously; 50 % and 12 %. Reduced glutathione given orally; 70 % and 0 %. *l*-Cystine given orally; 70 % and 0 %. The high figure in question, therefore, was certainly due to faecal contamination of the urine. The new figures support the argument in the discussion.