99. Metabolism of Sulphur. Cysteic Acid*

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The place of cysteic acid in metabolism has never been adequately determined. Earlier experiments [Schmidt & Clark, 1922; Andrews & Randall, 1935; White, Lewis & White, 1937; Medes, 1937] were designed to ascertain whether this substance is in the path of the oxidation of cystine to inorganic sulphate. In general it was concluded that while ingested cysteic acid may give rise to small amounts of inorganic sulphate, cysteic acid injected intravenously or subcutaneously does not contribute to sulphate formation. Hence, the conclusion was reached that cysteic acid cannot be an intermediate in the oxidation of cystine to inorganic sulphate.

That cysteic acid is a physiologically occurring product has been shown more recently by Bernheim & Bernheim [1939] and Medes [1939] who extracted an enzyme from liver which oxidizes the sulphur of cysteine to the sulphonc acid stage. However, no hepatic enzyme which acts on cysteic acid has been found.

Two possible clues to its fate have been suggested. (a) Virtue & Doster-Virtue [1939] showed that cysteic acid ingested with cholic acid increases the output of taurocholic acid in the bile of dogs, but the mechanism for its conversion into taurine has not been discovered. (b) Schmidt & Clark [1922] found that following oral administration of cysteic acid without accompanying cholic acid a deaminated product appeared in the urine. Such a product could not have been taurine. Very suggestive in connexion with this finding of Schmidt & Clark, is the report of Cohen [1940] that cysteic acid may participate in the transamination activities of muscle tissue according to the formulas: α -ketoglutaric acid + *l*-cysteic acid $\rightarrow$ *l*-glutamic acid + β -sulphopyruvic acid, and oxaloacetic acid + *l*-cysteic acid $\rightarrow$ *l*-aspartic acid + β -sulphopyruvic acid. If the β -sulphopyruvic acid is excreted unchanged the appearance of a deaminated product of cysteic acid would be accounted for; but since this would seem to be a very unstable substance, further investigation was advisable to determine whether it is excreted entirely in this form or whether part of it slowly gives rise in the tissues to inorganic sulphate. Medes [1937] had observed that there was a rise in the sulphate elimination beginning about 20 hr. after ingestion of 3.64 g. of cysteic acid and that this elevated level was continued for approximately 24 hr. The rise in sulphate excretion might be explained on the basis of intestinal putrefaction, but it might also be due to some delayed mechanism in the tissues, such as transamination to sulphopyruvic acid and subsequent oxidation of the sulphur to sulphate.

Metabolism of cysteic acid following ingestion of small amounts over a 42 hr. period

It was thought that if transamination of cysteic acid to sulphopyruvic acid occurred in the muscle, followed by sulphate formation, the process would probably be slow and the amount oxidized to sulphate would be limited by the rate of transamination. It is possible that this latter process could not be speeded up sufficiently to care for unphysiologically large amounts of cysteic acid (up to 15 g.) administered at one time in some of the experiments previously reported. In the following experiment the amount ingested was cut as low as was consistent with the sensitiveness of the several tests employed and was administered in a series of small doses over a prolonged period.

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The subject was the same as was used for previous observations [Medes, 1937]. 1.848 g. of cysteic acid were dissolved in such a volume of water that 2 ml. contained 22 mg. A 2 ml. portion was taken by mouth every $\frac{1}{2}$ hr. for 42 hr. except for a 6 hr. period during the night when double the dose was consumed hourly. 1.056 g. were therefore ingested during the first 24 hr. and 792 mg. during the following 18 hr. The subject was on the same low protein, low sulphur diet as was employed in previous experiments [Medes, 1937]. Urine was collected in 6 hr. periods and analysed for total and neutral S, ethereal and inorganic sulphates and total N. Fig. 1 shows the results for the various periods.

Fig. 1. Excretion of sulphur during an experiment in which 22 mg. of cysteic acid were ingested every half-hour for 42 hours, except during 6 hours, in which 44 mg. per hour were taken.

It may be seen that excretion of extra S begins immediately after ingestion of the cysteic acid and for 30 hr. is entirely in the neutral S class. Afterwards neutral S falls to the normal value and sulphate-S begins to increase. The average level of total S was 16.9 mg./hr. before the first experimental period, was maintained at a fairly even level of 19.5 mg./hr. during the ingestion period and immediately fell to an average of 18.8 mg./hr. during the following 6 hr., and to 17.2 mg. during the next 24 hr. Total sulphate, on the other hand, averaged 14.4 mg./hr. in the pre-ingestion period, 14.6 mg./hr. during the first 24 hr. of the ingestion period, then rose to an average of 16.1 mg./hr. during the subsequent 36 hr.; 32% of the extra S was recovered, of which 16.1% was determined as neutral S and 15.9% as sulphate-S.

Although the delayed, slow rise in sulphate occurred as in previous experiments, the total amount recovered as sulphate was not greater than when a single large dose was administered and did not constitute a greater percentage of the ingested cysteic acid. This evidence, therefore, while not conclusive, seemed to indicate either that sulphate formation following transamination did not take place, or that the normal amount could not be increased even by prolonged ingestion of small amounts of cysteic acid, and hence the process, if it occurred, could not be demonstrated by an experiment of this type.

Metabolism of cysteic acid in muscle

A second approach to the problem was through testing for sulphate formation by muscle and liver tissue in the presence of sulphopyruvic acid; the experiments were conducted as follows. Transaminase was prepared as described by Cohen [1940] and incubated in the presence of cysteic acid and oxaloacetic or α -ketoglutaric acid* for 1 hr.

* The authors are indebted to Dr P. P. Cohen for generously supplying α -ketoglutaric and oxaloacetic acids.

in 0.07 *M* phosphate buffer at pH 7.7, after which part of the incubated mixture was removed to test for activity of the transaminase. These tests were carried out in the Warburg apparatus with chloramine-T as described by Cohen. In two solutions tested, about 15 and 18 % transamination was found to have taken place.

To the remainder of the incubated enzyme and sulphopyruvic acid, was added muscle or liver tissue freshly ground in a Latapie mincer. A sample of the solution was removed immediately for sulphate estimation and the remainder incubated for 2 hr. in an atmosphere of oxygen. A second sample was then removed for sulphate estimation. A control tube containing all the components except substrate and keto-acid was treated similarly. Sulphate determinations were carried out by the method described by Medes & Floyd [1942]. In two experiments in which oxaloacetic acid was employed with cysteic acid as substrate in the transamination procedure and in three experiments in which α -keto-glutaric acid was used with cysteic acid, no sulphate production from sulphopyruvic acid by either liver or muscle tissue could be detected.

Tests for sulphopyruvic acid in urine following cysteic acid ingestion

The possibility that sulphopyruvic acid resulting from deamination of cysteic acid may be the form in which this S is excreted was the object of the next experiment. The same general procedure was employed as in the previous feeding experiments, with the subject on a constant diet of somewhat higher S content, beginning 6 days before the administration of cysteic acid. On the seventh day 4.488 g. of cysteic acid monohydrate (24 m.mol.) neutralized with NaOH were ingested in three portions at hourly intervals. The 24 hr. samples of urine were analysed for total S, total and inorganic sulphate and amino-N. Since it was hoped to detect the presence of sulphopyruvic acid in case cysteic acid was excreted in this form, keto-acids were estimated by the colorimetric procedure of Sealock [1941] with pyruvic acid as standard. Values are expressed in terms of pyruvic acid equivalents.

The results are given in Table 1. Total S rose from an average of 17.6 m.mol. daily to 27.5 on the first day after ingestion (day 7), to 21.8 on the second day, and returned to the normal value, 17.6 m.mol. on the third day. This represented a recovery of 58.1 % of the extra S ingested.

Table 1

Fate of ingested and injected cysteic acid by a normal human subject, wt. 60 kg.

Method of admin.	Day of exp.	Vol. ml.	Sulphur in m.mol.					Ketone m.mol.	Amino-N m.mol.
			Total S	Neutral S	Total SO ₄	Ethereal SO ₄	Inorg. SO ₄		
Ingested	1	775	18.56	2.21	16.35	0.89	15.46	0.41	4.32
	2	950	18.31	1.98	16.33	0.80	15.53	0.43	5.12
	3	670	17.66	2.10	15.56	0.82	14.74	0.39	4.27
	4	965	18.49	2.09	16.40	0.87	15.53	0.42	4.75
	5	1127	16.03	1.98	14.05	0.72	13.33	0.36	1.94
	6*	945	17.54	2.06	15.48	0.92	14.56	0.39	4.17
	Av.		17.74	2.07	15.69	0.84	14.88		
Injected	7	735	27.58	2.06	25.52	3.69	21.83	0.47	3.34
	8	630	21.86	3.59	18.27	2.51	15.76	0.41	3.78
	9	950	17.68	2.54	15.14	0.83	14.31	0.40	4.09
	3	680	10.66	1.92	8.74	0.98	7.76	0.56	2.96
	4†	685	10.10	2.02	8.08	0.96	7.14	0.39	2.50
	5	750	10.92	3.83	7.08	0.90	6.18	0.43	4.23
	6	600	9.23	2.15	7.08	0.92	6.16	0.43	3.51
	7	860	9.10	1.80	7.30	1.09	6.21	0.43	2.69

* 4.488 g. (24 m.mol.) neutralized cysteic acid were ingested in 3 portions at 8, 9 and 10 a.m.

† 1.318 g. cysteic acid (=7.5 m.mol.) neutralized with 19.42 ml. 0.3860 *N* NaOH injected at 9.15 a.m. Same amount injected at 10 a.m.

In this experiment, in contrast to the earlier one, sulphate-S rose early, attaining an elevation of 25.5 m.mol. on the first subsequent day, dropping to 18.3 m.mol. on the second day and reverting to the normal value on the third. This represents 51.7 % of the added S or 88.9 % of the recovered S.

The rise in neutral S was slight. There was no increase on the first day after ingestion; on the second day the output rose from the average of 2.0 to 3.5 m.mol. On the third day it was still somewhat elevated, 2.5 m.mol., which corresponded to 8.3 % of the added S or 14.3 % of the recovered S. There was no increase in amino-N, and keto groups also remained at the control level. Hence sulphopyruvic acid could not have been present in more than traces among the excreted products.

Test for sulphopyruvic acid in urine following injection of cysteic acid

On account of the large amount of sulphate excreted and the complication of its possible formation from the action of intestinal flora, the experiment was repeated with cysteic acid injected intravenously. 15 m.mol. (2.636 g.) were administered in two portions with an interval of an hour. The results are given in Table 1. Total S was still falling towards the level, afterwards attained on the fourth day, of 9.2 m.mol. Considering any above this as extra S derived from the injected cysteic acid, 1.6 m.mol. of extra S were excreted during the 24 hr. following administration, or 11.3 % of that injected. It was all in the form of organic S. Ketones did not rise, but amino-N increased by 1.7 m.mol., a value approximately equal to the increase in neutral S, or 1.8 m.mol. The compound excreted could have been either taurine or unchanged cysteic acid. This evidence shows a direct relationship between the rise in the amino-N and the increase in the neutral S. Since unphysiological amounts of cysteic acid have been injected the results should, if transamination has taken place and if both end-products are excreted, show an increase in the keto group equivalent to the rise in the amino-N. Since the keto group is constant, evidently sulphopyruvic acid is not excreted. The strikingly different picture obtained upon injection of cysteic acid from that presented following ingestion, indicates either that bacterial destruction is very extensive or that the intestinal mucosa acts in some way upon absorbed cysteic acid with extensive disruption of the molecule.

To summarize: about 60 % of the S of cysteic acid ingested without accompanying cholic acid was excreted partly in the neutral S fraction of urine and partly as sulphate. There was no simultaneous rise of amino-N or of the keto group. When cysteic acid was injected intravenously only about 10 % of the S was recovered and was entirely in the organic S fraction. Urinary amino-N increased to about the same extent, while ketones remained constant.

Excretion in the bile

Although it has been amply shown that taurine, administered either orally [Schmidt, Adelung & Watson, 1918] or intravenously [Foster, Hooper & Whipple, 1919] without accompanying cholic acid, is excreted by way of the urine, it does not follow that cysteic acid behaves similarly since at this preliminary stage the union with cholic acid may be effected, resulting in a different disposition of the end-product. Virtue & Doster-Virtue [1939] found that cysteic acid injected into dogs appeared as taurine in the bile. Their dogs, however, were supplied with cholic acid and no studies were made by them in its absence. To ascertain whether that portion of the S of cysteic acid which does not appear in the urine may be excreted in some form in the bile, the following experiments were carried out on two patients who had been operated on for bile obstruction 4 days before initiating the tests. They were placed upon diets of controlled S content, and bile and urine were collected in 24 hr. periods. Results are given in Table 2. As may be seen, the volume of bile was normal. Urine was analysed for the usual S distribution, for amino-N before and after hydrolysis, and for bile acids. For the latter, the method of Gregory & Pascoe [1929] was employed. It was used unmodified in determining bile

Table 2

Excretion of S in the urine and bile of two bile fistula patients, following oral administration of cysteic acid. All values are expressed in m.mol.

Table 1. All values are expressed in m.mol.												
Day	S in Diet	Vol. ml.	Crea- tinine	Sulphur				Amino-N			Bile salts	Ketone
				Inorg. SO ₄	Total SO ₄	Org. S	Total S	Before hydrol.	After hydrol.	Diff. (taurine + glycine)		
Patient R. Urine*												
1	13.1	570	5.1	9.71	10.40	1.87	12.27	13.48	18.26	4.78	0.39	0.59
2†	12.6	765	4.8	9.13	9.67	1.36	11.03	15.69	19.90	4.21	0.39	0.61
3	13.4	1250	4.6	9.22	10.17	6.16	16.33	12.46	17.44	4.98	0.44	0.59
Patient R. Bile*												
1	—	340	—	0.02	0.03	—	3.70	—	—	12.6	11.10	—
2†	—	407	—	0.03	0.05	—	3.45	—	—	15.2	15.10	—
3	—	320	—	0.34	0.47	—	3.54	—	—	9.3	9.72	—
Patient W. Urine*												
1	5.0	1350	5.7	3.99	4.43	3.11	7.54	2.98	10.06	7.08	0.51	0.37
2‡	8.7	840	5.8	6.05	6.44	3.22	9.66	4.21	11.50	7.29	0.53	0.38
3	9.1	575	5.5	5.00	5.33	10.11	15.44	3.38	14.81	11.43	0.63	0.37
4	9.6	583	5.5	5.01	5.35	4.59	9.94	4.99	15.64	10.65	0.37	0.39
Patient W. Bile*												
1	—	308	—	0.00	0.25	—	2.65	—	—	6.33	8.29	—
2†	—	420	—	0.00	0.27	—	2.64	—	—	6.78	7.18	—
3	—	390	—	0.17	0.32	—	2.67	—	—	7.32	8.19	—
4	—	355	—	0.14	0.29	—	2.62	—	—	8.75	7.82	—

* Collections closed daily at 8.30 a.m.

† 12.8 m.mol. of cysteic acid administered at 8.30 a.m. and 12.8 m.mol. at 9.45 a.m.

‡ 10.6 m.mol. of cysteic acid administered at 8.30 a.m. and 10.6 m.mol. at 9.45 a.m.

acids in bile. For urine, the preliminary extraction with alcohol was omitted, the reaction being carried out directly on urine, employing as a blank a urinary sample similarly treated except for the omission of furfuraldehyde. Readings were taken in a photoelectric colorimeter with a 6200 Å. filter and estimations made from a standardized curve obtained with graded amounts of cholic acid. Patient R received 4.50 g. (25.6 m.mol.) of cysteic acid divided into two doses, at 8.30 and 9.45 a.m. on the third morning, and patient W received 4 g. (21.2 m.mol.) similarly administered on the third day. Table 2 gives the urinary and bile analyses for 2 days before ingestion of cysteic acid and for 1 and 2 days respectively, immediately after (collection of urine started at the time of administration).

As may be seen no significant change in S excretion by way of the bile was ascertainable. The neutral S fraction of urine increased, representing 19 and 33 % of the ingested S respectively. Amino-N (confirming the finding of Schmidt & Clark [1922]), except for a slight rise in patient W, was not elevated, and the keto reaction was unchanged. Thus only 19 and 33 % respectively was accounted for, and that, except for a small amount in W, could not have been cysteic acid, taurine or sulphopyruvic acid.

Action of various tissues on cysteic acid

Since ingested cysteic acid behaves differently from injected cysteic acid, an attempt was made to find out if some other tissue besides liver acts on it. Slices of liver, heart, intestines and kidney were incubated, under conditions similar to those previously employed. CO₂ and amino-N were determined before and after incubation. Parallel tests were conducted with and without addition of cysteic acid. The results are given in Table 3. It will be seen that liver, heart or muscle had no effect on cysteic acid. Kidney tissue acted on the carboxyl group, but had no effect on the N. Under the action of intestinal tissue, both amino-N and carboxyl completely disappeared.

Table 3

Effect of various tissues upon cysteic acid. Tissue slices were incubated in 0.02 *M* phosphate buffer at pH 7.4 which was 0.02 *M* with respect to cysteic acid. Controls were similarly incubated without cysteic acid. Figures represent m.mol./ml.

Tissue	Cysteic acid added	Time (34°) hr.	CO ₂			Amino-N		
			In 0.002 <i>M</i> cysteic acid	Without cysteic acid	Difference	In 0.002 <i>M</i> cysteic acid	Without cysteic acid	Difference
Liver	0.02	0	0.0351	0.0150	0.0201	0.0227	0.0018	0.0209
		5	0.0335	0.0134	0.0201	0.0252	0.0045	0.0207
Heart	0.02	0	0.0335	0.0134	0.0201	0.0225	0.0020	0.0205
		5	0.0349	0.0148	0.0201	0.0258	0.0064	0.0194
Intestines	0.02	0	0.0335	0.0134	0.0201	0.0228	0.0027	0.0201
		5	0.0588	0.0620	0.0000	0.0619	0.0611	0.0008
Intestines	0.02	0	0.0335	0.0134	0.0201	0.0228	0.0027	0.0201
		5	0.0590	0.0600	0.0000	0.0426	0.0529	0.0000
Kidney	0.02	0	0.0351	0.0150	0.0201	0.0223	0.0021	0.0202
		5	0.0375	0.0219	0.0156	0.0248	0.0058	0.0190

To assure ourselves that this effect of intestinal enzymes was not due to the presence of intestinal bacteria, the experiment was repeated with the following additional procedures. After the regular incubation period, the medium surrounding the slices was strained through coarse filter paper to free it of tissue, and divided into two equal portions. To one was added cysteic acid so that the original concentration was restored. The other portion was passed through a Berkefeld filter to free it from any intestinal flora which might remain in the solution. To this portion, cysteic acid was also added in the same concentration. It was anticipated that if the disappearance of cysteic acid were due to the action of the intestinal flora, the substrate would be lost from the solution that had been strained through filter paper but be unaltered in the flask whose contents had passed through the Berkefeld filter. Results are given in Table 4. Actually, neither

Table 4

Action of intestinal tissue on cysteic acid. Concentration of cysteic acid was 2×10^{-2} m.equiv. per ml. of medium. Incubation was in 0.02 *M* phosphate buffer at pH 7.4 for 2 hr. Controls without cysteic acid were incubated similarly. Results are expressed in m.equiv. $\times 10^{-2}$ per ml. of medium.

Period	Time hr.	Carboxyl			Amino-N		
		With cysteic acid	Without cysteic acid	Difference	With cysteic acid	Without cysteic acid	Difference
Preliminary	0	0.0593	0.0388	0.0205	0.0247	0.0043	0.0204
	5	0.0587	0.0376	0.0211	0.0242	0.0046	0.0196
After straining through filter paper	0	0.0587	0.0376	0.0211	0.0239	0.0048	0.0196
	5	0.0523	0.0302	0.0223	0.0245	0.0051	0.0194
After passing through Berkefeld filter	0	0.0585	0.0377	0.0218	0.0246	0.0044	0.0201
	5	0.0558	0.0348	0.0210	0.0243	0.0041	0.0202

solution was changed, showing that the results were due to enzymes in the gut wall and not to the action of bacteria clinging to the intestinal surface. The fragments from the cysteic acid molecule were not identified. The possibility that the S fraction is ethyl sulphonic acid is now being investigated. Sulphate determinations were unsatisfactory owing to the relatively large amounts of sulphates in the tissue, but such assays as could be carried through indicated that sulphate was not formed.

Since kidney slices acted on the carboxyl group of cysteic acid without affecting the N, taurine apparently had been produced. An effort was made to extract the enzyme responsible. Table 5 gives the results of a series of experiments in which cysteic acid was incubated with kidney slices and kidney brei under both aerobic and anaerobic conditions.

Table 5

Decarboxylation and deamination of cysteine acid by liver and kidney of the rat. Substrate concentration was 2.00×10^{-2} m.equiv. per ml. of medium. Incubation was for 2 hr. in 0.02 *M* phosphate buffer at pH 7.4. Results expressed in m.equiv. $\times 10^{-2}$ per ml. of medium.

Tissue	Condition	Substrate	Carboxyl		Amino-N	
			Before	After	Before	After
Liver brei	Aerobic	Sodium cystinate	2.01	1.53	—	—
		Cysteic acid	2.03	2.04	—	—
		Leucine	2.03	1.82	—	—
		Histidine	2.03	0.92	—	—
Kidney slices	Aerobic	Cysteic acid	2.01	1.56	2.02	1.90
			2.01	1.53	2.00	1.98
			2.01	1.57	2.05	1.92
Kidney brei	Aerobic	Cysteic acid	2.01	1.42	2.05	1.21
			2.02	1.52	2.05	1.21
			2.01	1.52	1.92	0.00
Kidney brei	Anaerobic	Cysteic acid	2.02	1.35	2.05	2.07
			2.01	1.53	2.03	1.97

phosphate buffer 0.02 *M* at pH 7.4 was employed. It may be seen that while kidney slices convert cysteine acid into taurine, the brei, in the presence of oxygen, behaves apparently like the intestinal mucosa in that both amino and carboxyl groups disappear. In an atmosphere of N only the carboxyl radical is affected with resulting taurine formation.

DISCUSSION

It has been shown [Medes & Floyd, 1942] that taurine may arise in the liver from cystine, which is first oxidized to cystine disulphoxide and then decarboxylated to the disulphoxide of cystamine. By a subsequent oxygen uptake, taurine is produced. The experiments recorded here show a second pathway for taurine formation through an anaerobic decarboxylation of cysteine acid. Blaschko [1942] isolated the enzyme from liver, while the present authors* found it in kidney. The results are summarized in the accompanying scheme.

* Reported by Medes & Floyd at the Meeting of the Society of Biological Chemists, April, 1942.

The various oxidative steps are enzymic, as are the decarboxylations. There are, therefore, three enzymes concerned in the production of taurine from cystine and two in the conversion of cysteine into the same end-product. In either case, cystine or cysteine could appear quantitatively as taurine. A question of fundamental importance is the interconvertibility of the cystine and cysteine systems.

Since sulphhydryl may be oxidized quantitatively to disulphide by cytochrome oxidase, all of the cysteine could conceivably be converted into taurine through cystine and its disulphoxide. But starting with cystine, it could not all be transformed to taurine through cysteine. The latter is produced from cystine in the tissue by hydrolytic cleavage, and since cysteine sulphenic acid, formed as one of the products of hydrolysis, immediately dismutates to cysteine and cysteine sulphinic acid, theoretically only three-fourths of all cystine present could be converted into taurine through this channel. The remaining fourth, present as sulphinic acid, would be oxidized to inorganic sulphate. The sulphate portion might however be increased to include the entire product, if conditions in the tissues should be such that cytochrome oxidase was able to shunt all the cysteine back to the general reservoir, cystine, before the enzyme converting it into taurine could act.

At what stage do these reactions become irreversible? This question is answered better by feeding experiments which test the availability of the various end-products or of their metabolic intermediates for growth-promoting properties. Taurine cannot replace cystine in the diet [Lewis & Lewis, 1925; Beard, 1926] and hence it apparently cannot be converted by the body into cystine. This conclusion may also be drawn from the fact that taurine does not contribute to sulphate formation [Schmidt *et al.* 1918]. Cysteic acid does not increase urinary sulphate [White *et al.* 1937], hence its biological formation appears to be irreversible. Bennett [1937] reported that cystine disulphoxide is available for growth, although it is only one-third as effective as cystine. Lavine [1936] showed that cystine disulphoxide probably undergoes dismutative decomposition to cystine and cysteine sulphinic acid according to the reaction, $3R(SO)_2R \rightarrow RSSR + 4R(SO_2)H$, and since no enzyme present is capable of reducing the sulfoxide to the disulphide form, once the oxidation process has set in, it cannot be freely reversed. Since cysteine sulphinic acid does not support growth in place of cystine [Bennett, 1937], the tissues appear to be unable to reverse appreciably the series of reactions leading to its formation. Cystamine disulphoxide has not been tested, but our meagre knowledge of biological carboxylations does not lead to the expectation that this step is reversible. Hence we may conclude that while two pathways to taurine formation are open to cystine or to cysteine, once the initial steps are taken, reversibility is largely lost and has completely disappeared with formation of cysteic acid or taurine, and probably with cystamine disulphoxide. Experiments with tissue slices or with partially purified enzymes can scarcely present any criterion for deciding what governs a choice of pathways in the intact organism. The fact that the decarboxylation of cysteic acid must occur under anaerobic conditions unless further disruption of the molecule is to be brought about, presents an additional complicating factor. Since end-products begin to appear in the urine within a few minutes after the ingestion of cystine [Medes, 1937] the speeds of reactions in the liver or kidney must far exceed those in experiments done *in vitro*, and it is scarcely to be expected that relative rates will be maintained under conditions so unphysiological.

In all the experiments recorded above, the S recovered was only a portion of that ingested. In one case (Exp. 1, Table 1) 58 % of the ingested S was found, largely in the organic portion. In the other experiments recovery ranged from 11 to 40 %; Schmidt & Clark [1922] recovered about 58 %. White *et al.* [1937] recorded the appearance of all the S in the organic fraction of rabbit urine following ingestion of 1.58 m.equiv. of cysteic acid. This high recovery cannot be ascribed entirely to the relatively low dose, as in the experiment described in this paper, in which very small portions were ingested, total recovery amounted to 32 %. Nor can it be ascribed entirely to the fact that it was

injected, as in the experiment recorded here, in which the cysteic acid was similarly administered, only 11 % was recovered. It was hoped that in these detailed experiments some clue to the fate of the remaining S would be found, but there was no evidence of its delayed excretion either in urine or bile. Can cysteic acid be elaborated into some other S compound which is retained and utilized? Possibly the rabbit, in which 100 % was recovered, has some different mechanism or more powerful enzymes available for its conversion into taurine. However, apparently cysteic acid readily forms taurine when cholic acid is present in sufficient amounts to form taurocholic acid; hence under strictly physiological conditions, this S retention may never occur.

SUMMARY

Cysteic acid is decarboxylated anaerobically by an enzyme located in the kidney, with production of taurine.

Under aerobic conditions, further fragmentation of the molecule occurs, with loss of the amino group.

A similar aerobic breakdown takes place in the presence of intestinal mucosa.

Because of this activity of the intestinal wall, the form in which cysteic acid is excreted after ingestion is different from that which appears after intravenous injection. The non-S fraction of the molecule has not been identified.

No evidence could be found that sulphopyruvic acid, formed in transamination reactions, contributes to sulphate formation in the tissues.

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