CXCI. METABOLISM OF SULPHUR
VIII. OXIDATION OF THE SULPHUR-CONTAINING AMINO-ACIDS BY ENZYMES FROM THE LIVER OF THE ALBINO RAT[1]

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THE paths of oxidation of the sulphur-containing amino-acids in the animal body have been deduced largely from quantitative and qualitative studies of the end-products excreted under various metabolic conditions. An attempt has been made in this investigation to extract the enzymes concerned in these transformations and to study some of the conditions under which they operate. The present paper is concerned, for the most part, with the oxidation of cystine and cysteine and of some of their partially oxidized derivatives.

Methods

Cysteine and cystine were determined by the method of Shinohara & Padis [1935]. Protein-free filtrates were prepared by adding 1·5 ml. of the solution to be tested to 13·5 ml. of sodium tungstate-sulphuric acid mixture in a 15 ml. centrifuge tube.

Protein precipitant (modified from Folin and Svedberg, for preparation of protein-free filtrates): 15 g. of sodium tungstate are dissolved in about 200 ml. water, 30 ml. 2/3 *N* H_2SO_4 added and the volume made up to 250 ml.

By this method, cytochrome *c*, which interferes in the determination of cysteine with phosphotungstic acid, is precipitated along with the tissue proteins. When the precipitate has begun to flocculate, the tubes are centrifuged and 5 ml. of the supernatant fluid are transferred to each of two 25 ml. volumetric flasks for the determination of SH and SS groups. For the former, 6·5 ml. of sodium acetate-acetic acid buffer at *p*H 5·2 are added, followed by 2 ml. of the phosphotungstic acid uric acid reagent. For determination of disulphide, 2 ml. of 1 *M* sodium sulphite are added just before the phosphotungstic acid reagent. For the standard, 4 ml. of 0·002 *M* cysteine, 6·5 ml. of buffer and 2 ml. of reagent are prepared in a third 25 ml. volumetric flask. The contents of the flasks are then made up to 25 ml., and colorimetric readings are taken at the end of 20 min.

Sulphate was determined by three different methods developed during the course of the investigation. Repeated checks indicated agreement in the values obtained. The methods were as follows.

(*a*) Pirie's [1934] modification of Cuthbertson & Tompsett's [1931] method.

(*b*) A slight modification of Denis & Reed's [1926–7] procedure for the nephelometric determination of sulphate in blood serum. 8 ml. of the solution con-

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taining the tissue slices or tissue extract are added to 2 ml. of 20% sulphate-free trichloroacetic acid, and centrifuged after about 20 min. To 2 ml. of the filtrate are added 5 ml. water and 1 ml. of 5% gelatin. Finally, 2 ml. 1% $BaCl_2$ are added with continuous mixing with a fine glass rod. A blank is prepared substituting water for the $BaCl_2$ and a series of $(NH_4)_2SO_4$ standards containing 12, 24 and 36 μg. S respectively. The amount of sulphate present as sulphur is read, after deducting the reading of the blank, from a curve constructed by comparing graded amounts of the standard sulphate solution with each of the several standards.

(*c*) An adaptation of the nephelometric method for use with the photelometer [Medes & Stavers, 1939].

Experiments with tissue slices

Pirie [1934] first succeeded in demonstrating that slices of liver and kidney of the rat are capable of oxidizing the sulphur of cystine, cysteine, methionine and glutathione to inorganic sulphate. He states that finely ground tissue is inactive. Pirie concerned himself only with sulphate formation. Experiments conducted similarly were carried out in this investigation to obtain a survey of other reactions which may be occurring in liver tissue. Albino rats were also employed, the entire procedure being carried out as described by Pirie, using the organ-slice method of Warburg. A constant temperature bath at $38{\cdot}5° \pm 0{\cdot}2$ was used. All experiments, unless otherwise stated, were continued for a 2 hr. period with a 95% O_2-5% CO_2 mixture bubbling continuously through the solutions. The carbonate buffer of Krebs with substitution of magnesium chloride for magnesium sulphate, as suggested by Pirie, was employed for the most part. In later experiments, especially with some of the extracted enzymes, $0{\cdot}2M$ KH_2PO_4-NaOH buffer at *p*H 7·5 was substituted. In this case pure O_2 was used for aeration in place of the O_2-CO_2 mixture. Sulphate formations by different tissues are recorded in Table I.

The results agree in general with those reported by Pirie. Kidney and liver oxidize the compounds most readily; blood, spleen and lung tissue are inactive, while cardiac and heart muscles possess a low degree of activity. Duodenum rapidly gives rise to sulphate; even without added cysteine, its output was high as compared with those of the other tissues, except kidney and liver. In general, sulphate formation from cystine was somewhat less in a given period than from cysteine; cystine disulphoxide yielded about the same amount in the 2-hr. period as did cystine, while the most rapid conversion was from cysteinesulphinic acid. The slow production of sulphate from cystine as compared with that from cysteine tends to confirm the finding of Pirie that cystine does not constitute the immediate source, but reduction to cysteine must take place. The same conclusion would apply to cystine disulphoxide. The yield of sulphate from cysteine, as seen from Table I, varied, under the conditions of these experiments, being around 3% of the added sulphur. When phosphate buffer was substituted for carbonate buffer, sulphate formation from cysteinesulphinic acid was undiminished, whereas no sulphate was produced from cysteine.

A second reaction also occurs. The greater portion of the cysteine is converted into cystine under these conditions. In Table II, the yields of cystine in a series of experiments are recorded; 53–89% appeared as cystine at the close of the 2-hr. periods. This oxidation to cystine takes place with equal facility in phosphate and carbonate buffers.

It may be seen also that a third reaction is taking place. The recovery of cysteine plus cystine (final column) varies from 65 to 89% of the initial cysteine.

Table I. *Conversion of organic S into inorganic SO_4 in carbonate buffer at* p*H 7·4*

10 mg. (8·26 m. equiv.) of the compound to be tested in 10 ml. of the buffer were aerated throughout by a 95 % O_2-5 % CO_2 gas mixture. Time, 2 hr. Temp. 38°·5 ± 0·2.

Compound	Tissue slices	Sample no.	Tissue av. wt. mg.	Compound used m$M \times 10^2$	SO_4 formed as S m$M \times 10^3$	SO_4 formed as S μg.
l-Cysteine	Liver	26	56·97	8·26	3·49	79·90
,,	Kidney	12	36·61	8·26	2·02	64·94
,,	Blood	4	61·07	8·26	0·19	6·37
,,	Spleen	5	26·84	8·26	0·38	12·30
,,	Duodenum	1	14·90	8·26	1·67	53·80
,,	Lung	4	22·32	8·26	0·48	15·67
,,	Heart muscle	6	47·66	8·26	0·91	29·31
,,	Muscle (striped)	4	78·92	8·26	1·24	40·05
l-Cystine	Liver	8	58·73	4·13	1·83	58·7
,,	Kidney	7	46·04	4·13	1·15	37·1
,,	Muscle	4	79·70	4·13	1·07	34·4
,,	Heart muscle	3	57·53	4·13	1·25	40·1
l-Cystine disulphoxide	Liver	8	59·48	4·13	1·85	59·5
,,	Kidney	7	45·62	4·13	1·42	45·6
,,	Muscle	4	78·20	4·13	1·19	38·2
,,	Heart muscle	3	59·30	4·13	1·22	39·3
,,	Spleen	1	31·70	4·13	0·55	17·7
l-Cysteinesulphinic acid	Liver	8	58·42	8·26	2·30	73·9
,,	Kidney	7	47·50	8·26	3·01	96·6
,,	Muscle	4	77·85	8·26	2·32	74·4
,,	Heart muscle	3	57·23	8·26	1·66	53·5
,,	Spleen	1	29·00	8·26	0·38	12·5
Control	Liver	15	52·54	—	0·47	15·1
,,	Kidney	8	30·72	—	0·51	16·6
,,	Blood	4	61·67	—	0·22	7·2
,,	Spleen	5	28·47	—	0·38	12·3
,,	Duodenum	1	14·80	—	1·38	44·4
,,	Lung	4	22·62	—	0·42	13·7
,,	Heart muscle	3	34·43	—	0·24	8·0
,,	Muscle	2	76·20	—	0·33	10·7

Table II. *Cystine formation from cysteine in the presence of liver slices in carbonate and phosphate buffers at* p*H 7·6. Temp. 38·5° ± 0·2*

Buffer	Cysteine mg.	*C*	Time min.	SH mg.	SS mg.	Total mg.	Time min.	SH mg.	SS mg.	Total mg.
Carbonate	10	+	60	2·25	4·74	6·99	120	1·71	5·32	6·45
Carbonate	10	+	60	6·40	3·86	10·26	120	2·12	6·14	8·26
Carbonate	10	+	60	5·03	4·24	9·45	120	0	8·88	8·88
Phosphate	10	−	60	9·1	0·7	9·8	120	2·4	6·0	8·40
Phosphate	10	+	60	3·04	6·02	9·06	120	0·51	7·38	7·89

Since only about 3 % of the sulphur appears as inorganic sulphate, some reaction is taking place by which neither cystine nor inorganic sulphate is produced. In the present investigation attempts were made to study, through the use of liver extracts, these three reactions as well as sulphate formation from cysteinesulphinic acid.

Studies with liver extracts

As a preliminary to the study of the several reactions, a general survey was made by carrying out a series of experiments substituting liver brei for liver slices.

Preparation of liver brei. The rat was killed by a blow on the head and the liver immediately perfused with physiological salt solution, first by way of the arterial system from the heart, then by way of the venous system through the hepatic portal and the hepatic veins. When the deep reddish colour was superseded by a uniform pale greyish cast, the liver was removed and ground in phosphate or carbonate buffer either in a Latapie mincer or in a mortar with sand. After standing with occasional stirring for 30 min., it was centrifuged and the supernatant liquid filtered through a Jena glass Büchner funnel of medium porosity (3 G 3). Repeated examinations failed to demonstrate any intact liver cells in the filtrate. 4 ml. of the liver extract were used in each tube, usually 0·5 ml. of cytochrome *c*, 1 ml. of water, buffer to make a total volume of 10 ml. and 10 mg. of cysteine or its sulphur equivalent. At this concentration, as observed by Pirie, cystine formed from cysteine just fails to precipitate out, and hence aliquot parts may be removed at intervals for determination of thiol and disulphide.

Table III. *Oxidation of cysteine to cystine in the presence of (a) crude liver brei, filtered through a sintered glass filter, and (b) oxidase from brei purified similarly to beef-heart cytochrome oxidase*

Brei no.	Treatment of brei	Cyto-chrome *c*	No. of exp. av.	45 min. SH mg.	45 min. SS mg.	90 min. SH mg.	90 min. SS mg.	Total SH + SS 45 min. total mg.	Total SH + SS 90 min. total mg.
1	Crude	+	8	4·45	3·92	0·39	6·55	8·37	6·94
2	Crude	−	6	5·29	3·15	0·73	5·90	8·44	6·63
3	Crude, heated	+	5	7·39	2·53	5·96	4·14	9·92	10·10
4	Purified	+	8	4·70	5·29	1·11	8·36	9·99	9·47
5	Purified	−	5	7·22	2·43	3·28	6·81	9·65	10·09
6	Purified, heated	+	8	7·39	2·51	3·64	6·26	9·90	9·90

In Table III, the first three items record a typical experiment in which crude liver brei in phosphate buffer was employed. As with slices, the most vigorous reaction was that concerned in the oxidation of cysteine to cystine. When the extract was not heated a loss of cysteine plus cystine occurred, only 66 and 69 % being recovered. When the extract was heated for 5 min. at 80° before addition of the cysteine no loss of cystine plus cysteine was seen, demonstrating that the reaction involving loss is enzymic. Sulphate formation occurred only in carbonate buffer and then to a much reduced degree. It also failed to occur when the extract had been heated. In general, then, the reactions which take place upon substitution of the brei for liver slices, were qualitatively and, except for sulphate formation, quantitatively similar to those in which the tissue itself was used.

(*a*) *Cytochrome oxidase.* Cytochrome oxidase, formerly designated indophenol oxidase, is well known to catalyse the oxidation of cysteine to cystine. Since this enzyme is apparently present in all tissues, it seemed most probably the enzyme responsible for this oxidation.

Cytochrome oxidase for comparative studies was prepared from beef-heart by the method of Stotz & Hastings [1937] and purified by three precipitations in 0·05 *M* acetate buffer at *p*H 4·5 as described by Stotz *et al.* [1938] with subsequent dialysis for at least 18 hr. in frequent changes of glass-distilled water. Cytochrome *c* was prepared according to the procedure of Keilin & Hartree [1937].

As a preliminary to the study of the enzymic oxidation of cysteine to cystine its oxidation in oxygen-saturated phosphate buffer at *p*H 7·5 and 38·5° was

followed. In Fig. 1 is plotted a group of such curves (*a* to *g*). When temperature and oxygen saturation were maintained uniformly from the start, rate of disappearance of cysteine was represented by a straight line as in curves *e*, *f* and *g*. Therefore rate of oxidation is independent of concentration. The degree of slope varied widely in different experiments, since traces of impurities greatly influence

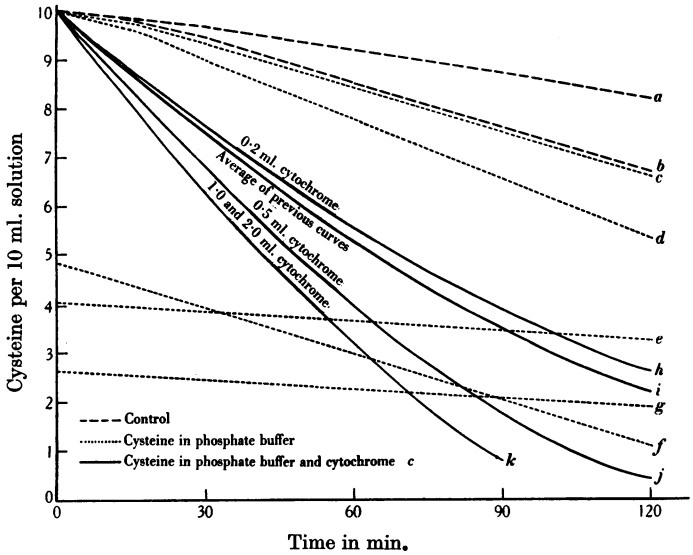

Fig 1. Rate of oxidation of cysteine to cystine in 0·04 *M* phosphate buffer at *p*H 7·5 at 38·5° ± 0·2 in a constant stream of oxygen. Abscissae, time in minutes; ordinates, mg. cysteine per 10 ml. solution. Curves *a* and *b* are controls from previous experiments; *c* to *g* similarly are pure cysteine in phosphate buffer; *h* to *k* contain cytochrome *c* in addition.

Table IV. *Oxidation of cysteine to cystine*

48·4 mg. cysteine were introduced into a 100 ml. volumetric flask containing 0·04 *M* phosphate buffer at *p*H 7·5. The buffer had been saturated previously with oxygen, and the volume was adjusted with additional oxygenated buffer. Oxygen was bubbled through the solution throughout the experiment. Temp. 38·5° ± 0·2.

Time min.	SH mg.	SS mg.	Total determined mg.
0	48·5	0	48·5
15	44·4	3·9	48·3
30	39·8	8·6	48·4
45	34·2	14·2	48·4
60	29·5	19·1	48·6
90	21·3	27·2	48·5
120	11·5	37·2	48·7
150	2·4	46·0	48·4
180	0	48·7	48·7

the rate, as has been amply brought out by previous investigators. In Table IV is recorded a typical experiment in which 48·3 mg. were dissolved in 0·04 *M* phosphate buffer previously saturated at 38·5° with oxygen. The volume was adjusted to 100 ml. with additional oxygenated buffer and the solution maintained at 38·5° ± 0·2 in a stream of oxygen. After 3 hr. all the cysteine was

converted into cystine. Recovery of cystine plus cysteine was at all times 100 % of the initial cysteine: hence the reaction conformed to the equation

$$2RSH + \tfrac{1}{2}O_2 \rightarrow RSSR + H_2O.$$

In another series of experiments, cytochrome *c* was added to ascertain its effect on the rate of oxidation of cysteine and its effect on the type of curve. With cytochrome *c* present, the rate of reaction was always increased. In an experiment conducted simultaneously with that recorded in Table IV, complete oxidation of cysteine to cystine had occurred after 120 min. instead of 160 min. (as read from the curve) in the experiment without cytochrome *c*. The type of curve was also changed (Fig. 1, *h* to *k*), the rate of oxidation no longer being independent of cysteine concentration. Addition of increasing amounts of cytochrome *c* also influenced the rate up to a certain maximum, after which further addition produced no more increase. Recovery of cysteine plus cystine was 100 % of the initial cysteine.

When liver extract was purified by the same procedure as was used in the preparation of cytochrome oxidase, all the initial cysteine was recovered as cysteine plus cystine, as shown by the last three items in Table III. The increased rate upon addition of cytochrome *c* to the system (item 4), above the rate in its absence (item 5), may be taken as further indication that the enzyme is cytochrome oxidase. Further evidence in this direction may be obtained by its behaviour in the presence of cyanide. As shown by Keilin [1929] and later by Stotz *et al.* [1938], cyanide prevents the reoxidation of cytochrome *c* and hence should inhibit the oxidation of cysteine by cytochrome oxidase. Fig. 2 shows an

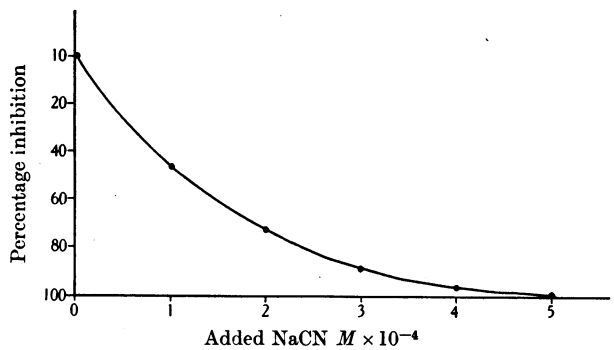

Fig. 2. Inhibition of oxidation of cysteine to cystine by sodium cyanide. The solutions contained 4 ml. purified cytochrome oxidase from rats' liver, 0·5 ml. cytochrome *c*, 1 ml. water and 4·5 ml. phosphat ebuffer at *p*H 7·5. Abscissae, added NaCN $M \times 10^{-4}$; ordinates, percentage inhibition of SH to SS.

experiment in which graded amounts of sodium cyanide were added to 10 mg. of cysteine in phosphate buffer in the presence of the purified enzyme and cytochrome *c*. As may be seen, the accelerating effect of cytochrome *c* was more and more inhibited in the presence of increasing amounts of cyanide, until nearly 100 % inhibition was attained. A similar effect was produced by urethane.

It may be concluded then, that the enzyme which is present in the liver of the albino rat and which oxidizes cysteine to cystine is cytochrome oxidase.

This enzyme has no effect on methionine. In one experiment in which cysteine was replaced with 1·25 mg. methionine per ml., 100 % of the methionine was recovered after 2 hr., as determined by Kassell & Brand's [1938] modification of

Baernstein's method.[1] No sulphydryl was detectable with nitroprusside, and no sulphate with barium chloride. Iodimetric titration indicated that the enzyme does not affect cysteinesulphinic acid either.

(*b*) *An enzyme oxidizing cysteine to cysteic acid.* In the procedure for extracting and purifying cytochrome oxidase, the enzyme is precipitated from the tissue extract in sodium acetate buffer at *p*H 5·0. The precipitation is incomplete. In the supernatant liquid containing small amounts of cytochrome oxidase is to be found a second enzyme responsible for the loss of sulphydryl plus disulphide. In the first experiment recorded in Table V it may be seen that oxidation of cysteine to cystine is occurring, both in the heated and in the unheated specimens—in the latter, with much greater rapidity. In the unheated tube, the loss of sulphydryl plus disulphide is 3·32 mg. after 1 hr., whereas none has disappeared from the heated tube. After 5 hr. in the unheated tube all the sulphydryl plus disulphide has disappeared but none from the other. Since at the close of the 2 hr. period 4·74 mg. had been present as disulphide in the unheated tube, disulphide as well as sulphydryl must be acting as substrate.

To determine if cystine is an intermediate in this oxidation reaction of cysteine or if cysteine is the immediate source, the rates of disappearance of cystine and cysteine were compared in the next two experiments in Table V. Cystine was used as sodium cystinate since this dissolves in the phosphate buffer and at the concentration of about 1 mg. per ml. is approximately at the point of saturation. As may be seen there is a slow conversion of cysteine into cystine, while at the same time a gradual loss of the total amino-acid occurs. The rate of loss is about the same from the cystine solution as from the cysteine. The fourth experiment is similar to Exp. 3 except that crude filtered suspension of liver was employed instead of the partially purified. In this instance loss of cystine plus cysteine is even more rapid in the tube in which cystine acted as substrate. These results suggest either that cystine is the immediate substrate or that here there is some mechanism rapidly reducing cystine to cysteine. If this reduction is enzymic, here is the only indication of enzymic reduction of disulphide to thiol which this investigation has revealed.

The end-product of the reaction appears to be cysteic acid as indicated by iodimetric titration. Amino-nitrogen is unchanged. Since it has been shown by Virtue & Doster-Virtue [1939] that cysteic acid may serve as the precursor of taurine, it seems probable that we are dealing with an enzyme concerned in the production of taurine.

This enzyme appears to be identical with that described by Bergheim & Bergheim [1939], who employed a suspension of liver tissue in phosphate buffer, at *p*H 6·7. They state that it does not oxidize cystine. As suggested by them, the difference in the behaviour of cystine may be due to the more alkaline reaction at which these experiments were carried out. That there is a difference in behaviour at these two reactions is further demonstrated by the suppression of this reaction by sodium pertitanate at *p*H 6·7, as shown by Bergheim & Bergheim, although no inhibition occurs at *p*H 7·5. The final experiment in Table V was carried out in phosphate buffer at *p*H 6·7. The enzyme had been purified as described above except that phosphate buffer at the lower *p*H was used throughout. Sodium pertitanate, prepared as described by Bergheim & Bergheim was added. The results show the inhibition of cysteic acid formation.

An attempt was made to determine whether the enzyme could act on cysteinesulphinic acid since the latter compound might be an intermediate in this oxidation of cysteine. Three test tubes were set up, each containing 4 ml. of

[1] I am indebted to Mr Thomas Callan for estimation of methionine.

Table V. *Oxidation of cysteine to cysteic acid*

10 mg. of cysteine (or its equivalent of sodium cystinate) were dissolved in phosphate buffer in a total volume of 10 ml. and maintained in an oxygen atmosphere. Temp. 38·5°.

Exp. no.	1 hr. SH mg.	1 hr. SS mg.	1 hr. Total mg.	1 hr. Loss mg.	2 hr. SH mg.	2 hr. SS mg.	2 hr. Total mg.	2 hr. Loss mg.	5 hr. SH mg.	5 hr. SS mg.	5 hr. Total mg.	5 hr. Loss mg.	SH mg.	SS mg.	Total mg.	Loss mg.
1						10 mg. cysteine + cytochrome										
	3·78	2·90	6·68	3·32	0	4·74	4·74	5·26	0	0	0	10·0	—	—	—	—
						10 mg. cysteine + cytochrome, enzyme heated										
	8·03	2·11	10·14	0	6·05	4·16	10·21	0	0	10·11	10·11	0	—	—	—	—
2						10 mg. cysteine + cytochrome										
	3·43	4·73	8·22	1·78	0	6·51	6·51	3·49	—	—	—	—	—	—	—	—
						10 mg. cystine + cytochrome										
	0	7·07	7·07	1·57	0	6·46	6·46	3·54	—	—	—	—	—	—	—	—
						10 mg. cysteine										
	6·45	1·98	8·43	2·93	3·19	3·50	6·69	3·31	—	—	—	—	—	—	—	—
	30 min.				60 min.				90 min.				2 hr.			
3						10 mg. cysteine + cytochrome										
	6·45	1·37	7·82	2·18	4·40	1·55	5·90	4·10	3·29	1·64	4·93	5·07	0	2·29	2·29	7·71
						10 mg. cystine + cytochrome										
	0	7·65	7·65	2·35	0	5·75	5·75	4·25	0	5·00	5·00	5·00	0	3·06	3·06	7·94
						10 mg. cysteine										
	6·11	1·50	7·61	2·39	4·00	1·69	5·69	4·31	2·60	1·79	4·39	5·61	1·54	2·23	3·77	6·23
	45 min.				90 min.											
4						10 mg. cysteine + cytochrome											
	4·11	3·29	7·40	2·60	0	4·94	4·94	5·06	—	—	—	—	—	—	—	—
						10 mg. cystine + cytochrome										
	0·52	5·03	5·55	4·45	0	4·55	4·55	5·45	—	—	—	—	—	—	—	—
						10 mg. cysteine + cytochrome (enzyme boiled)										
	8·68	1·45	10·13	0	6·20	3·63	9·83	0·17	—	—	—	—	—	—	—	—
5						10 mg. cysteine + cytochrome										
	7·74	0·92	8·66	1·34	5·66	2·12	7·78	2·22	—	—	—	—	—	—	—	—
						10 mg. cysteine + cytochrome (enzyme heated)										
	8·45	1·55	10·00	0	7·14	2·84	9·98	0·02	—	—	—	—	—	—	—	—
						10 mg. cysteine + cytochrome + Na pertitanate										
	8·06	2·00	10·06	0	4·16	4·76	9·92	0	—	—	—	—	—	—	—	—

the partially purified brei, 4 ml. phosphate buffer, 0·5 ml. cytochrome *c*, 1·5 ml. water and 30 mg. cysteinesulphinic acid. From tube *a*, 8 ml. were removed at once for iodimetric titration. Tube *b* was heated in a boiling bath for 2 min., then incubated at 38·5° as a control for tube *c*, for 3 hr. At the close of this period, 8 ml. were removed from tubes *b* and *c* for iodimetric titration. To each of these three 8 ml. portions in 15 ml. centrifuge tubes, 2 ml. of 20 % trichloroacetic acid were added, the solutions centrifuged, and 8 ml. of the supernatant liquid titrated with sodium thiosulphate. The results of two such experiments are given in Table VI. It may be seen that a loss of about 17 % of cysteinesulphinic acid occurred in the presence of the active enzyme (tubes *c*). During a third 3-hr. period 20 % of an equivalent amount of cysteine (23·7 mg.) was oxidized to the cysteic acid stage. The close agreement of these two figures, 17 and 20 % respectively, indicates that cysteinesulphinic acid may be a stage in this oxidation of cysteine.

Table VI. *Recovery of cysteinesulphinic acid*

(*a*) Before, (*b*) after (heated), and (*c*) after (unheated) incubation with purified enzyme for 3 hr. at 38·5° ± 0·2. Each portion finally titrated with thiosulphate contained 0·1254 m.equiv. or 19·2 mg. cysteinesulphinic acid.

	Cysteinesulphinic acid mg.	Recovery %
Exp. 1*a*. Before incubation	19·2	—
,, 1*b*. After incubation (heated)	18·9	98·3
,, 1*c*. After incubation (unheated)	16·0	83·4
Exp. 2*a*. Before incubation	19·3	—
,, 2*b*. After incubation (heated)	19·1	98·6
,, 2*c*. After incubation (unheated)	16·1	83·7

(*c*) *Sulphinic acid oxidase.* The enzyme which oxidizes the sulphur of cysteinesulphinic acid to inorganic sulphate is active in crude brei obtained by grinding liver in either carbonate or phosphate buffer. Its activity is retained after filtration of the brei through a sintered glass funnel with medium size pores which retain all intact cells. When the crude filtered brei is brought to *p*H 5·0 with an acetate buffer the sulphinic acid oxidase remains in the supernatant liquid above the precipitated cytochrome oxidase and is active even after dialysis. Table VII records a typical experiment. It may be seen that compared with the enzyme which oxidizes the sulphur of cystine to inorganic sulphate, this oxidase is exceedingly active, 1167 μg. S being recovered as sulphate, representing 56 % of the sulphur of the added cysteinesulphinic acid. The loss in purifying was about 75 %.

Table VII

Partial purification of the enzyme from liver of the albino rat, which oxidizes the sulphur of sulphinic acid to inorganic sulphate: (*a*) Crude brei in phosphate buffer. (*b*) The cytochrome oxidase precipitated at *p*H 5·0 ± 0·2 with acetate buffer, the precipitate made up with phosphate buffer to the same volume as the supernatant liquid and both dialysed against repeated changes of glass-distilled water. All experiments were made in phosphate buffer. Results are expressed in μg. S recovered from initial 10 mg. sulphinic acid.

Exp.	Enzyme	Sulphinic acid (mg.)	Treatment	Sulphate-S μg.
a	Crude	10	—	1167
a	,,	10	Heated	0
b	Precipitate	10	—	19
b	,,	10	Heated	0
b	Filtrate	10	—	268
b	,,	10	Heated	0

(*d*) *Enzyme oxidizing cystine with the production of inorganic sulphate.* The enzyme which oxidizes the sulphur of cysteine to inorganic sulphate is active in the brei obtained by grinding liver tissue in carbonate buffer and filtering through the Büchner funnel. It is readily adsorbed on permutit either from the carbonate buffer or from a dilute neutral salt solution, but cannot be eluted by any methods attempted. It may be precipitated by $MgSO_4$ or Na_2SO_4, but the dialysed precipitate was inactive. While repeated microscopic examinations of the active brei have failed to reveal the presence of intact liver cells, occasional nuclei from disrupted cells and a few blood cells were seen. It is possible that they may carry the active enzymes, as these formed elements were always lacking after first steps of purification when the enzymic activity was lost.

Discussion

Conclusions as to the path of oxidation of cysteine in the liver must be more or less tentative, since the several enzymes are imperfectly separated and may even be contaminated with other enzymes attacking the same substrate at different positions in the molecule. In the production of inorganic sulphate from cysteine there is no evidence of step-wise oxidation, viz. addition of each oxygen atom separately, although the possibility is not excluded. It may be that cysteinesulphinic acid is the end-product of the reaction, and that the oxidation of the latter is carried out by the sulphinic acid oxidase. This possibility is suggested, since otherwise it is difficult to postulate a function for the latter enzyme, which seems to be present in larger amounts than any of the others studied. Cysteine and cystine disulphoxide are definitely excluded as intermediates, since sulphate-production from them is slower than from cysteine. It appears probable, then, that oxidation of the sulphur of cysteine to inorganic sulphate occurs exclusively along the line of the sulphydryl series.

There is somewhat more evidence of a step-wise oxidation of cysteine to cysteic acid. Cystine seems to be oxidized with about equal facility, leaving some doubt as to which is the immediate substrate. If cystine must be reduced to cysteine before being oxidized along the thiol series, the reduction would seem to be enzymic, since it occurs with great rapidity. There is no other evidence of the presence of an enzyme in the liver of the rat which can accomplish this reduction, as shown by the slowness of sulphate-formation from cystine. Therefore the evidence seems to indicate that cystine itself may be the immediate substrate in the oxidation to cysteic acid. Cysteinesulphinic acid is oxidized by the same enzyme, or by a second enzyme present in the same preparation. It may therefore be a stage in the oxidation of cysteine to cysteic acid. An attempt will be made to settle some of these problems with more highly purified enzyme preparations.

Summary

Four enzymes have been extracted from liver tissue of the albino rat.

1. Cytochrome oxidase, which oxidizes cysteine quantitatively to cystine. It has no effect on methionine or cysteinesulphinic acid.

2. An enzyme which oxidizes the sulphur of cysteine to inorganic sulphate. An active crude liver brei has been obtained but attempts at further purification proved unsuccessful. It is possible that this enzyme carries the oxidation only to the sulphinic acid stage. Disulphides cannot act as substrates.

3. An enzyme which oxidizes the sulphur of cysteinesulphinic acid to inorganic sulphate.

4. An enzyme which oxidizes cysteine to cysteic acid (according to iodimetric titration). This oxidation probably represents the first step in the production of taurine. Cysteinesulphinic acid is possibly an intermediate.

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