7.5% polyvinylpyrrolidon (PVP) solution containing 0.03% saponine and homogenized (dilution factor 125 ×). 1 g of liver was diluted with 11.5 vol of PVP-saponine solution and homogenized (dilution factor 12.5 ×). Both suspensions were allowed to stand at room temperature for 30 min with occasional stirring; they were then centrifuged at 18000 *g* for 20 min and the supernatants preserved for analysis.

In appropriate aliquots of the supernatants the haemoglobin content was determined by the specific method of HAVEMAN et al.[2], using triethanolamine buffer at pH 7.5. With this method the increase in absorption (attributed specifically to haemoglobin) was measured at 546 mμ (or 570 mμ) after adding $K_3Fe(CN)_6$ (extinction 1) and then KCN (extinction 2). The differences in extinctions (ΔE) multiplied by the dilution factors represent the amount of haemoglobin in the undiluted original samples. The haemoglobin concentration in liver compared to that of the whole blood can be used to calculate an index of the contamination of the liver by blood.

The results of a representative experiment are seen in the Table.

The ΔE^{546} of 1 ml diluted whole blood sample was 0.200. Therefore the ΔE^{546} of 1 ml undiluted whole blood would be $0.200 \times 125 = 25$.

The ΔE^{546} of 1 ml diluted liver homogenate was 0.060. Therefore the ΔE^{546} of 1 g 'undiluted' liver (wet weight) would be $0.060 \times 12.5 = 0.75$.

Because 0.75 is 3% of 25, this would indicate that 3% of 1 ml whole blood (that is 30 μl) was found as a contaminant in 1 g of liver (wet weight), in this experiment.

Values of 1.5–5% (average 4.2%) have been obtained in a series of ten experiments. This variation appears to be dependent upon the circumstances of death.

After these, the analysis of the cathepsine and peptidase activity of liver, and of whole blood, was carried out. 1 g of liver, and 0.2 ml whole blood (many times the amount found as liver contaminant), was homogenized in 0.25 *M* saccharose or 0.1% of Triton X-100. Aliquots of the homogenates were mixed with buffer and substrate and the determinations carried out as described by RADEMAKER[3].

	After $K_3Fe(CN)_6$	After KCN	ΔE^{546}
E 546 mμ blood	0.370	0.570	0.200
E 546 mμ liver	0.360	0.420	0.060

The following enzymes were measured: (1) Cathepsine A activity with the specific substrate carbenzoxy *l*-glutamyline-*l*-tyrosine (0.04 *M*) at pH 5.1 (found as optimal) in citric acid-phosphate buffer. (2) Cathepsine B with 0.04 *M* benzoyl-*l*-argininamide at pH 5.5 in Michaelis buffer (veronal-acetate). (3) Cathepsine C with 0.04 *M* glycyl-*l*-phenyl-alanylamide at pH 5.4 in Michaelis buffer in which 0.02 *M* cystein was dissolved. (4) Carboxypeptidase with 0.04 *M* carbobenzoxy-glycyl-*l*-phenylalanine at pH 5 in citric acid-phosphate buffer. (5) Dipeptidase with 0.25 *M* diglycine at pH 7.7 in a phosphate buffer containing 0.002 *M* $CoCl_2$. (6) Tripeptidase with 0.16 *M* triglycine at pH 7.2 in phosphate buffer containing 0.002 *M* versene.

In experiments 2 and 3 ammonia was determined using the slightly modified method of VAN SLYKE and CULLEN[4]; the free acidic groups in experiments 1, 4, 5 and 6 were determined by the method of RADEMAKER and SOONS[5]. Mikrokjeldahl method was used for protein determinations.

With these methods, under the conditions specified, blood samples had no activity in the experiments 1, 5 and 6. In experiments 2, 3 and 4, the activity of 0.2 ml sample of whole blood was less than 5% of that for 1 g of liver (wet weight).

These findings, the presence of small amounts of blood and the insignificant activity of blood samples, indicate that the cathepsine and peptidase activity of rat liver homogenate is due to liver enzymes *per se*.

Zusammenfassung. Es wurde die Blutmenge in der Rattenleber sowie die Kathepsinaktivität der Leberhomogenate und des Blutes bestimmt. Unter den beschriebenen Bedingungen war die Kathepsinaktivität der Leberhomogenate nicht durch Blutkontamination beeinflusst.

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[2] R. HAVEMAN, F. JUNG, and B. v. ISSEKUTZ JR., Biochem. Z. *301*, 116 (1939).
[3] W. RADEMAKER, Dissertation, Utrecht (1959).
[4] D. D. VAN SLYKE and G. E. CULLEN, J. biol. Chem. *24*, 117 (1916).
[5] W. RADEMAKER and J. B. J. SOONS, Biochim. biophys. Acta *24*, 209 (1957).

The Transulfuration of Sulfinates by Polysulfides

Organic polysulfides, produced either enzymically or by incubation of thiols with elementary sulfur, are known to transfer spontaneously an atom of sulfur to sulfinates[1–4]:

$$R\text{–SSH} + R'\text{–SO}_2\text{H} \longrightarrow R'\text{–SO}_2\text{SH} + R'\text{–SO}_2\text{SH}$$

This reaction is of biological importance since it may explain the production of thiosulfonates found in biological material as cysteine and cysteamine metabolites (see [3] for bibliography). Attempts to prepare the polysulfide analogue of cysteine (thiocysteine) demonstrated that this compound is unstable in aqueous solutions and is partially converted into cystine and inorganic polysulfides[1]:

$$2\ R\text{–SSH} \longrightarrow R\text{–SS–}R + \text{H}_2\text{S}_2$$

Instability in water solution is a general property of organic polysulfides and their life span is still unknown. Since inorganic polysulfides are expected to transulfurate sulfinates as well as organic polysulfides, the question rises whether the transulfurating agent is the organic or the inorganic polysulfide, produced through the decomposition of the former.

[1] D. CAVALLINI, C. DE MARCO, B. MONDOVÌ, and G. B. MORI, Enzymologia *22*, 161 (1960).
[2] D. CAVALLINI, D. DE MARCO, and B. MONDOVÌ, Arch. Biochem. Biophys. *87*, 281 (1960).
[3] C. DE MARCO, M. COLETTA, and D. CAVALLINI, Arch. Biochem. Biophys. *93*, 179 (1960).
[4] B. MONDOVÌ and C. DE MARCO, Enzymologia *23*, 156 (1961).

Transulfuration of sulfinates by inorganic polysulfides

Procedure for the preparation of polysulfide	Sulfinate added	μ-moles of thiosulfonate produced	
		1 h	2 h
(a) H_2S + S	HT	31.3	43.3
	CSA	33.5	43.1
(b) H_2S + rat liver sulfide oxidase	HT	7.1	9.0
	CSA	6.1	9.2
(c) H_2S + horse kidney sulfide oxidase	HT	7.3	13.9
	CSA	5.9	11.3
(d) H_2S + Cu^{++}	HT	10.8	14.0
	CSA	12.3	17.6

Experimental conditions: 100 μ-moles of H_2S were added in the form of $Na_2S \cdot 9\,H_2O$; S was added in the form of finely powdered elementary sulfur (80 mg); Cu^{++} was added as $CuCl_2$ (10 μ-moles); rat liver sulfide oxidase was prepared as indicated in [5] (5 ml of the final solution was used); horse kidney sulfide oxidase was prepared by a method to be published (40 mg of freeze dried powder were used); sulfinates were added in 100 μ-moles amount (HT = hypotaurine; CSA = cysteinsulfinic acid). The mixtures contained 3 ml Tris buffer pH 7.2, 0.5 *M*, except for the rat liver sulfide oxidase preparation which contained a phosphate buffer; the final pH was adjusted to 7.2 when necessary by addition of 2 *N* HCl; the final volume was 6 ml. The vessels were shaken in a bath at 38°. Thiosulfonates were determined in aliquots of the solutions by the procedure reported in [3].

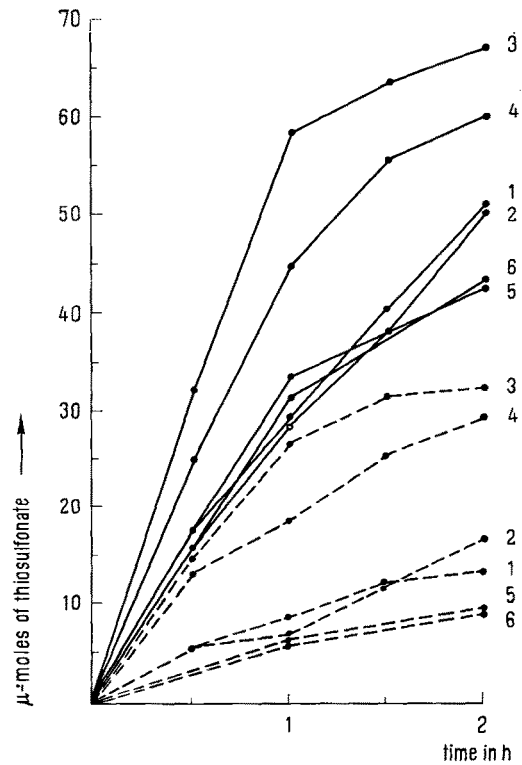
The Table shows that inorganic polysulfides transulfurate hypotaurine and cysteinesulfinic acid yielding the respective thiosulfonate. Polysulfides have been produced in the incubation mixture (see Table) by the following reactions: (a) by addition of sulfur with sulfide; (b) by oxidation of sulfide by rat liver sulfide oxidase[5,6]; (c) by oxidation of sulfide by a horse kidney extract containing sulfide oxidase; (d) by oxidation of sulfide by copper ions.

In order to establish the contribution of organic and inorganic polysulfides to the mechanism of transulfuration of sulfinates, we have produced the organic polysulfide by incubating a thiol with elementary sulfur as in our earlier work[3], and have compared the amount of thiosulfonates produced in the system flushed with a stream of nitrogen with the amount produced when the system was not flushed with nitrogen. Since at the pH near neutrality, as used in this work, inorganic polysulfides decompose by the reaction:

$$H_2S_n \rightleftharpoons H_2S + S_{n-1}$$

the removal of volatile H_2S by degassing with nitrogen pushes the equilibrium towards the right with the result of decreasing the concentration of inorganic polysulfide. The organic polysulfides used in the present work are not expected to be volatile, and any depression of transulfuration caused by flushing with nitrogen is thus a measure of the extent of transulfuration going through the inorganic polysulfides.

As can be seen in the Figure, the extent of transulfuration is strongly depressed by bubbling nitrogen through the solution, indicating that a substantial part of the reaction is actually produced by inorganic polysulfides. When the decrease of transulfuration caused by bubbling nitrogen is compared in systems containing different thiols, the ratio of the reaction removed by degassing with that surviving this procedure is not the same. With H_2S, cysteine and cysteamine as thiols the percentage of depression caused by degassing is respectively 80, 72.5 and 52 at the end of the incubation. This finding suggests that



Effect of bubbling nitrogen on the transulfuration of sulfinates by polysulfides. The same conditions as those reported in the Table. Thiols = 100 μ-moles, sulfur = 80 mg; sulfinates = 100 μ-moles. Full lines-reaction performed in stoppered vessels. Broken lines = reaction performed in vessels flushed with a stream of nitrogen. 1 = cysteine + S + hypotaurine; 2 = cysteine + S + cysteinesulfinic acid; 3 = cysteamine + S + hypotaurine; 4 = cysteamine + S + cysteinesulfinic acid; 5 = H_2S + S + hypotaurine; 6 = H_2S + S + cysteinesulfinic acid.

only a part of the transulfuration is produced by inorganic polysulfides and that another variable portion goes directly through the organic polysulfide. The extent of this second portion may be appreciated by the difference of percentage of depression caused by bubbling nitrogen in the system containing H_2S and S with that of the other systems. This difference is obviously related to the degree of stability of the organic polysulfide which appears higher for the polysulfide of cysteamine than for that of cysteine[7].

Riassunto. È stato dimostrato che la transulfurazione che consegue alla incubazione dei solfinati con zolfo ed un composto tiolico è dovuta in parte alla produzione di un polisolfuro organico, formato dal tiolo e zolfo, ed in parte al polisolfuro inorganico, formato dalla decomposizione di quello organico. L'entità della transulfurazione prodotta direttamente dal polisolfuro organico varia con il tipo di polisolfuro e dipende dalla sua stabilità nelle condizioni usate.

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[5] C. F. BAXTER, R. VAN REEN, P. B. PEARSON, and C. ROSENBERG, Biochim. biophys. Acta *27*, 584 (1958).

[6] B. SÖRBO, Biochim. biophys. Acta *58*, 349 (1961).

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