search, United States Air Force, through its European Office under Contract No. AF 61 (052)-40. Facilities for use of the electronic digital computer BESK were granted by the *Swedish Board for Computing Machinery.*

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Received December 19, 1961.

On the Acceptor Specificity of Rhodanese

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It has previously [1] been shown that rhodanese catalyzes the reaction between thiosulfonates and sulfite to give the corresponding sulfinate and thiosulfate. It could then be expected that rhodanese should also catalyze and exchange reaction between thiosulfate and sulfite

$$S_2O_3^{2-} + {}^{35}SO_3^{2-} = S^{35}SO_3^{2-} + SO_3^{2-}$$

This has now been verified (Table 1), which is of special interest in connection with the experiments on thiosulfate metabolism recently reported by Szczepkowski *et al.*[2,3] These authors injected ^{35}S-labelled cystine into rats and found that most of the radioactivity was excreted into the urine as sulfate, with only a small fraction as thiosulfate. If, however, unlabelled thiosulfate was injected together with the labelled cystine, much more radioactivity was excreted as thiosulfate and less as sulfate. This was interpreted as indicating that thiosulfate is an important metabolic precursor of sulfate, which is in contrast to current opinions[4] on the metabolism of sulfur compounds. The most important precursor of sulfate is usually considered to be sulfite, which is formed from cysteine or cystine with cysteinesulfinate as an intermediate. The results of Szczepkowski *et al.* can in fact be explained by a rhodanese catalyzed dilution of labelled sulfite by unlabelled thiosulfate.

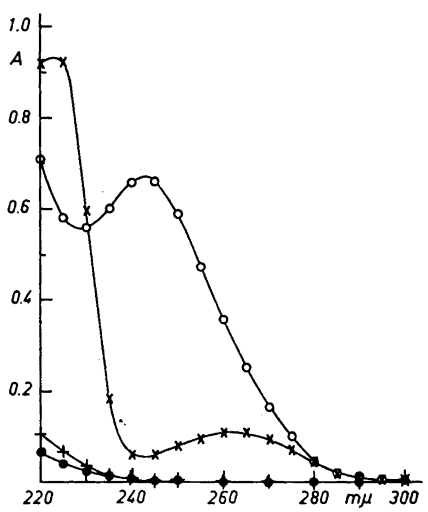

Fig. 1. Absorption spectrum of thiosulfonates and sulfinates. O *p*-toluenethiosulfonate, × *p*-toluenesulfinate, + thiotaurine, ● hypotaurine. Each compound at 1 × 10^{-4} M concentration in 0.05 M phosphate buffer, pH 7.4.

As sulfite is an acceptor for rhodanese, it was of interest to investigate if the structurally related sulfinates (RSO_2^-) could also participate in rhodanese catalyzed transsulfuration reactions. This could be demonstrated to be the case by a spectrophotometric technique, that was based on the fact that arylthiosulfonates (which are very active sulfur donors for rhodanese [5]) show a much stronger light absorption around 240 mμ than do the corresponding sulfinates or alkyl thiosulfonates (Fig. 1). It is thus possible to demonstrate a transsulfuration between an aromatic thiosulfonate and an aliphatic sulfinate or between an aliphatic thiosulfonate and an aromatic sulfinate by following the change in light absorption at 240 mμ. (Other sulfur acceptors, such as sulfite or cyanide, which absorb weakly at 240 mμ, can also be studied in this way). This spectrophotometric technique for following rhodanese catalyzed reactions seems to be of potent value for studies on the reaction mechanism of the enzyme, since it is now possible to rapidly determine what effect different substituents in the donor or acceptor molecule have on the reaction velocity. The rhodanese cata-

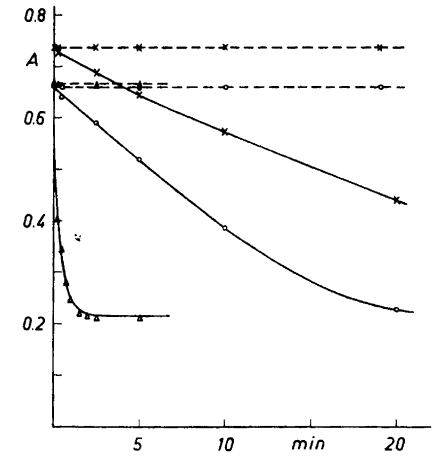

Fig. 2. Reaction between toluenethiosulfonate and sulfinates or sulfite. The test system contained 1×10^{-4} M *p*-toluenethiosulfonate, 5×10^{-4} M sulfinate or sulfite, 0.05 M phosphate buffer pH 7.4 and rhodanese as indicated (3 RU/ml in case iof the sulfinates and 0.6 RU/ml in case of sulfite). Temp. 23°C. O hypotaurine, × cysteinesulfinate, △ sulfite. Dashed curves are without added enzyme.

lyzed reactions between *p*-toluenethiosulfonate and hypotaurine or cysteinesulfinate are shown in Fig. 2. The identity of the reaction products (toluenesulfinate and thiotaurine or thiocysteate, respectively) was established by paper chromatography. Is is also evident from Fig. 2 that sulfite is a better sulfur acceptor than the sulfinates studied. The reaction between a thiosulfonate and sulfinate is reversible as shown in Fig. 3. The equilibrium constant

$$K = \frac{[\text{toluenesulfinate}]\,[\text{thiotaurine}]}{[\text{toluenethiosulfonate}]\,[\text{hypotaurine}]}$$

was calculated to be 1.2 and thus the amino alkylsulfinate has about the same affinity for sulfur as the arylsulfinate. Rhodanese also catalyzed the reaction between thiotaurine and cysteinesulfinate to give hypotaurine and thiocysteate, as demonstrated by paper chromatography. (The test system of pH 7.4 contained 4×10^{-3} M substrate plus 1.4 RU/ml rhodanese, and was incubated 30 min. at 20°C).

Some thiol compounds were also tested as sulfur acceptors for rhodanese at pH 7.4 with *p*-toluenethiosulfonate as the donor. It was found that rhodanese had no catalytic effect with inorganic sulfide, cysteine, cysteamine or glutathione, but that a slow enzyme-catalyzed reaction occurred with mercaptoethanol and thioglycolate. The reaction products are presumably toluenesulfinate and a persulfide of mercaptoethanol and thioglycollate, respectively, but the latter compounds have not yet been identified and are probably rather unstable.

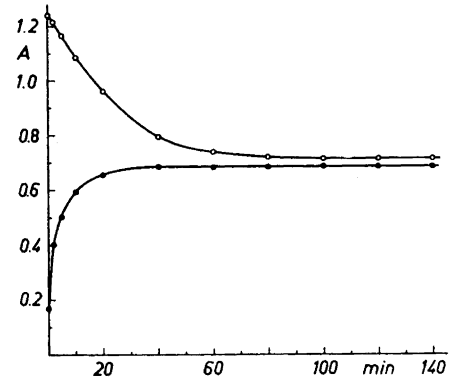

Fig. 3. Reversible reaction between thiosulfonate and sulfinate. The test system contained 2×10^{-4} M substrates and 6 RU/ml rhodanese. Other conditions as in Fig. 2. O *p*-toluenethiosulfonate and hypotaurine, ● *p*-toluenesulfinate and thiotaurine.

Experimental. Crystalline rhodanese, sodium *p*-toluenethiosulfonate, thiotaurine and hypotaurine were prepared as described in earlier publications [5-7], whereas sodium *p*-toluene-

Table 1. Exchange reaction between thiosulfate and sulfite. The test system, at pH 7.4 contained 125 μmoles each of $Na_2S_2O_3$ and $Na_2{}^{35}SO_3$ (7.2×10^3 c.p.m. per μmole), 28 μmoles of NaH_2PO_4 and rhodanese as indicated. Final volume 2.5 ml. The reaction mixtures were incubated for 5 min. at 20°C.

Rhodanese RU*	$S^{35}SO_3^{2-}$ formed μmoles
0	0
1.5	5.7
16.0	48.2

* As defined in Ref.[6]

sulfinate [8] and ^{35}S-labelled sulfite [9] were prepared as described elsewhere. Cysteine sulfinic acid was obtained from the California Corporation for Biochemical Research. Thiosulfate, as the nickel-etylenediamine complex, was isolated from the reaction system of Table 1 by a procedure which was slightly modified from that described by Ames and Willard [10]. Its radioactivity was determined in a windowless gas-flow counter (Frieseke & Hoepfner FH 407), with suitable corrections for self-absorption being applied. Spectrophotometric measurements were made in a Beckman DU spectrophotometer in 1 cm cuvettes. Paper chromatography was carried out with ascending development on Whatman No. 1 paper with acetone-water (4:1) as the solvent. R_F-values in this system are as follows: toluenethiosulfonate 0.87, toluenesulfinate 0.53, thiotaurine 0.42, hypotaurine 0.10, thiocysteate 0.15, cysteinesulfinate 0.07. The samples were dried slowly on the paper with cold air, as this was found to minimize the apparent »spontaneous» reaction described in the case of the reaction between thiotaurine and cysteinesulfinate by De Marco and Coletta [11]. Spots were revealed by iodoplatinate or ninhydrin.

Acknowledgements. This work was supported by a grant from the *Swedish Medical Research Council.* Excellent technical assistance was given by Mr. Ingvar Hedenborg.

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Received December 28, 1961.

N-Pyrrolidinomethyltetracyclin. Stabilität in Lösungen und Papierchromatographische Prüfung

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N-Pyrrolidinomethyltetracyclin (PMT) ist als ein in Wasser leichtlösliches Tetracyclinderivat mit gewissen Vorteilen gegenüber anderen, früher zugänglischen Tetracyclinen [1] introduziert worden. Es wird als Trockensubstanz zur Bereitung von wässrigen Lösungen angeboten. Die Kontrolle der Stabilität des Derivates ist pharmazeutisch und analytisch von besonderem Interesse.

Verschiedene Methoden zur papierchromatographischen Identifizierung von Tetracyclinen sind veröffentlicht worden, von denen hier die von Selzer und Wright [2] als die von verschiedenen Gesichtspunkten aus am besten geeignete hervorgehoben werden soll. Ein wesentlicher Vorteil ist die Schnelligkeit dieser Methode, wodurch die Epimerisierung beim C(4) des Tetracyclins, Chlortetracylins u.s.w. nur in sehr begrenztem Umfange geschehen kann. Von unseren Erfahrungen mit dem System von Selzer und Wright bezügl. Tetracyclin, Chlortetracyclin, Demethylchlortetracyclin und Oxytetracyclin haben wir in anderem Zusammenhang berichtet [3]. Beim Chromatographieren von PMT erschien es uns, als ob sich diese Substanz leicht zersetzte.

Beim Arbeiten nach Selzer und Wright unterschieden sich somit die Chromatogramme, die durch Absetzen von frisch zubereiteter und älterer Lösung in Wasser erhalten wurden. Bei Lagerung der Lösung verschob sich der Schwerpunkt von einem Fleck mit R_F ca. 0.07 (Fleck 1) zu einem Fleck mit R_F ca. 0.28 (Fleck 2) und gleichzeitig erschien ein dritter Fleck mit R_F ca. 0.12 (Fleck 3), der mehr und mehr sichtbar wurde. Die Flecke 2 und 3 stimmten mit denen des Tetracyclins und dessen Epimerisierungsproduktes Quatrimycin überein und deshalb wurde Fleck 1 dem PMT zugeschrieben. Mit frisch zubereiteter wässriger Lösung wurde ein sehr schwacher Tetracyclinfleck und kein Quatrimycinfleck erhalten. Mit frisch zubereiteter methanolischer Lösung wurden dagegen ein