SODIUM THIOSULFATE #94B

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Sodium Thiosulfate-Volume 2 #94B 6/21/73

APPENDIX A

(full text copy of all papers used in the preparation of the monograph)

to

SODIUM THIOSULFATE

A Monograph

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Dr. Jon E. Villaume

and

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THE FRANKLIN INSTITUTE RESEARCH LABORATORIES
BENJAMIN FRANKLIN PARKWAY • PHILADELPHIA, PENNA. 19103

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REDUCTION OF CERTAIN SULFUR COMPOUNDS TO HYDROGEN SULFIDE BY THE INTESTINAL MICROORGANISMS OF THE DOG

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(Received for publication, October 22, 1937)

A number of papers have dealt with hydrogen sulfide formation from various sulfur compounds by the agency of specific cultures. Among the more recent ones may be cited those of Muesow and Paine (1), Zorkendorfer (2), Tarr (3), and Starkey (4). Most investigators of the effect of specific strains of bacteria on sulfur compounds are in agreement that whereas H_2S is easily produced by many microorganisms from compounds representing an intermediate state of oxidation of the sulfur, completely oxidized forms (sulfates and sulfonates) are, as a rule, not reduced to H_2S. Kochman (5), however, reports formation of H_2S from Na_2SO_4 by the microorganisms of both cat and rabbit feces; Muesow and Paine (1) found H_2S production in certain cases from K_2SO_4 (but not from Na_2SO_4), while Zorkendorfer (2) reports some production of H_2S from sulfates in the intestine. Other investigators have reported negative results with sulfates.

In the present investigation, no attempt has been made to work with pure cultures, since our interest lay mainly in determining the practical results of bringing various sulfur compounds into contact with the mixed bacteria of the intestinal mucosa. In all cases the fresh mucosa of the small intestine of dogs was removed and hashed very shortly after death. A weighed amount of the mucosa (50 gm.) was placed in a 29 × 300 mm. test-tube. To this was added an amount of the sulfur compound to be tested corresponding in sulfur content to 0.002 mole (0.480 gm.) of cystine.

J Biol Chem 122(3):687-692, Feb. 1938

When necessary to produce an approximately neutral solution, the substance was added as the sodium salt. This tube, provided with inlet and outlet tubes for aeration, was connected to a second tube containing 50 cc. of an ammoniacal solution of cadmium nitrate (3.5 gm. of $Cd(NO_3)_2 \cdot 4H_2O$ + 25 cc. of concentrated NH_3 + 25 cc. of H_2O) for absorption of the hydrogen sulfide. A third tube, containing 5 N H_2SO_4, served to remove ammonia from the stream of air. This was necessary, since from two to four such groups were always run in series at one time in order to produce as uniform results as possible. It was usually found practicable to run a series of three or four determinations at one time with different portions of the same sample of intestinal mucosa. In each series, a charge of *l*-cystine was used to provide a reference substance, while other sulfur compounds were used in the other groups. Thus variations in the content of microorganisms in different lots of intestinal mucosa were always standardized against one reference substance (*l*-cystine) and the data obtained were calculated as ratios of the degree of decomposition of *l*-cystine, the latter being taken as unity (see Table I).

The apparatus was placed in a constant temperature room regulated at 38° and air was slowly bubbled through the entire train for 48 hours. The cadmium sulfide produced from each substance was then filtered, washed, covered with excess standard 0.100 N iodine solution, and acidified. The excess iodine remaining after the reaction with the hydrogen sulfide was titrated with 0.100 N sodium thiosulfate. The sulfur compounds used were of a satisfactory degree of purity, as evidenced by sulfur and nitrogen determinations. *l*-Cystine was prepared from human hair; *i*-cystine, by boiling *l*-cystine in hydrochloric acid until racemization was complete; dibenzoylcystine, by the method of Morrow (6); cystine hydantoin and cysteic acid hydantoin, as described by Andrews and Andrews (7); cystine phenylhydantoin and cysteic acid phenylhydantoin, as described by Andrews and Andrews (8); and cysteic acid, by oxidation of cystine with bromine (9). The taurine and methionine were commercial samples of practically theoretical purity. The inorganic salts and the free sulfur were of ordinary C.P. grade. Dibenzoylcystine and all compounds containing a sulfonic acid group were used in the form of their sodium salts.

In Table I are summarized the data obtained. Column 1 represents the actual percentage decomposition of each individual substance as compared with the percentage decomposition of cystine shown in Column 2. Decomposition is expressed in terms

TABLE I

Effect of Intestinal Bacteria on H_2S Production from Various Sulfur Compounds

Compound	Percentage decomposition		Average corrected ratio (*l*-cystine = 1.00)
	Compound (1)	*l*-Cystine control (2)	(3)
dl-Cystine	46.4	39.8	
	44.2	48.0	1.03
Dibenzoylcystine	4.3	38.0	
	4.9	49.1	0.00
Cystine hydantoin	12.0	25.4	
	30.6	49.1	0.47
" phenylhydantoin	0	25.4	
	0	69.8	0.00
Cysteic acid hydantoin	0	19.9	
	0	55.8	0.00
" " phenylhydantoin	0	19.9	
	0	48.0	0.00
" "	2.2	25.4	
	2.0	58.0	0.00
Taurine	8.2	58.0	
	7.7	39.8	0.08
Methionine	11.1	38.0	
	16.5	55.8	0.21
Sodium sulfate	6.0	68.9	
	6.9	60.9	0.03
" sulfite	15.3	66.6	
	11.9	41.5	0.18
" thiosulfate	18.6	66.6	
	15.4	41.5	0.26
Free sulfur	43.9	55.8	
	35.3	60.9	0.65

of that percentage of the charge represented by the hydrogen sulfide produced. Since each separate train of experiments was conducted with a different sample of fresh mucosa, the variations in percentage decomposition of cystine represent variations in the activity of microorganisms in different lots of mucosa. It will

be noted that in every instance the same *l*-cystine control was used for two different experimental substances; the same percentage decomposition for cystine therefore always appears twice in Table I.

Table I also contains (in Column 3) the results of recalculating the data in terms of the ratios of the percentage decomposition of *l*-cystine, the latter being taken as unity. In this calculation a blank correction for the hydrogen sulfide produced from the mucosa itself has been applied. Three such blank determinations, expressed in cc. of 0.1 N iodine solution, amounted to 2.5, 6.0, and 3.0 cc. The average (3.8 cc.) has therefore been used as a blank correction on all determinations. All figures in Column 3 of Table I have been so corrected. In spite of the fact that a number of different dogs were used for these experiments, these ratios are remarkably constant.

With certain compounds (cystine phenylhydantoin, cysteic acid hydantoin, and cysteic acid phenylhydantoin) even less hydrogen sulfide was obtained than in the blank determination. This inhibiting action on the part of the two phenyl derivatives is sufficiently plausible but to observe such an effect on the part of cysteic acid hydantoin is somewhat surprising. Other experiments with these substances, not recorded in Table I, confirmed this result. A degree of decomposition corresponding roughly to the blank was obtained with dibenzoylcystine, while, as would be expected, nether the sulfonic acid group (cysteic acid and taurine) nor the hydantoin ring (cystine hydantoin) showed any inhibiting properties.

Although no significant difference was observed between the degree of decomposition of *l*- and of *i*-cystine, we have further tested the possibility of preferential bacterial action on the individual isomers by treating a 2 gm. sample of *i*-cystine with a correspondingly larger amount of mucosa as described above for several days. After decomposition had reached nearly 50 per cent, as indicated by titration of the cadmium sulfide produced, the mixture of mucosa was acidified and filtered. The cystine was then purified by three recrystallizations and dried. It was found to be optically inactive. It must be concluded that the mixed microorganisms of the intestine of the dog show no special preference for the natural isomer of cystine. Assuming that similar results would apply to the intestine of the rabbit *in vivo*,

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it would seem that the differences in metabolic oxidation reported by du Vigneaud, Craft, and Loring (10) for *d*- and *l*-cystine definitely represent a real difference in intracellular oxidation rather than the result of a greater degree of production and subsequent oxidation of hydrogen sulfide from the natural isomer. This suggests the possibility that the differences in oxidation reported by du Vigneaud, Craft, and Loring may have been considerably dulled by production and metabolic oxidation of hydrogen sulfide from these compounds, and that if their results could have been corrected for this factor, still more striking differences between the rate of metabolic oxidation of the isomers of cystine might have been apparent.

Similar possibilities arise in comparing hydrogen sulfide production from *l*-cystine with that from *dl*-methionine. Du Vigneaud, Loring, and Craft (11), as well as others, have reported that oral administration of *dl*-methionine and of *l*-cystine produces similar degrees of oxidation of the sulfur administered as measured by the production of urinary sulfate. The data in Table I show a definitely lower degree of decomposition of methionine and thus imply a correspondingly more rapid metabolic oxidation for this amino acid. The ability of the normal organism to oxidize rapidly the sulfur of methionine is further emphasized by the greater speed of its absorption from the small intestine (12).

The effect of intestinal microorganisms on cysteic acid and taurine is of interest in connection with the studies of White, Lewis, and White (13). These authors report production of a limited amount of urinary sulfate after oral administration of cysteic acid and of considerably more sulfate after oral administration of taurine. They suggest that intestinal production of hydrogen sulfide may be responsible for a portion of this sulfate. The data in Table I indicate some decomposition of taurine but not of cysteic acid and, if we may assume that the effect of intestinal microorganisms in rabbits is similar to that in the dog, lend support to the conclusion of White, Lewis, and White that there is some evidence of sulfate production from cysteic acid in the metabolism of the rabbit.

The results obtained with inorganic sulfur compounds as well as with free sulfur correspond to those previously reported by most investigators (*cf.* (14)), particularly as regards the very slight evidence of sulfide production from sodium sulfate.

SUMMARY

The ability of a mixture of microorganisms from the mucosa of the small intestine of the dog to produce hydrogen sulfide from the following compounds has been quantitatively compared: *l*-cystine, *i*-cystine, dibenzoylcystine, cystine hydantoin, cystine phenylhydantoin, cysteic acid hydantoin, cysteic acid phenylhydantoin, cysteic acid, taurine, methionine, sodium sulfate, sodium sulfite, sodium thiosulfate, and free sulfur.

Of the above, the following gave no evidence of producing hydrogen sulfide: cystine phenylhydantoin, cysteic acid, cysteic acid hydantoin, and cysteic acid phenylhydantoin. The apparent production of hydrogen sulfide from sodium sulfate was negligibly small.

A definitely inhibiting action was exhibited by cystine phenylhydantoin, cysteic acid hydantoin, and cysteic acid phenylhydantoin.

No appreciable difference in the rate of decomposition of *l*- and *i*-cystine was observed. Furthermore, no evidence was obtained that these microorganisms decompose the *l* isomer in preference to the *d* form.

The possible bearing of these and other findings on the interpretation of metabolic studies of sulfur compounds is discussed.

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C.R. 106
mar. 6, 1931

SOCIÉTÉ DE BIOLOGIE DE BELGRADE

SÉANCE DU 23 DECEMBRE 1930

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Présidence de M. J. Georgevitch.

Augmentation du glutathion dans le foie et dans le sang après administration d'hyposulfite de soude par voie entérale,

par Vojislav Arnovljevitch.

Le taux du glutathion dans les différents organes est assez stable. Mais on peut le modifier de plusieurs façons, surtout en augmentant ou en diminuant les apports du soufre dans l'organisme. L. Melon (1) a montré qu'une alimentation riche en cystine provoque une augmentation du glutathion hépatique, sans faire varier, ou très peu, la quantité du glutathion des autres organes. D'autre part, N. C. Laclau et A.-D. Marenzi (2) ont trouvé que chez le Rat, avec un régime pauvre en cystine, la quantité du glutathion diminue dans le foie et dans le muscle.

(1) *C. R. de la Soc. de biol.*, 1929, t. *101*, p. 1167.
(2) *C. R. de la Soc. de biol.*, 1930, t. *103*, p. 1287.

Nous avons été ainsi amenés à rechercher si le taux du glutathion augmente dans le foie et dans le sang après apport de soufre inorganique. Il nous a semblé préférable d'employer l'hyposulfite de soude, qui n'est pas toxique, et qu'on peut donner aux animaux par voie buccale. A partir de l'hyposulfite il se formerait

Pigeons Témoins	Poids au début de l'expérience (en gr.)	Quantité de $Na_2S_2O_3$ administrée par jour (en gr.)	Durée du traitement (en jours)	Poids à la fin de l'expérience (en gr.)	Taux du glutathion en mgr. pour 100 gr.	
					sang 45 à 62,5	foie 280 à 295
I	480	1	16	405	70	310
II	450	2	16	365	82,4	320
III	390	2	14	320	80	337,5
IV	430	1	25	380	76,3	328
V	460	2	25	395	84,5	335
VI	395	1	30	315	74	

dans l'organisme du soufre à l'état naissant, car sous l'influence de l'acide chlorhydrique de l'estomac, l'hyposulfite se décompose suivant la formule :

$$Na_2S_2O_3 + 2\ HCl \longrightarrow 2\ NaCl + H_2O + SO_2 + S$$

L'hyposulfite a été donnée à des Pigeons, à raison de 1 et 2 gr. par jour, pendant 17 à 30 jours. Les animaux supportaient très bien cette dose : quelquefois seulement les selles étaient diarrhéiques. En outre, nous avons noté régulièrement une diminution du poids des animaux pendant l'expérience.

Nous avons tout d'abord déterminé sur plusieurs Pigeons témoins le taux normal de glutathion dans le sang et dans le foie (3). Chez le Pigeon neuf le sang contient 45 à 62,5 mgr. de glutathion pour 100 c.c. de sang, tandis que le foie en contient 280 à 295 mgr. pour 100 gr. de substance. Ensuite, nous avons déterminé le taux de glutathion chez les Pigeons qui ont reçu tous les jours, par voie entérale 1 ou 2 gr. de $Na_2S_2O_3$. Dans ces conditions la quantité de glutathion dans le foie et dans le sang augmentait régulièrement, comme le montre le tableau ci-joint.

Conclusion. — Après administration d'hyposulfite de soude à des Pigeons par voie entérale, le taux de glutathion augmente régulièrement dans le sang et dans le foie.

(*Clinique médicale propédeutique de l'Université.*)

(3) Le glutathion a été dosé par la technique de Tunnicliffe : le tissu trituré avec du sable est extrait par l'acide trichloracétique à 10 p. 100 ; on titre avec de l'iode n/100 en présence d'amidon comme indicateur.

AUGMENTATION OF GLUTATHIONE IN FAT AND BLOOD AFTER ADMINISTRATION OF SODIUM THIOSULFATE PARENTERALLY

The level of glutathione in different organs is rather stable. But one is able to modify it in several manners, especially by augmenting or lowering the sulfur supplied to the organism. L. Melon (1) has shown that a diet rich in cysteine raises the level of hepatic glutathione with very little variation in the quantity of glutathione in other organs. On the other hand, N. C. Laclau and A.-D. Marenzi (2) have found that feeding rats a diet low in cystine causes a diminution of glutatione in fat and muscle.

We have been thus led to investigate whether the level of glutathione rises in fat and blood after giving inorganic sulfur. It seemed preferable to employ sodium thiosulfate, which is not toxic and which one can administer orally.

Pigeon Groups	Wt at start	$NNaS_2O_3$ daily	Wt. at end	Duration (days)	Glutathion level (mg/100 gm) blood	fat
I	480gm	1gm	405gm	16	70	310
II	450	2	365	16	82.4	320
III	390	2	320	14	80	337.5
IV	420	1	380	25	76.3	328
V	460	2	395	25	84.5	335
VI	395	1	315	30	74	-

Naissant sulfur forms in the animal, because at the acidity of the stomach thiosulfate decomposes according to the following formula:

$$Na_2S_2O_3 + 2HCl \longrightarrow 2\ NaCl + H_2O + S + SO_2$$

Sodium thiosulfate has been given to pigeons at a level of 1 and 2 gm/day for 17 to 30 days. The animals took this dose very well; only sometimes the salt was diarrhetic. Further, we have noted regularly a diminution of the weight of the animal during the experiment.

At first we determined in several pigeons the normal level of glutathione in the blood and fat (3). With an untreated pigeon the blood contains 45-62.5 mg glutathione/100 ml, while the fat contains 280-295 mg/100 gm fat. We then determined the level of glutathione in pigeons who received 1 or 2 gm of sodium thiosulfate every day parenterally. Under these conditions it rises regularly in fat and blood, as the table above illustrates.

CONCLUSION

After administration of sodium thiosulfate parenterally to pigeons the level of glutathione augments regularly in fat and blood.

(1) Comp. Rend. Soc. Biol. 101:1167 (1929)
(2) Comp. Rend. Soc. Biol. 103:1287 (1930)
(3) Glutathione has been analyzed by the method of Tunnicliffe: tissue triturated with sand is extracted with 10% trichloroacetic acid; then titrate with N/100 iodine in the presence of amidon indicator.

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SULFIDE OXIDATION IN RAT TISSUES*

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INTRODUCTION

The enzymic pathways involved in the catabolism of sulfur-containing amino acids have been reviewed by SINGER AND KEARNEY[1], and more recently by CHATAGNER AND BERGERET[2]. Two distinct routes are recognized by which the sulfur of cysteine is oxidized to inorganic sulfate; one via cysteine sulfinic acid and the other through either the cysteine desulfhydrase or transaminase-desulfurase mechanism to hydrogen sulfide, with subsequent oxidation to sulfate.

The cysteine desulfhydrase mechanism of this latter pathway has been the subject of numerous studies. In mammalian tissues the formation of hydrogen sulfide from cysteine can be demonstrated under anaerobic conditions[1,2,3,4]. Microorganisms of the intestine also produce hydrogen sulfide[5], and in the case of *Escherichia coli* this process is greatly enhanced by aerobic conditions[6]. Relatively little is known about the ability of mammalian tissue to oxidize hydrogen sulfide, or the pathways and mechanisms by which this is accomplished. *In vivo* experiments by DZIEWIATKOWSKI[7] have shown that small doses of ^{35}S-labeled sodium sulfide injected into rats are partially converted to ^{35}S-labeled sulfate. DER-GARABEDIAN AND FROMAGEOT[8] reported that in tissue homogenates, sulfide is oxidized to thiosulfate. In subsequent publications TER-KAREPETIAN[9] *alias* DER-GARABEDIAN reported some of the characteristics of this "enzymic" system which he named sulfide oxidase. Recently SORBO[10] reported the heme-catalyzed oxidation of sulfide to thiosulfate.

The studies reported here represent an extension of some of this earlier *in vitro* work. Our results confirm the formation of thiosulfate by rat-liver extracts, but differ from the earlier reports[8] with respect to some of the characteristics ascribed to the catalytic system involved in this oxidation.

METHODS AND MATERIALS

Rat liver extracts

Young adult male rats of the Wistar strain, ranging from 200 to 300 grams in weight were maintained on pelleted Purina small animal chow. They were starved for 24 hours prior to sacrificing§.

* Contribution No. 204 of the McCollum-Pratt Institute. Aided by a grant from the Nutrition Foundation. A preliminary report of a part of this paper was presented at the Federation Meeting in Atlantic City, 1956.

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*** Present address: Naval Medical Research Institute, National Naval Medical Center, Bethesda 14, Maryland.

§ The stimulating effect of starvation on the sulfide oxidase system is described elsewhere[11,12].

References p. 591.

TISSUES*

, PAUL B. PEARSON

altimore, Md., (U.S.A.)

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for 24 hours prior to sacrificing[8].

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system is described elsewhere[11,19].

To obtain rat-liver extracts, the animals were lightly anesthetized with ether and decapitated. The livers (and occasionally the kidneys) were immediately exsanguinated by introducing a cannula into the inferior vena cava and perfusing *in situ* with ice cold $M/7.5$ phosphate buffer pH 7.2. The perfused livers were rinsed, blotted, weighed, resuspended in cold phosphate buffer (pH 7.2) and homogenized in a Waring blender for 1 minute. The final homogenates contained 120 mg liver (wet weight) per ml. The rat-liver homogenates were spun at 12000 *g* for 15 min in a refrigerated centrifuge. The precipitate was discarded. Routinely the supernatant extracts were used immediately after preparation for the experiments described below.

The protein [illegible] extract was determined by the method of LOWRY *et al.*[12].

Substrate and test system

Washed sodium sulfide crystals ($Na_2S \cdot 7H_2O$) were dissolved in water to make a $4 \cdot 10^{-2} M$ solution. The test system, consisting of 22 ml of liver extract in buffer and contained in a closed Erlenmeyer flask, was first preincubated for 5 min at 37°C, 3 ml of the sulfide substrate was then added, the mixture shaken and the zero time sample withdrawn immediately. Occasionally larger flasks with proportionally larger volumes were employed. Both the addition of substrate and the withdrawal of aliquots for sulfide and thiosulfate assay, was accomplished through a short length of plastic tubing attached to a glass tube which extended into the reaction mixture. Except for the times when additions were made or samples were withdrawn, the tube was kept tightly closed. This minimized the loss of substrate through hydrogen sulfide escaping to the outer atmosphere during the sampling process. All incubations were conducted (unless indicated to the contrary) in an atmosphere of air in a water bath shaker at 37°C.

Assay for sulfide and thiosulfate

Sulfide oxidation in the system could be followed both by the disappearance of substrate and by the formation of thiosulfate in the reaction mixture. The sulfide remaining in the reaction mixture was measured colorometrically by the methylene blue method [illegible]cribed for inorganic systems by FOGO AND POPOWSKY[13] and used previously for the assay of alkaline phosphatase in biological materials by DOYLE[14]. A 0.3 ml aliquot of the incubation mixture was required for this assay. Thirty minutes after the addition of the N,N-dimethyl-*p*-phenylenediamine sulfate and prior to the assay of the colored solution (in a D. U. Beckman spectrophotometer at 670 mμ), a portion of it was spun to precipitate all cloudiness due to biological material.

The method was standardized both by comparison with the less specific iodine titration technique for hydrogen sulfide in solution[13] and by the theoretical values of sulfide present (based on the original weight of the sodium sulfide crystals). The relationship of the sulfide present and color formation was linear and reproducible in the range tested (Fig. 1).

Thiosulfate formation was determined by chemical and enzymic assay procedures. Routinely KURTENACKER's titration technique[15] for thiosulfate in the presence of sulfide, sulfite and sulfate was employed with the following modifications:

(1) The $10^{-3} M$ iodine solution used for titration was kept in a colored automatic burette under a nitrogen atmosphere, thus no change in strength of the solution occurred over extended periods of time. (2) The titration endpoints were greatly improved by removing residual proteins and phosphates from the aliquot with uranyl acetate.

An aliquot of 10 ml of the reaction mixture was shaken with 5 ml of a neutral $ZnCO_3$ slurry and 2 ml glycerol in a 50 ml volumetric flask. After 10 minutes, 20 ml of an uranyl acetate solution (0.5 g/100 ml) were added and the mixture again shaken. Following another 10 minutes, the mixture was diluted to 50 ml final volume, and filtered. 10 ml of the filtrate were rapidly titrated against $0.001 M$ iodine in the presence of 1 ml formol and 3 ml of a 10% acetic acid solution. Potato starch in borate was used as indicator. All samples were run in duplicate.

The method was reproducible and accounted for from 94 to 100% of any thiosulfate added directly to a homogenate before deproteinization. This indicated that adsorption of thiosulfate to the precipitate was negligible.

The method employed for measuring thiosulfate formation using rhodanese, was based on SORBO's assay technique for rhodanese[16]. A system was employed in which the thiocyanate formed by the reaction $CN^- + S_2O_3^= \xrightarrow{\text{rhodanese}} CNS^- + SO_3^=$ was *limited only* by the amount of thiosulfate present in the sample. To 2 ml of the incubation mixture and 0.5 ml of $0.2 M$ KH_2PO_4, 8 mg of rhodanese* dissolved in 0.5 ml of $5 \cdot 10^{-1} M$ thiosulfate with a trace of albumin was added. The reaction was started by the addition of 0.5 ml of $0.25 M$ KCN, and after five minutes of shaking at room temperature, stopped by the addition of 0.5 ml of formol. Subsequently 5 ml of ferric nitrate reagent was added**. The volume of the mixture was increased to 20 ml with distilled water and immediately filtered. The optical density of the filtrate was determined in a Beckman DU

* 100 g $Fe(NO_3)_3 9H_2O$ plus 200 ml concentrated HNO_3 made up to 1 l.

** Obtained from Mann Biochemical Corp. U.S.A.

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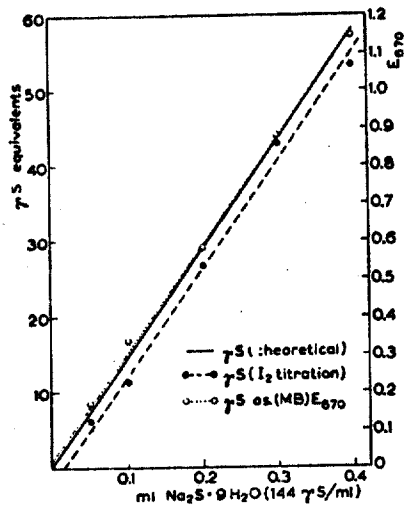

Fig. 1. Iodometric and colorimetric determination of sulfide in tissue extracts.

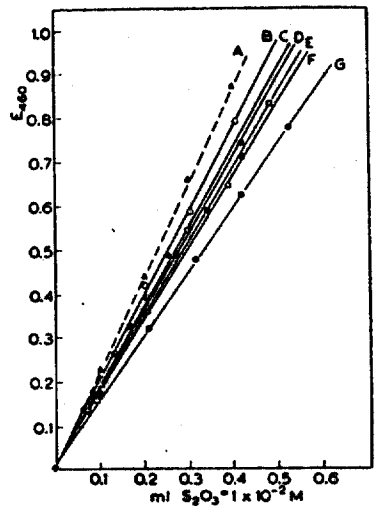

Fig. 2. Standard curves for thiosulfate assay with Rhodanese in the presence of various rat-liver extracts.

spectrophotometer at 460 mμ. Thiosulfate formation was measured as the change in optical density from that of the zero time aliquot. Standard curves were constructed with different rat-liver extracts and known amounts of thiosulfate. The relationship between the thiosulfate added and the optical density at 460 mμ was always linear. However, the slope of standard curves in different liver preparations did not always coincide (Fig. 2), so that to quantitate results, it was necessary to obtain a standard curve for every liver preparation used. It was found experimentally that the intrinsic rhodanese of the crude liver preparation or sulfide substrate was not the modifying factor involved. The presence of sulfide or sulfur in the reaction mixture did not measurably modify the results under the assay conditions employed.

RESULTS AND DISCUSSION

Two parallel systems

When fresh extracts of perfused rat livers in phosphate buffer were incubated at pH 7.3 in a closed system at 37°C with ample sodium sulfide as substrate, thiosulfate was formed at a uniform rate over a 2 h period. This is shown in Fig. 3. For any particular preparation the formation of thiosulfate was proportional to the amount of rat liver extract present. Buffer by itself had no appreciable activity. By exposing the preparation to approximately 100°C in a boiling water bath for 3 min, 50 to 90% of its oxidative ability was destroyed. The residual activity remained despite prolonged boiling. When fresh and boiled homogenate were mixed, the resulting activity represented the mathematical sum of the two. Resuspension and rehomogenization of the heat-denatured precipitate did not reactivate the system. Thus incomplete destruction, heat-activated inhibition, or occlusion of just one system, seemed unlikely.

The presence of two separate systems was supported by several experiments. Figs. 4, 5, and 6 show the effects of pH, incubation temperature and substrate concentration respectively on the heat-labile and heat-stable components of the sulfide-

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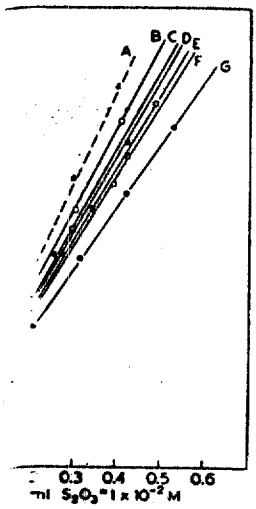

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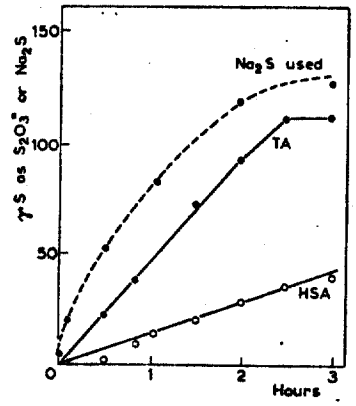

Fig. 3. Thiosulfate formation and sulfide disappearance in the sulfide-oxidizing system of rat liver. TA = total activity (combining heat-stable and heat-labile activity). HSA = heat-stable activity.

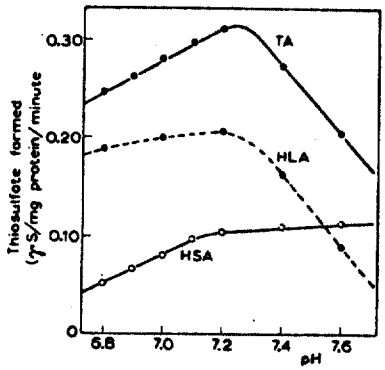

Fig. 4. The effect of pH on the sulfide-oxidizing system. TA = total activity. HSA = heat-stable activity. HLA = heat-labile activity.

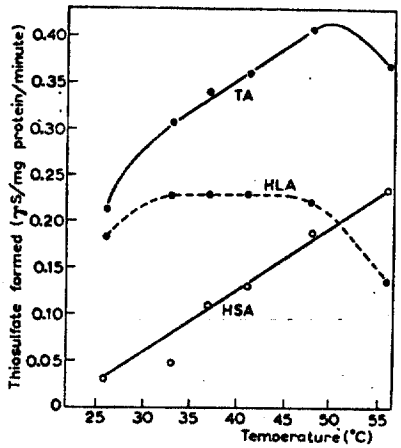

Fig. 5. Effect of temperature on components of sulfide-oxidizing system. TA = total activity. HSA = heat-stable activity. HLA = heat-labile activity.

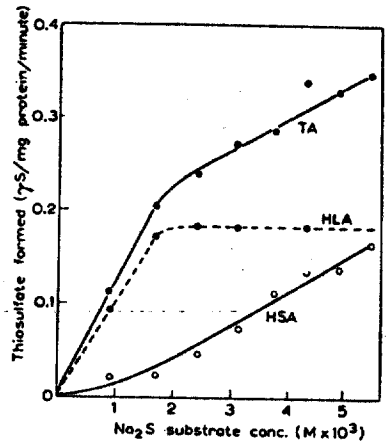

Fig. 6. Effect of substrate concentration on sulfide-oxidizing system. TA = total activity. HSA = heat-stable activity. HLA = heat-labile activity.

oxidizing system. The heat-labile activity in each case, was evaluated as the total activity minus the activity remaining after boiling the preparation for 3 min. The heat-labile component had both a pH optimum around pH 7.2 to 7.3 and a temperature optimum from 35°C to 50°C. The heat-stable component showed neither (Fig. 4 and 5). Furthermore, the heat-labile component of the system became saturated with respect to substrate, yet no substrate saturation could be demonstrated for the heat-stable component within the range of sulfide concentrations compatible with the analytical

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techniques (Fig. 6). Thus it would appear likely that two parallel systems exist in rat liver, both oxidizing sulfide to thiosulfate, one nonenzymic and heat stable and the other with some characteristics of an enzymic system. The two systems may in some way be related to each other. The name "sulfide oxidase" was assigned to the heat-labile system. It corresponds in nomenclature to a heat-stable system described by DER-GARABEDIAN AND FROMAGEOT[6,8].

Masking effect of blood

The heat-labile component of the system was completely masked if the livers were not exsanguinated by perfusion prior to homogenization and extraction. (Table I). If blood was added back to the perfused rat-liver extracts, no apparent decrease in activity was observed upon boiling the preparation as the decreased sulfide-oxidizing ability of the blood-free boiled extract was more than compensated for by the increased activity of the boiled blood; hence the masking effect.

TABLE I

THE MASKING EFFECT OF BLOOD ON THE HEAT-LABILE SULFIDE OXIDASE SYSTEM

Preparation from	γS of $S_2O_3^{=}$ formed ml rx mix/min			γS of $S_2O_3^{=}$ formed/mg protein min		
	Fresh T.A.	Boiled H.S.A.	(2)–(3) H.L.A.	Fresh T.A.	Boiled H.S.A.	(5)–(6) H.L.A.
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Non-perfused liver*	(16.8)	(18.3)	(—1.5)	(0.08)	(0.09)	—(0.01)
Perfused liver	35.1	6.9	28.2	0.31	0.06	0.25
Blood	11.2	40.0	—28.8	0.09	0.31	—0.22
Perfused liver and blood**	46.0	43.5	2.5	0.19	0.18	0.01

* This is a preparation from a different animal to the one used below and thus no direct comparison with reconstituted liver and blood can be made.

** Mixture of 11.3 mg blood protein for every 10 mg of liver protein. Amounts are equivalent to those used for perfused liver and blood, tested separately. T.A. = Total activity. H.S.A. = heat-stable activity. HLA. = heat-labile activity.

Oxygen requirement

Formation of thiosulfate virtually ceased in the absence of oxygen. Very small amounts of thiosulfate formed in a nitrogen atmosphere could be attributed to residual oxygen dissolved in the extract or buffer.

Substrate specificity

No detectable thiosulfate formation could be measured when sulfur or sulfite were used as sole substrates.

End products and stoichiometry

The thiosulfate formed by this system was identified chemically and enzymically as outlined above. Additional qualitative rests were run with solutions free of residual sulfide. The mercury salt techniques, as well as some other tests suggested by KURTENACKER[15] confirmed thiosulfate formation. Sulfide disappearance from the reaction mixture was also measured (Fig. 3). After an initial loss of substrate, presumably to the atmosphere, and equilibration with the vapor phase of the reaction vessel, the rate of sulfide disappearance and thiosulfate appearance corresponded quite closely.

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IDE OXIDASE SYSTEM

of $S_2O_3^{=}$ formed mg protein min		
H.S.A.	Boiled H.S.A. (6)	(5)-(6) H.S.A. (7)
58)	(0.09)	—(0.01)
1	0.06	0.25
9	0.31	—0.22
9	0.18	0.01

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The stoichiometry of the sulfide-oxidizing system was studied with rat-liver extracts incubated in two-armed Warburg vessels. Into the main compartment 1.5 ml of extract and 1.5 ml phosphate buffer pH 7.3 were introduced. The two sidearms contained 0.4 ml of the sodium sulfide substrate in 0.001 *M* sodium hydroxide and 0.4 ml of 0.001 *M* hydrochloric acid respectively. Oxygen uptake was measured manometrically 5 min after the start of the reaction and every 5 min thereafter for one hour or longer. Due to the escape of some sulfide substrate to the atmosphere of the vessel, the oxygen uptake for the first 5–10 min appeared depressed. It continued at a constant rate thereafter. In calculating oxygen uptake in the usual manner[17], this artifact was eliminated by extrapolating back to zero time from the linear plot representing the time period from 10 to 60 min.

Thiosulfate formation was measured by the rhodanese method. A separate flask was used for each time period. Suitable controls were also run. The correction for endogenous carbon dioxide production was found to be negligible.

TABLE II

STOICHIOMETRY OF SULFIDE-OXIDIZING SYSTEM

Experiment	*Sulfide loss*	*Oxygen uptake*	*Thiosulfate* formed*	*Sulfide S recovered as thiosulfate S*
	μmoles hour/ml of reaction mixture			
1	4.5	5.0	1.9	86%
Ratio:	(2.3) :	(2.6) :	(1.0)	
2	4.2	4.1	1.6	
Ratio:	(2.7)	(2.6)	(1.0)	76%
Theoretical ratio:	(2.0)HS^- +	(2.0)O_2	(1.0) $S_2O_3^{=}$ + H_2O	100%

* Rhodanese assay method.

The results of this study are recorded in Table II. The values obtained in two successive experiments were consistent and indicated a comparatively close correspondence to the theoretical ratio of oxygen uptake, sulfide disappearance, and thiosulfate formation. Such discrepancies as do exist, consist of excessive sulfide disappearance and oxygen utilization above that indicated by the thiosulfate formed. This may be due to a variety of reasons, among them (a) a lag period in the system, (b) the formation of by-products other than thiosulfate, or (c) technical difficulties in the assay technique. Thus the escape of sulfide to the outer atmosphere while transferring aliquots would have increased apparent sulfide utilization, and the small return into solution of the hydrogen sulfide of the gaseous phase during the reaction period would have tended to increase the apparent oxygen consumption as measured manometrically while decreasing the apparent sulfide loss.

Distribution of sulfide oxidase activity in rat organs and cell constituents

The heat-labile activity was not confined to the liver, but was also demonstrated in the perfused kidney. Owing to the virtual impossibility of ridding the spleen of blood hemoglobin, no heat-labile system could be demonstrated in this organ. These results are shown in Table III.

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TABLE III

A COMPARISON OF THE SULFIDE-OXIDIZING SYSTEMS IN LIVER, KIDNEY AND SPLEEN

No. of rats pooled	*Organ*	*Perfused*	*γS of $S_2O_3^{=}$ T.A.*	*formed mg H.S.A.*	*prot./min H.L.A.*
3	Liver	yes	0.40	0.10	0.30
3	Kidney	yes	0.51	0.17	0.34
3	Spleen	no	0.19	0.38	—
4	Kidney	yes	0.35	0.03	0.32
4	Spleen	no	0.26	0.45	—

T.A. = Total activity (H.S.A. + H.L.A.). H.S.A. = heat-stable activity. H.L.A. = heat-labile activity.

Fractionation of rat-liver cells into their constituent fractions according to a slightly modified Hogeboom and Schneider technique* gave results recorded in Table IV. Although no clear-cut separation was achieved, the sulfide oxidase was found preferentially in the mitochondrial fraction. If rat-liver homogenates prepared in the usual way in phosphate buffer were spun at 100,000 *g* for one h, the greater part of the activity was found in the supernatant fraction indicating that sulfide oxidase was basically a soluble system.

TABLE IV

DISTRIBUTION OF SULFIDE-OXIDIZING ACTIVITY IN RAT LIVER CELLS*

Cell fraction	*Activity in fraction (% of total activity)*	*Heat-labile activity (% of total activity)*
All components	100	47
Nuclei	12	1
Mitochondria	36	16
Microsomes	16	8
Cytoplasmic supernatant	16	7
Recovery (per cent)	81	32

* Cell fractionation by modified Hogeboom method.

Preliminary purification of rat-liver extract

The crude extract in phosphate buffer (pH 7.3) was heated rapidly to 67°C and held at this temperature for 2 min. It was immediately cooled in an ice bucket. The supernatant of the cooled spun preparation contained most of the sulfide oxidase system (Table V). A five- to ten-fold increase in specific activity was consistently noted. Of this, the heat-stable component had the greater share. The sulfide oxidase properties of the crystalline protein ferritin, a component of this system, are being reported separately[18].

Further purification of the system by means of salting-out techniques have, to date, met with only sporadic success. If the preparation was separated into two fractions by salting out with ammonium sulfate (pH 6) to a final concentration of 2 *M*, both the precipitated and supernatant fractions, after redissolving and dialysis against distilled water at 4°C, contained approximately the same specific activity. The two fractions differed in that the heat-stable activity (HSA) remained preferentially in the

* B. K. JACOBSEN. Personal communication.

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VER, KIDNEY AND SPLEEN

med. mg H.S.A.	prot. min H.L.A.
0.10	0.30
0.17	0.34
0.38	—
0.03	0.32
0.45	—

eat-stable activity. H.L.A. = heat-

fractions according to a slightly
e results recorded in Table IV.
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IN RAT LIVER CELLS*

Heat-labile activity (% of total activity)
47
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SA) remained preferentially in the

TABLE V

PURIFICATION OF SULFIDE OXIDIZING SYSTEM

Fraction	Specific activity*		
	Total activity	Heat-stable activity	Heat-labile activity
Crude homogenate	1.0	1.0	1.0
[illegible]	1.1	1.0	1.1
70°C Supernatant	5.5	12.6	3.9
70°C ppt.	0.01	0.4	0

* Activity of components in crude homogenate = 1.0/mg protein. Of the total initial activity, 16% is heat-stable activity and 84% is heat-labile activity.

supernatant portion of the ammonium sulfate-treated preparation, while the reverse was true for the heat-labile fraction (HLA) of the sulfide oxidase system.

All properties described in this communication for the crude rat-liver extract were substantially duplicated in the partially purified system. The only exception was the ratio of heat-labile to heat-stable system, due doubtlessly to some destruction of the former during the purification procedure.

SUMMARY

1. Extracts of rat liver and kidney were shown to catalyze the oxidation of sulfide to thiosulfate. The system responsible for this oxidation contained both a heat-stable and heat-labile component, the former non-enzymic and the latter probably enzymic. Some of the dual characteristics and other properties of the system have been described.

2. The presence of blood masked the heat-labile activity in rat-liver tissue.

3. The greater part of oxygen uptake and sulfide disappearance observable in the system could be accounted for by the thiosulfate formed.

4. Sulfide oxidation to thiosulfate, is most closely associated with the mitochondrial fraction of rat-liver cells. Its relative heat-stability has made possible some purification of the system.

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THE ABSORPTION AND ELIMINATION OF METABISULPHITE AND THIOSULPHATE BY RATS

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Rats eliminated 55 per cent of the sulphur of metabisulphite and 23 per cent of that of thiosulphate into the urine in the 4 hours after the oral administration of these compounds. This sulphur was excreted almost entirely as inorganic sulphate.

A search of the literature yielded much information about the formation of sulphite and its detoxification in the animal body, but little evidence about the absorption of sulphite from the gastrointestinal tract and the manner of its excretion. Strong indication exists that orally administered metabisulphite solution is absorbed by rats, for Lockett and Natoff[1] found that the ingestion of 11·1 to 25·5 mg. of sodium metabisulphite per 100 g. weight per day did not increase the wet weight of the faeces excreted. Such metabisulphite would have required 0·57 to 1·3 ml. of water for its isotonic solution. Less than a third of this volume of water would have caused a significant increase in the wet weight of the faeces if added to them, for the average wet faeces excreted was 2·31 ± 0·01 g. per 100 g. weight per day. The object of the present work has been to make some estimate of the speed and degree of this absorption and discover in what form ingested metabisulphite is excreted by this species.

Experimental

Female Wistar rats were fed diet 41 b of Stein and were housed in the laboratory for at least 1 week before use.

Experiments of Group I

Eight rats weighing 147·8 ± 1·8 g. were divided into pairs matching in weight. Two pairs drank only sodium metabisulphite (2,000 p.p.m. as SO_2) and two pairs water, for 3 days: all were deprived of food for the last 24 hours of this period. Then each animal received a volume of its drinking fluid equivalent to 5 per cent of weight by stomach tube. Immediately afterwards the bladders were emptied by gentle suprapubic pressure, each pair was put into a separate metabolism cage, and the urine was collected for 4 hours. A cross-over test was made 4 days later in which those pairs which had previously drunk the metabisulphite solution drank the water, and *vice versa*. The whole procedure was repeated.

Experiments of Group II

Twelve rats, weighing 260·5 ± 1·6 g., were assigned to four groups for tests in which oral treatments were allocated to groups according to

a 4 × 4 Latin Square design and were administered by stomach tube at intervals of 3 days. On each occasion an hydrating volume of water equivalent to 2·5 per cent body weight was given after a fast of 18 hours and was followed, 1 hour later, by a volume of test solution equal to 5 per cent of body weight. Urine was collected from each group separately throughout the next 4 hours (see Experiments Group I). The test solutions used were NaCl, 0·74 per cent; $Na_2S_2O_3$, 5 H_2O, 4·51 per cent; Na_2SO_4,10 H_2O, 5·86 per cent; $Na_2S_2O_5$, 3·46 per cent; all w/v in water.

Experiments of Group III

These differed from those of Group II in that the rats used weighed 267·5 ± 2·4 g., and were assigned to six groups. Treatments were given by intraperitoneal injection, in volume equivalent to 3 per cent of body weight, and consisted of 0·9 per cent NaCl w/v; 13·4 ml. 1·46 per cent anhydrous Na_2SO_4, 8·0 ml. 1·95 per cent $Na_2S_2O_5$ and 8·0 ml. 2·54 per cent $Na_2S_2O_3$,5 H_2O all w/v in water, each diluted to 100 ml. with 0·9 per cent NaCl w/v, administered to each group in turn in a four-part cross over test according to a random block design.

TABLE I

THE EFFECT OF THE INGESTION OF 5 PER CENT WEIGHT OF A SOLUTION OF SODIUM METABISULPHITE ON THE URINARY EXCRETION OF SULPHUR BY RATS IN THE SUCCEEDING 4-HOUR PERIOD

	Water load	Metabisulphite load	t calc. n = 14
Urine volume, ml.	8·1 ± 0·68 (16)	9·3 ± 0·64 (16)	1·36
Inorganic sulphur as $SO_4^{=}$ in mg.	10·7 ± 2·13 (16)	33·2 ± 3·35 (16)	5·56
Reducing power as $SO_4^{=}$ in mg.	1·3 ± 0·27 (16)	2·0 ± 0·29 (16)	2·53
Organic sulphur as $SO_4^{=}$	0·3 ± 0·03 (16)	0·4 ± 0·12 (16)	0·70

The values shown are means for pairs of rats ± S.E. of the mean (number of observations).

Chemical Methods

The total and inorganic sulphur of urine was estimated as sulphate by the method of Bray, Humphris, Thorpe, White and Wood[2], modified only by substitution of a Gallenkamp photoelectric colorimeter N.3615 for a Spekker photoelectric absorptiometer, and replacement of the Chance Neutral Filter H 508 by one with a neutral density of 1·15. Since sulphite, thiosulphate and thiocyanate reduce iodine, they were collectively estimated in urine as an increase in the reduction of iodine above control levels. 1 ml. urine was slowly added to 5 ml. of 0·1N aqueous iodine. After 2 minutes 0·2 ml. HCl (B.P.) was added and the excess iodine was titrated with 0·01N thiosulphate using mucilage of starch as indicator. Blank tests were made throughout.

RESULTS

Changes Induced in the Urinary Excretion of Sulphur by the Oral Administration of Sodium Metabisulphite in Rats

Comparison was made of the sulphur-containing compounds excreted in the urine of rats in the 4-hour period immediately following the

administration by stomach tube of either water or a solution of sodium metabisulphite in Experiments of Group I. The results of the eight tests made are summarised in Table I. The sulphate equivalent of the mean oral dose of metabisulphite administered per rat was 44·4 mg. Of this, 53 per cent was excreted in the urine in the first 4 hours after its administration. Much the larger part of this sulphur was in inorganic form, and was sulphate. There was also a small but significant increase in the reducing power of the urine attributable to the presence of sulphite, thiosulphate or thiocyanate.

Comparison of the Changes Induced in the Urinary Excretion of Sulphur by the Oral Administration of Sulphate, Thiosulphate and Metabisulphate to Rats

Experiments of Group II were used to make comparison of the sulphur containing compounds excreted in the urine in the 4 hours immediately after the administration by stomach tube of solutions of sodium chloride,

TABLE II

THE URINARY EXCRETION OF WATER AND SULPHUR BY GROUPS OF THREE RATS IN THE 4 HOURS AFTER THE ORAL ADMINISTRATION OF AQUEOUS SOLUTIONS OF SODIUM CHLORIDE, SULPHATE, METABISULPHITE AND THIOSULPHATE IN A VOLUME EQUIVALENT TO 5 PER CENT WEIGHT

Salt in loading fluid	NaCl	Na_2SO_4	$Na_2S_2O_5$	$Na_2S_2O_3$
Water load excreted per cent	46·5 ± 9·32 (4)	6·9 ± 0·82 (4)	65·5 ± 4·40 (4)	45·2 ± 11·61 (4)
Inorganic sulphur as SO_4^{--} in mg.	8·9 ± 0·62 (4)	22·2 ± 3·94 (4)	197·4 ± 6·47 (4)	79·2 ± 11·11 (4)
Organic sulphur as SO_4^{--} in mg.	1·5 ± 0·21 (4)	3·4 ± 0·37 (4)	2·1 ± 1·61 (4)	4·0 ± 1·42 (4)
Sulphur load excreted per cent	—	7·1 ± 1·15 (4)	55·1 ± 6·24 (4)	23·1 ± 3·11 (4)

The values shown are means ± S.E. (number of groups).

metabisulphite, thiosulphate and sulphate in a volume equivalent to 5 per cent of body weight. Equivalent amounts of sodium were present in all these solutions and equal amounts of sulphur in the last three. The results of these experiments are summarised in Table II. The differences between the proportions of the sulphur load excreted in the 4 hours immediately after the ingestion of the three sulphur compounds were highly significant ($P = 0·01$ by t test). Approximately 55 per cent of the sulphur load of metabisulphite, 23 per cent that of thiosulphate and 7 per cent that of sulphate were eliminated. The sulphur load was excreted solely in inorganic form in each case.

Comparison of the Changes Induced in the Urinary Excretion of Sulphur by the Intraperitoneal Injection of Isotonic Solutions of metabisulphite, thiosulphate, and sulphate

Intraperitoneal injections of isotonic aqueous sodium metabisulphite in volume equivalent to 3 per cent of body weight caused an immediate restlessness rapidly followed, in each of 12 rats, by cyanosis, prostration and cardiovascular collapse. Half the animals died at this stage, within 35 minutes of the injection. The remainder had begun to recover at 40 minutes and appeared normal half an hour later. No such toxic

effects resulted from similar intraperitoneal injections of sodium thiosulphate or sulphate. The toxic symptoms of intraperitoneal metabisulphite were not seen when the dose was reduced to one-quarter by dilution of the original isotonic solution 1 part in 4 with isotonic sodium chloride. After trial, one-twelfth the LD50 concentration of metabisulphite was selected for use in comparison of the effect of intraperitoneal injections of isotonic solutions of metabisulphite, thiosulphate and sulphate in volume equivalent to 3 per cent of weight on the urinary excretion of sulphur. The results of one such experiment, typical of three, are shown in Table III. In these Group III experiments the total sulphur injected, expressed as mg. of sulphate per 100 g. weight, amounted to 4·81 when metabisulphite or thiosulphate, and 3·95 when sulphate solutions were used. Neither the form of excretion nor the extent of the excretion of this sulphur differed whether metabisulphite, thiosulphate or sulphate had been given by the intraperitoneal route. In the first 4 hours after injection

TABLE III

COMPARISON OF THE URINARY SULPHUR OUTPUTS IN THE 4 HOURS FOLLOWING THE INTRAPERITONEAL INJECTION OF SODIUM METABISULPHITE, THIOSULPHATE, SULPHATE AND CHLORIDE IN ISOTONIC SOLUTIONS, IN VOLUME EQUIVALENT TO 3 PER CENT WEIGHT

Loading solution	Sodium metabisulphite	Sodium thiosulphate	Sodium sulphate	Sodium chloride
Water load per cent	36·7 ± 5·23 (6)	44·3 ± 9·38 (6)	39·6 ± 4.42 (6)	37·2 ± 5·8 (6)
S.G.	1030 ± 3·43 (6)	1020 ± 3·59 (6)	1022 ± 2·94 (6)	—
Inorganic sulphur as SO_4'' in mg.	28·4 ± 1·56 (6)	27·6 ± 2·88 (6)	21·3 ± 6·73 (6)	6·50 ± 3·11 (6)
Total sulphur as SO_4'' in mg.	30·0 ± 1·35 (6)	29·2 ± 2·95 (6)	23·2 ± 1·84 (6)	7·70 ± 1·58 (6)
Reducing power as SO_4 in mg.	2·0 ± 0·90 (6)	2·4 ± 0·42 (6)	2·4 ± 0·33 (6)	—
Sulphur load excreted per cent	88·6 ± 5·29 (6)	84·9 ± 11·7 (6)	87·7 ± 14·16(6)	—

The values shown are means + S.E. (numbers of groups).

80 to 90 per cent of the administered sulphur was eliminated as inorganic sulphate; this was accompanied by very small amounts of iodine reducing substances. The diureses evoked by these sulphur containing solutions matched that caused by nearly equivalent sodium loading (Table III).

DISCUSSION

The rates of the urinary elimination of sulphur, provided in the forms of metabisulphite, thiosulphate or sulphate, were indistinguishable when these compounds were given by intraperitoneal injection (Table III) but differed when the oral route was substituted (Table II). The form in which the sulphur appeared in the urine was the same whether metabisulphite, thiosulphate or sulphate was administered. Therefore it may be inferred that the rates of absorption of these compounds from the gastrointestinal tract differ. Rats absorb metabisulphite more rapidly than thiosulphate, and sulphate is, as expected, absorbed only to a very limited extent.

Administered thiosulphate and metabisulphite are not excreted as such by rats because the ingestion or injection of these two iodine reducing substances caused no greater increase in the iodine reducing power of the urine during their renal excretion than did that of sulphate (Tables I and

III) and that increase was very small (Table I). All three compounds were excreted in the urine almost solely in the form of sulphate. It must therefore be concluded that the only important detoxification system for metabisulphite and thiosulphate in the rat is an oxidative one (Table III). Fridovich and Handler[3,4], have shown that the oxidation of sulphite by rat livers does take place. An intermediate thiosulphonate is formed and hydrolysed to yield a suphydryl compound and a sulphate. These reactions were shown to involve the reversible participation of a flavoprotein and hypoxanthine proved an obligatory co-factor at pH values of and in excess of 7·6.

The small amount of iodine reducing substance in normal rat urine may be, by analogy, thiosulphate (Table I) for small amounts of thiosulphate are usually present in the urine of man[5] and in that of cats and dogs[6]. This thiosulphate of normal urine probably arises from the sulphur containing amino acids of the diet[7]. Since rat liver converts mercaptopyruvate rapidly and quantitatively to thiosulphate in the presence of sulphite[8] the slight increase in the iodine reducing power of rat urine during the elimination of the metabisulphite should probably be attributed to the excretion of slightly more than the usual trace of thiosulphate.

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mucopolysaccharase activity which it has not so far been possible to remove.

3. The chondrosulphatase did not attack a number of other carbohydrate sulphate esters or esters which are known to be substrates for other types of sulphatases.

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Formation of Ammonia from Glutamine by Rat-brain Particles

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Weil-Malherbe (1953) showed that in washed or dialysed rat-brain homogenates the rate of production of ammonia from glutamine was accelerated in the presence of inosine triphosphate (ITP) or of both inosinic acid and creatine phosphate. He suggested that the amide group of glutamine was transferred to ITP or another inosine phosphate to give the corresponding adenine compound, possibly at the expense of high-energy phosphate, followed by its deamination.

Reinvestigation of the problem with an enzyme preparation from rat brain apparently confirmed Weil-Malherbe's findings. However, further experiments failed to support his interpretation of the results.

It was noted that phosphate and sulphate stimulated production of ammonia under the conditions of the test (cf. Greenstein & Leuthardt, 1948). The apparent stimulation of production of ammonia from glutamine by inosine phosphates can be satisfactorily explained by the presence of phosphate and sulphate. These would be present in Weil-Malherbe's preparations. The effect of various ions on the reaction glutamine → glutamic acid + NH_3 was measured.

MATERIALS AND METHODS

Barium creatine phosphate was prepared by the method of Ennor & Stocken (1948), and the barium salt of inosinic acid (IMP) according to Ostern (1932) (see Lehninger, 1951). The barium salt of ITP was prepared according to Kleinzeller (1942). The preparation of the sodium salts from the barium salts was achieved by decomposition with sodium sulphate. Barium creatine phosphate was dissolved in water, and barium inosine mono- and tri-phosphates were dissolved in chilled N-HCl; the barium was precipitated by the addition of a slight excess of 0·5 M-Na_2SO_4, the precipitate centrifuged off and the supernatant neutralized with NaOH (Eggleston

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& Hems, 1952). Adenosine 5′-monophosphate (AMP), as the free acid, was a commercial sample (Roche Products Ltd.). Glutamine, prepared from mangolds (Hughes, 1950), was given by Dr D. E. Hughes. All solutions were brought to pH 7·4 before use.

^{32}P-Labelled IMP (Eggleston, 1954) was supplied by Mr L. V. Eggleston. The material was present on a square of chromatogram paper and gave about 30000 counts/min. The ^{32}P-labelled IMP was eluted from the paper for 10 min. by the reaction mixture in the vessel before the experiment, and the paper removed before addition of the enzyme.

Determination of ammonia. The microdiffusion method of Conway (1947) was used, the transfer time being 1 hr. at room temperature. When not in use the units were stored in 0·01 N-HCl. After diffusion of the NH_3 into the acid in the centre well, the contents were transferred to a glass-stoppered test tube, the well being washed out three times with 0·5–0·6 ml. of NH_3-free water, distilled in the apparatus described by Parnas & Heller (1924). The units were placed in a manipulator box (J. W. Towers Ltd., Widnes, Lancs.) containing an open dish of concentrated H_2SO_4, to remove traces of NH_3 from the atmosphere, during the transfer of the contents of the centre well to the test tubes. This precaution was necessary because atmospheric NH_3 caused inconsistencies. The volume was made up to 3 ml. after the addition of 0·03 ml. of 0·0003 M-$MnSO_4$. The NH_3 was estimated colorimetrically by the method of Russell (1945); 1 ml. of the alkaline phenoxide reagent and 1 ml. of the hypochlorite solution were added, and the colour was developed by standing the tubes in a boiling-water bath for 5 min. and then rapidly cooling them. The optical density at 625 mμ. was determined with a Unicam spectrophotometer against water.

Determination of phosphate compounds. Inorganic phosphate, ITP, IMP and AMP were separated and estimated by the method of Krebs & Hems (1953). The two solvents used were *iso*propyl ether–90% (w/v) formic acid (60:40, v/v) (Hanes & Isherwood, 1949) and *iso*butyric acid–NH_3 (*iso*butyric acid–N-NH_3 soln.–0·1 M disodium ethylenediaminetetraacetate; 100:60:1·6, by vol.).

Creatine phosphate (CP) was separated from inorganic phosphate by propan-1-ol–NH_3 [propan-1-ol–NH_3 soln. (sp.gr. 0·880)–0·1 M disodium ethylenediaminetetraacetate–water; 240:120:0·8:60, by vol.] (Oliver, personal communication). The solutions containing the CP were made alkaline after the precipitated protein had been removed by centrifuging and before 'spotting' on the paper.

Separation of glutamine and glutamic acid. This was achieved by the *iso*butyric acid–NH_3 solvent after 16–18 hr. on a descending chromatogram at room temperature. The compounds were located by spraying with 0·4% ninhydrin in *n*-butanol saturated with water, and heating.

Enzyme preparation. The brain of a freshly killed rat was removed and cooled in crushed ice. It was then weighed and homogenized with 5 ml. of cold water in a glass homogenizer of the Potter–Elvehjem type. To the homogenate was added sufficient water to make a concentration of 1 g. wet wt. of tissue to 10 ml. of water.

The mixture was spun in a Lusteroid centrifuge tube at 600 *g* for 3 min. in the high-speed head of an International refrigerated centrifuge. The supernatant was then spun at 8500 *g* for 15 min., the temperature being kept at 0°. The clear supernatant fluid was discarded and the particulate material resuspended in the original volume of water and spun again at 8500 *g* for 15 min. The process was repeated and the resulting particles were suspended in water to serve as enzyme preparation. Dry weights of particle preparations were obtained at 105° after 4 hr. (5·2–5·8 mg./ml.).

Samples were incubated at 30°, in 20 ml. conical flasks fitted with rubber bungs and glass tubes for gassing. Each 4 ml. of reaction mixture contained 1·3 ml. of enzyme suspension and 0·4 ml. of 0·35 M-$NaHCO_3$ buffer, pH 7·4. The final concentration of IMP, AMP and CP was 0·004 M, of glutamine, 0·008 M. The gas space contained N_2+CO_2 (95:5).

Samples (1 ml.) were removed from the vessels at the desired times and deproteinized by adding to conical centrifuge tubes containing 0·1 ml. of 30% (w/v) trichloroacetic acid. The solutions were centrifuged to remove protein and the supernatant was stored at −14°.

The initial concentration of NH_3 in all reaction mixtures, 1–2 μg./ml., introduced by addition of the enzyme suspension and solutions, was subtracted from the values obtained after incubation.

RESULTS

Production of ammonia from glutamine in the presence of ITP or IMP and CP together

ITP or IMP and CP together stimulated formation of NH_3 on incubation with glutamine and the enzyme preparation (Table 1) (cf. Weil-Malherbe, 1953). No production of NH_3 occurred in the absence of glutamine, indicating that this substance is the primary source of the NH_3. The rates of hydrolysis of ITP, IMP and CP were measured and also the rates of production of NH_3 from these incubated mixtures. The results are shown in Figs. 1 and 2. Fig. 1 shows that the reaction rate decreased considerably when only a small proportion of the glutamine had been broken down.

Paper chromatography of samples of each reaction mixture showed that there was no perceptible formation of AMP or adenosine during the incubation. In those reaction mixtures where IMP was initially present, inorganic phosphate increased and a quantitatively corresponding amount of IMP

Table 1. *Production of ammonia from glutamine by washed brain particles*

Volume of reaction mixture was 4 ml., including 1·3 ml. of enzyme suspension. ITP, IMP and CP, 0·004 M; glutamine, 0·008 M; $NaHCO_3$, 0·035 M; pH 7·4. Temperature of incubation, 30°.

	NH_3 produced in 60 min. (μg./ml.)
ITP + glutamine	28·2
IMP + CP + glutamine	20·1
IMP + glutamine	13·8
CP + glutamine	10·6
Glutamine	2·9
IMP + CP	0·0
IMP	0·0
CP	0·0
Nil	0·0

disappeared. In chromatograms from the same incubation mixture a spot appeared which absorbed the 257 mμ. Hg line and ran a little behind the AMP area in the *iso*butyric acid–NH_3 solvent. This spot on elution gave an ultraviolet absorption curve with a peak at 248 mμ., similar to that of hypoxanthine or inosine. The material ran in the same position as an inosine marker spot, but was different from both xanthine and hypoxanthine markers. Similar chromatographic evidence was obtained when propan-1-ol–NH_3 was the developing solvent. These findings were taken to indicate that the products of hydrolysis of IMP were inosine and inorganic phosphate. Chromatograms also showed that complete hydrolysis of the IMP by the enzyme preparation occurred after incubation for 4 hr. (see also Fig. 2).

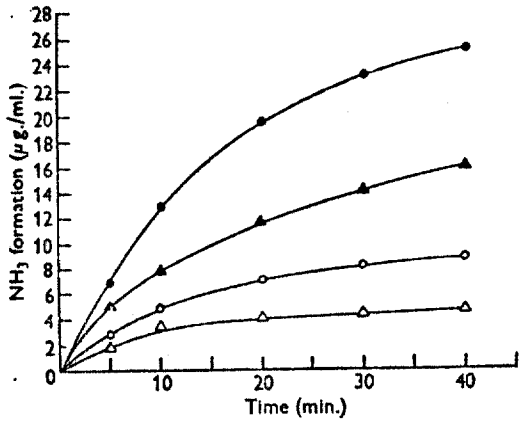

Fig. 1. Rate of formation of ammonia from glutamine. Each vessel contained 4 ml. of reaction mixture, including 1·3 ml. of enzyme suspension, 0·008 M glutamine and 0·035 M-$NaHCO_3$; pH 7·4. △, No addition; ○, 0·004 M CP; ▲, 0·004 M IMP; ●, 0·004 M ITP.

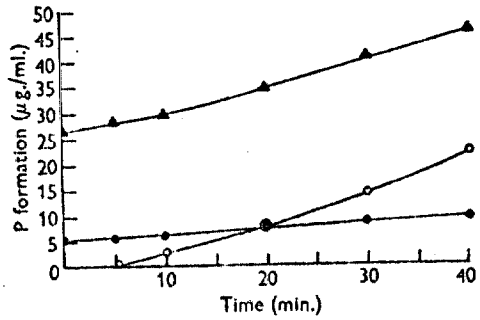

Fig. 2. Rate of formation of orthophosphate. Each vessel contained 4 ml. of reaction mixture, including 1·3 ml. of enzyme suspension, 0·008 M glutamine and 0·035 M-$NaHCO_3$; pH 7·4. No addition resulted in no phosphate present. ●, 0·004 M CP; ○, 0·004 M IMP; ▲, 0·004 M ITP.

Further chromatographic examination with the *iso*butyric acid–NH_3 solvent showed that as the incubation proceeded glutamine decreased in amount and glutamic acid appeared.

Possibility of AMP being an intermediate in the reaction

Effect on adenylic deaminase activity of washing the brain particles. If AMP is an intermediate in the reaction, the activity of the adenylic deaminase present in the enzyme preparation must be sufficiently high to produce NH_3 from AMP at a rate equal to, or greater than, the rate of production from glutamine and ITP (or IMP and CP). To measure the rate of this formation of NH_3, the crude homogenate, the once-washed particles and the twice-washed particles, were each incubated with AMP. (final concn. 0·004 M) under the test conditions. The results given in Table 2 show that the washing procedure reduced formation of NH_3 from AMP to negligible proportions. The rate and magnitude of production of NH_3 from a mixture of glutamine, IMP and CP, under the same conditions, were not affected.

Experiments with ^{32}P-labelled IMP

^{32}P Labelled IMP (0·004 M), CP (0·004 M), carrier AMP (0·004 M) and glutamine (0·008 M) were incubated with the suspension for 60 min. Samples of the reaction mixture at zero time and at 60 min. were run on chromatograms and the AMP areas cut out, wet-ashed and counted as described by Eggleston & Hems (1952). There was no increase with time of the radioactivity associated with the AMP area. This showed that IMP had not been aminated, although 16 μg. of NH_3/ml. had been formed.

If the hydrolysis of AMP was inhibited, any AMP formed from IMP and glutamine would accumulate and could be detected. Heppel & Hilmoe (1951) showed that low concentrations of NaF inhibited 5′-nucleotidase. Paper chromatography indicated that NaF at a final concentration

Table 2. *Effect of washing rat-brain particles on adenylic deaminase activity and production of ammonia from glutamine*

Volume of reaction mixture was 3 ml., including 1·3 ml. of enzyme suspension; AMP, IMP and CP, 0·004 M; $NaHCO_3$, 0·035 M; pH 7·4. Temperature of incubation, 30°.

Enzyme suspension	NH_3 (μg./ml.) produced in 60 min. from AMP	Glutamine + IMP + CP
Whole homogenate	12·1	20·6
Once-washed particles	3·0	19·8
Twice-washed particles	0·6	19·5

of 0·02 M inhibited the hydrolysis of AMP and IMP completely, under the test conditions. No increase in inorganic phosphate was detected even after incubation for 4 hr. The previous experiment was repeated with NaF added to a final concentration of 0·02 M. After incubation for 4 hr. there was no increase in radioactivity associated with the AMP area.

Effect of L-*glutamic acid on production of* NH_3 *from glutamine*

Krebs (1935) showed that production of NH_3 from glutamine by brain glutaminase was inhibited equally by addition of either L- or D-glutamic acid. This observation might explain the decline in the rate of production of NH_3, with time, described above. Table 3 shows that formation of NH_3 from glutamine in the presence of IMP, CP and the brain preparation is inhibited 97 % by L-glutamic acid.

Effect of phosphate and sulphate on production of NH_3 *from glutamine*

Greenstein & Leuthardt (1948) found that certain ions, especially phosphate and sulphate, activated the glutaminase of rat-brain homogenates.

The preparation of solutions of CP, IMP and ITP involved decomposition of the barium salts with an excess of sodium sulphate. Thus solutions of these substances always contain sulphate. During incubation IMP undergoes hydrolysis and yields phosphate. It was considered that the increased production of NH_3 during incubation of glutamine with IMP and CP might therefore be due to glutaminase activation by sulphate and phosphate.

To investigate this possibility, phosphate equivalent in amount to the IMP usually added was incubated with glutamine and the enzyme. A second vessel also contained sulphate (0·002 M final concn.). This amount was added to allow for sulphate that had been added for complete precipitation of barium from IMP and CP, and was about 17 % in excess. Table 4 shows the results of production of NH_3 in a typical experiment.

The figures show that both phosphate and sulphate stimulate production of NH_3 at low concentration and could explain the effect of addition of IMP and CP.

Effect of various ions on production of NH_3 *from glutamine*

Phosphate. A range of phosphate concentrations, 0·00125–0·08 M, was tested; the results in Fig. 3 show that formation of NH_3 increased with phosphate concentration and had not reached a maximum at 0·08 M.

The effect of glutamic acid on this stimulation was also tested. Two levels of phosphate concentration were used and incubated with glutamine and enzyme under the test conditions. At the same time a similar experiment was run where glutamic acid was also added at half of the concentration of the glutamine present. Fig. 4 shows that doubling the concentration of phosphate increased the initial rate of formation of NH_3, over the blank value, by a factor of 2·7. In the presence of the glutamic acid the rate of formation of NH_3 was much reduced. In the absence of added inorganic phosphate glutamic acid inhibited production of NH_3 completely.

Other ions. A variety of other ions was tested at a concentration of 0·013 M. The results are given in

Table 3. *Effect of glutamic acid on production of ammonia*

Volume of reaction mixture was 4 ml., including 1·3 ml. of enzyme suspension. IMP and CP, 0·004 M; glutamine, 0·008 M; L-glutamic acid, 0·004 M; $NaHCO_3$, 0·035 M; pH 7·4. Temperature of incubation, 30°.

	NH_3 produced in 60 min. (μg./ml.)
IMP + CP + glutamine	18·9
IMP + CP + glutamine + glutamic acid	0·6

Table 4. *Effect of phosphate and sulphate on production of ammonia from glutamine*

Volume of reaction mixture was 4 ml., including 1·3 ml. of enzyme suspension. IMP and CP, 0·004 M; Na_2HPO_4, 0·004 M; Na_2SO_4, 0·002 M; $NaHCO_3$, 0·035 M; pH 7·4. Temperature of incubation, 30°.

	NH_3 produced in 60 min. (μg./ml.)
IMP + CP + glutamine	15·9
CP + glutamine	10·6
PO_4^{3-} + SO_4^{2-} + glutamine	14·3
PO_4^{3-} + glutamine	9·4
Glutamine	2·9

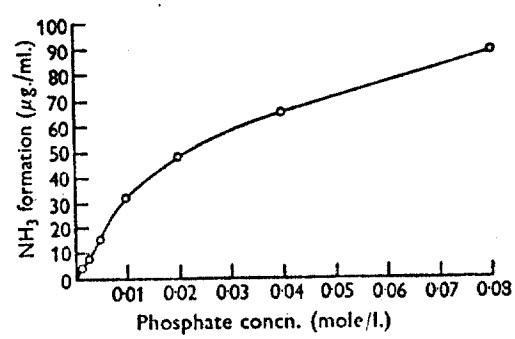

Fig. 3. Effect of increasing phosphate concentration on production of ammonia from glutamine. Each vessel contained 4 ml. of reaction mixture, including 1·3 ml. of enzyme suspension, 0·008 M glutamine, 0·035 M-$NaHCO_3$; pH 7·4. Phosphate concentrations varied as shown. Time of incubation, 60 min.

Table 5. Pyrophosphate caused an increase in production of NH_3 nearly double that caused by the same concentration of phosphate. The pyrophosphate was analysed by paper chromatography with formic acid–*iso*propyl ether as the developing solvent; no hydrolysis to inorganic phosphate occurred.

Soluble enzyme

Several attempts were made to prepare a soluble enzyme preparation. The suspension of rat-brain particles in either water or bicarbonate buffer (0·035 M) was shaken with ballotini beads in a Mickle vibrator for 15 min. in the cold room. The clear supernatant obtained on centrifuging was tested for enzyme activity under the conditions described above but was found inactive. The sediment was also inactive, as was the recombined supernatant and sediment.

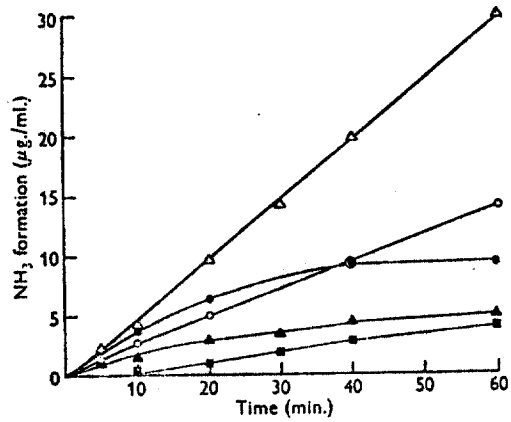

Fig. 4. Effect of L-glutamic acid on the rate of production of ammonia from glutamine in the presence of different concentrations of orthophosphate. Each vessel contained 4 ml. of reaction mixture, including 1·3 ml. of enzyme suspension, 0·008 M glutamine and 0·035 M-$NaHCO_3$; pH 7·4. ■, No additions; ○, 5 μmoles of phosphate/ml.; △, 10 μmoles of phosphate/ml.; 4 μmoles of L-glutamic acid resulted in no ammonia production; ▲, 4 μmoles of glutamic acid + 5 μmoles of phosphate/ml.; ●, 4 μmoles of glutamic acid + 10 μmoles of phosphate/ml.

Table 5. *Effect of various salts on glutaminase of rat brain*

Enzyme suspension, 1·3 ml. Substrate, glutamine. Concentration of salt, 0·013 M; HCO_3^-, 0·035 M; pH 7·4. Temperature of incubation, 30°.

Salt added	NH_3 formation (μg./ml./120 min.)
Nil	3·7
Na_2HPO_4	40·6
Na_2SO_4	23·6
$NaBO_2$	12·8
$Na_2S_2O_3$	16·3
KIO_3	5·5
$Na_4P_2O_7$	77·0
NaF	5·1
Na_2HAsO_4	14·7
$K_4Fe(CN)_6$	22·2
$K_3Fe(CN)_6$	1·1
Sodium ethylenediaminetetraacetate	8·3
$MgCl_2$	8·6

DISCUSSION

The incubation of IMP and CP or ITP (Table 1) with glutamine in the presence of washed rat-brain particles gives a greater production of ammonia than is found with glutamine alone. It has also been shown that the added glutamine is the primary source of the ammonia.

Washing the rat-brain particles resulted in a very marked decrease in ability to deaminate AMP (Table 2), whereas the rate and magnitude of production of ammonia from glutamine, CP and IMP under the same conditions was not affected. Further, when these twice-washed particles were incubated with glutamine, CP, ^{32}P-labelled IMP and carrier AMP there was no incorporation of radioactivity into the AMP, but production of ammonia was unaffected. In fact, no evidence for the amination of IMP to form AMP was found under conditions where IMP and CP stimulated the formation of ammonia from glutamine. This was taken to imply that the mechanism suggested by Weil-Malherbe (1953) for this stimulation, involving the intermediate amination of IMP, was not operative. Another explanation was sought.

Greenstein & Leuthardt (1948) had shown that rat-brain glutaminase was activated by very low concentrations of ions, especially phosphate and sulphate. Production of ammonia from glutamine under the test conditions could be explained satisfactorily in this manner.

The solution of ITP contained a little inorganic phosphate initially as can be seen from Fig. 2. It was found that both IMP and ITP were hydrolysed by the enzyme preparation to yield inorganic phosphate. The rate of hydrolysis of CP was very much slower.

The preparation of the solutions of the sodium salts of these compounds is achieved by precipitating the barium from solution with a slight excess of sodium sulphate, as a result of which these solutions always contain some sulphate.

The effect of inorganic phosphate and sulphate at low concentrations (0·004 M) was tested on the system deaminating glutamine; both were found to stimulate production of ammonia. The combined stimulation of these ions at this concentration was approximately equal to that of adding the same concentration of IMP and CP.

The effect of addition of glutamic acid to the enzyme system deaminating glutamine in the

presence of IMP and CP resulted in about 97% inhibition of production of ammonia. This result would be expected if the enzyme responsible were the brain glutaminase described by Krebs (1935). With inorganic phosphate (5 or 10 μmoles/ml.) replacing the IMP and CP in the system, addition of glutamic acid (4 μmoles/ml.) was accompanied by a very marked decrease in production of ammonia.

Greenstein & Leuthardt (1948) found that bicarbonate buffer did not activate glutaminase. Further evidence for this has been found in the low blank values. They measured the stimulatory effect of various ions on glutaminase, and some organic derivatives of phosphate and arsenate. The present study has included more multivalent ions, since Greenstein & Leuthardt (1948) had shown that univalent ions were poor stimulators of the reaction. It was concluded that, in general, as the ionic charge increased so did the effectiveness in stimulating production of ammonia (Table 5).

The effect obtained by Weil-Malherbe (1953) can be readily explained on the basis of an anion activation of brain glutaminase by phosphate formed by hydrolysis during the incubation, or by phosphate and sulphate added initially in these solutions.

SUMMARY

1. The formation of ammonia from glutamine by rat-brain particles in the presence of inosine triphosphate, or inosinic acid and creatine phosphate, is explained by the presence of low concentrations of phosphate and sulphate which activate the glutaminase present.

2. The reaction is 97% inhibited by the addition of L-glutamic acid.

3. The effect of various other ions on the reaction was studied. The results indicated that, as the ionic charge increases, so does the efficacy in stimulating production of ammonia.

4. The synthesis of adenosine 5′-monophosphate from inosinic acid does not occur in this system under these conditions.

I wish to thank Professor H. A. Krebs, F.R.S., and Dr G. Weber for their interest and advice in this work. I am also indebted to the Agricultural Research Council for the award of a grant for training in research methods.

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The Purification and Properties of Arginine Phosphokinase

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(*Received* 16 *May* 1956)

Phosphocreatine and phosphoarginine have been known for many years, but in spite of their importance in the process of muscular contraction little attention has been paid to the enzymes involved in their metabolism. The first detailed studies of creatine phosphokinase were made by Noda, Kuby & Lardy (1954), Kuby, Noda & Lardy (1954) and Embor & Rosenberg (1954). The enzyme arginine phosphokinase was first found to be present in crude extracts of crab muscle by Lohmann (1935), who showed that it was responsible for catalysing the equilibrium as expressed by the reaction phosphoarginine + adenosine diphosphate ⇌ arginine + adenosine triphosphate. The presence of the enzyme in extracts of octopus muscle and the jaw muscles of the echinoid *Sphaerechinus granularis* was demonstrated by Lohmann (1936) and by Baldwin & Needham (1937) respectively. Soroni, Dvornikova

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THE EXCRETION OF INTRAVENOUSLY INJECTED SODIUM THIOSULPHATE DURING UNCOMPLICATED HUMAN PREGNANCY.

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INTRODUCTION.

NINE years ago Nyiri[1] introduced the use of sodium thiosulphate as a means of estimating the renal function. In brief, the principle of the test, which found a good deal of favour, is as follows. In health, after the intravenous injection of sodium thiosulphate, a certain proportion is excreted unchanged by the kidneys, the remainder most probably being oxidized in the body to sodium sulphate.[2] In the presence of an impaired renal function the percentage excretion becomes impaired to an extent varying with the severity of the lesion. During an experimental study of the test by one of us,[3] it was observed, so far as we can ascertain for the first time, that in the pregnant animal there also occurred an impairment of excretion, to account for which no discoverable element of renal dysfunction was present. A further and more detailed study of this aspect of the test was subsequently carried out in the pregnant bitch[4] and there was found a constant depression of the thiosulphate excretion, commencing about three weeks after conception and lasting till term, after which in uncomplicated

pregnancy it quickly returned to normal. In view of these findings it was decided to carry out a series of estimations on the human subject, with the object of discovering whether a similar depression would be encountered, and, if so, to ascertain its extent, significance and possible clinical application.

Technique.

Type of Patients Studied.

The investigation was carried out on a series of pregnant patients, su[illegible] admitted to the Royal Prince Alfred Hospital some time before the expected date of confinement. Both before and after confinement estimations of the thiosulphate excretion were carried out as regularly as was practicable. A series of non-pregnant patients from the same ward was also studied for the purpose of establishing controls. All of these patients were under treatment according to the indications present.

Preparation of Thiosulphate Solution.

As in previous work more than one dosage was employed. In order to obtain quickly a clear and sterile solution of sodium thiosulphate the following procedure was adopted.

One hundred cubic centimetres of sterile distilled water were boiled for a few minutes in a sterile flask. The heating was then stopped and either ten, twenty or thirty grammes of crystalline sodium thiosulphate B.P. ($Na_2S_2O_3.5H_2O$) were added, and boiling was resumed for a few more minutes. The bottle was then stoppered with a piece of sterile cotton wool. Cloudiness of the solution occasionally necessitated filtration, after which it was once more brought to the boil. After cooling, the solution was ready for injection. A fresh solution was always prepared immediately before each batch of injections.

On account of the increase in volume due to the addition of the salt, ten cubic centimetres of the above solutions respectively contain approximately 0·95 gramme, 1·8 grammes and 2·6 grammes of crystalline sodium thiosulphate. The last mentioned, of which most use was made, represents a solution of about twice normal strength.

Procedure and Chemical Technique.

Ten cubic centimetres of one of the solutions of sodium thiosulphate prepared as described were injected intravenously on the occasion of each test. The injection must be entirely intravenous, since, besides vitiating the results, any escape of solution into the subcutaneous tissues was found to cause a good deal of pain, not accompanied, however, by any subsequent objective signs. After a proper intravenous injection no ill effects were ever observed.

Each patient was given about two hundred cubic centimetres of water at the time of injection, one and two hours after which specimens of urine were collected for thiosulphate estimation. Catheterization was not carried out, the patients merely being instructed to empty the bladder completely.

For the purpose of estimating the sodium thiosulphate output, approximately a teaspoonful of Fuller's earth, or preferably Lloyd's reagent (a proprietary preparation of purified and concentrated Fuller's earth), was added to the urine, diluted with water to a convenient volume, usually five hundred cubic centimetres. The mixture was then shaken and filtered, and a known fraction of the filtrate (ten to fifty cubic centimetres) acidified with normal sulphuric acid and titrated against a one-hundredth normal iodine solution, starch being used as the indicator. After each series of injections ten cubic centimetres of the thiosulphate solution left over were similarly diluted and titrated.

The number of cubic centimetres of one-hundredth normal iodine solution decolorized by the thiosulphate content of the urine, divided by the number decolorized by the amount of thiosulphate injected and multiplied by 100, gives the percentage of the injected thiosulphate which has been excreted. That is:

$$X = \frac{\text{C.cm. N/100 I required by urine} \times 100}{\text{C.cm. N/100 I required by } Na_2S_2O_3 \text{ injected.}}$$

On account of the iodine-combining substances normally present in the urine, the figures so obtained do not quite represent the true percentage excretion of sodium thiosulphate, and minor corrections should preferably be made by small deductions. Assuming that the iodine-combining substances normally present remain stationary, their significance will naturally diminish in proportion as the dosage of sodium thiosulphate is increased. We made deductions from our own figures for the two-hours' excretion as follows:

After 0·95 gramme = 2·2%
" 1·8 grammes = 1·2%
" 2·6 grammes = 0·8%

These figures represent the mean value obtained by titrating a series of normal two-hour excretions of urine against a one-hundredth normal iodine solution, expressed as percentages of the values obtained by similarly titrating the standard thiosulphate solutions. The total two-hours' percentage excretion, arrived at by the above means, is expressed throughout this paper as the nearest whole number.

Experimental Findings.

The Excretion of Sodium Thiosulphate in Non-Pregnant Patients.

It has been previously observed by one of us that in dogs[4] the amount of thiosulphate injected has some influence on the percentage excreted. This was also observed by Holboll[5] in the human subject after the administration to five normal individuals of amounts of thiosulphate representing from 0·78 gramme to 6·28 grammes of the crystalline salt. With other workers the dosages used varied little. Table I summarizes the results obtained by these various workers and by ourselves. To facilitate comparison with our own figures, their dosages are uniformly expressed as grammes of crystalline sodium thiosulphate ($Na_2S_2O_3.5H_2O$).

In a large series of one hundred and sixty-two normal and non-renal subjects, Nyiri[1], after the injection of 1·57 grammes, found a two-hour excretion of sodium thiosulphate ranging from 20% to 43%.

TABLE I.

Summary of Results obtained up to the present by Different Authors following Intravenous Injection of Sodium Thiosulphate.

Author.	Number of Cases.	Grammes of $Na_2S_2O_3.5H_2O$ Injected.	Range of 2-hour Excretion (%).	Mean 2-hour Excretion (%).
Nyiri[1]	162	1·57	20–43	
Iverson and Fasting-Hansen[6]	24	1·57	20–41	
Eskelund[7]	10	1·57	23·3–34·8	
Humbert and Finck[8] ..	?	1·24	23·4–30·7	25·9
Silberstein[9]	?	1·24		29·9
Holboll[5]	5 5 6 4 5	0·78 1·57 3·14 4·71 6·28	16·0–21·3 25·4–29·8 33·4–46·3 40·4–57·3 48·7–60·8	18·9 27·6 37·5 45·3 57·7
Bolliger and Earlam	14 13 12	0·95 1·82 2·58	19·0–31·2 25·0–40·5 25·1–45·9	23·7 30·6 31·9

The findings in five communications from other workers (Iverson and Fasting-Hansen,[6] Eskelund,[7] Humbert and Finck,[8] Silberstein[9] and Holboll[5]) who injected either the same amount or somewhat less, namely, 1·24 grammes, fall within the same limits. Authors dealing only with healthy individuals (Humbert and Finck, Silberstein, Eskelund and Holboll) obtained a considerably narrower range of values. Holboll demonstrated on his small series of normal individuals that the percentage of thiosulphate excreted increases with increasing amounts injected. Our group of patients also demonstrates this principle. It will also be observed that although the amounts injected by us differ from those used by other workers, our findings are within limits in accord with those obtained by them on normal and non-renal cases. Similarly we found also that the bulk of the excretion always took place during the first hour.

In order to evaluate our subsequent findings in the presence of pregnancy, we laid down, in accordance with the observations made by ourselves and others, the following figures as the lowest normal values:

After injection of 0·95 gramme, lowest normal value = 19%
" " " 1·8 grammes, " " " = 24%
" " " 2·6 grammes, " " " = 26%

At this stage it should be mentioned that out of forty-six non-pregnant patients suffering from venereal disease who were examined, seven showed an excretion which was below the established normal, some even showing less than half of the minimum normal excretion. These patients were all suffering from various complications of their original venereal infection, and do not fall within the scope of this paper. They will be discussed elsewhere.

The Excretion of Sodium Thiosulphate in Uncomplicated Pregnancy.

We examined the thiosulphate excretion in thirty-seven pregnant patients both before and after delivery of a full-term child. In nine of these we were able to make only one determination, and two determinations only in five. But in nine cases observations were made on from six to thirteen occasions. These cases necessarily supply the body of our observations, while those where only a few determinations were made amplify the points of interest. According to the results we obtained, we subdivided our cases into the following two groups:

(I) Pregnancies with no or only short-lived depression in the thiosulphate excretion.
(II) Pregnancies with marked and prolonged depression in the thiosulphate excretion.

(I) *Pregnancies with No or only Short-lived Depression in the Thiosulphate Excretion.*

Table II represents the sixteen cases in which at least two estimations were made, and which we classed into this group. The findings have been arranged according to the *ante partum* and *post partum* weeks in which they were obtained. The child in every case was full-term, and for the sake of simplicity labour has been listed in the table as occurring at the thirty-ninth week. An asterisk (*) opposite any figure denotes an injection of 0·95 gramme, a circle (°) one of 1·8 grammes, while all other figures were obtained after the injection of 2·6 grammes.

The findings before the twenty-third week are not incorporated in the table, and will be briefly mentioned. In Case XIV six determinations were made between the twelfth and twenty-third weeks of pregnancy. The injections were of 2·6 grammes and resulted in the excretion, in chronological order, of 38%, 34%, 39%, 36%, 24% and 23%, the last two figures being slightly subnormal. In Case IX, 27% was excreted in the twenty-first week, following the injection of 0·95 gramme. In three other cases, not appearing in Table II, high normal values were obtained between the tenth and twenty-first weeks.

But of the sixty-two tests listed in Table II, sixteen showed subnormal values in the *post partum*, as well as in the *ante partum* period. None of these subnormal values were found to occur from the twenty-third to the thirty-first week. But from the thirty-second to the thirty-fourth week, in eight cases five instances of definitely subnormal values were found. For example, Cases X and XIII showed a depression of 10% below the minimum normal value in the thirty-fourth and thirty-third weeks respectively. In three cases (III, VII and VIII) in which determinations were made during this period there were no subnormal values; in two of these only one estimation was carried out. During the next three weeks there is a tendency towards normal excretion. Depressed values are met with (Cases II, IV, XI and XV), but the depressions are distinctly less outspoken than those previously occurring.

In five instances determinations were made less than a week before confinement. These results are listed between the thirty-eighth week and confinement,

Table II.

Excretions of Sodium Thiosulphate during Pregnancy and the Puerperium in Patients of Group I.

Case.	No.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	1	2	3	4	
K.M.	1	27*	37*	23*		34*																	
W.H.	2														16*					19*			
H.H.	3												25*				24*	19*[1]					
G.E.	4															18*			29*				
W.D.	5															37*						15*	
H.F.	6								23*		10*		28*						25*	15*			22* (9/10)
K.K.	7	29*											31*	30*	27*	30*	30*						
W.M.	8							27*		31*	35	28	32	27		31		22[1]					
S.B.	9						34*	34*	30														
P.S.	10			34*									16	29		25		23[1]					
B.A.	11												21		35	24	20	32[1]			35		
P.V.	12														33							30	
R.J.	13										30	16	30		30				31				
M.A.	14													24	26								
C.A.	15										19		29			22	30						
K.B.	16																33	36[1]			14	33	

Table III.

Excretions of Sodium Thiosulphate during Pregnancy and the Puerperium in Patients of Group II.

Case.	No.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	1	2	3	4	
E.O'N.	17						20*			14*						11*							
N.N.	18													12*		11*				22*			
I.A.	19				23*								15*		12*			9*[1]	12*[1]	8*	7*		13(11/12) 27(10/10)
B.F.	20						5*	12*				6*	8*	11*		12*	9*	16*[1]	19*[1]	24*	26*	14*	27*
S.L.	21								14*		25*	13*	22*	22*	16*	18*		18*[1]					
B.V.	22						21		20	26	21	12	17	16		16		10[1]		9		8	8(9/10)
S.E.	23				31	27	9	7	22	20		14	6	13		8			10	8	12		

The double vertical line indicates the time of confinement.

the figure accompanying each result indicating the number of days before confinement at which the test was carried out. The figures obtained in this critical period do not show a clear-cut tendency. Cases VIII and X, six and two days respectively before delivery, demonstrate slightly subnormal values, while others (Cases XI and XVI) show somewhat elevated normal results. In Case XII a test was performed one day before delivery. Compared with previous findings the thiosulphate excretion was depressed, but still within normal limits.

In this group the number of determinations during the puerperium was small, eleven determinations in eight cases. The tests were performed from one week to four months after confinement, but mostly in the first four weeks. Eight of the findings were within normal limits, the remaining three (Cases V, VI and XVI) very definitely subnormal.

As in the normal patient, the bulk of the thiosulphate excretion in this group occurred in the first hour, during which the excretion in every case quite overshadowed that of the second hour, which ranged from nil to 9%. The great majority of the second hour excretions were between 1% and 2%.

To facilitate comparison of the patients of this group with those of Group II, to be discussed below, the thiosulphate excretion in Case VIII is depicted graphically in Chart I.

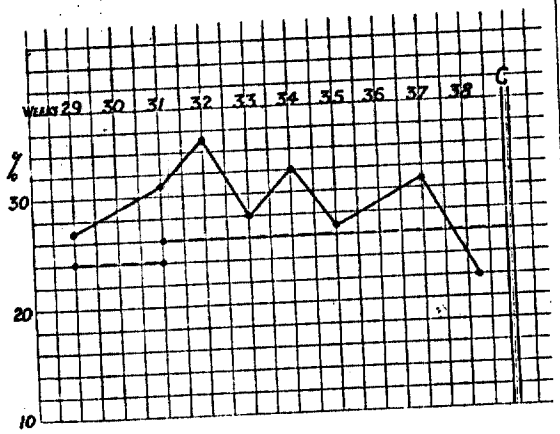

CHART I.
W.M., Case VIII. Thiosulphate excretion from twenty-ninth week of pregnancy till just before confinement. Dotted line indicates minimum normal excretion. C = confinement.

This patient, a *primipara* aged nineteen, was suffering from secondary syphilis, for which she was receiving treatment with "Novarsenobenzol" and "Mutbanol." Pregnancy and labour were uneventful, and a healthy full-term child was born and survived.

Apart from the presence of venereal disease, all other pregnancies in this group were similarly uneventful.

(II) *Pregnancies with Marked and Prolonged Depression in the Thiosulphate Excretion.*

The second group is composed of seven cases, which are listed in Table III, where the findings are tabulated in a similar manner to those in Table II. In the large majority of these cases fairly numerous estimations were carried out from the twenty-sixth week of pregnancy till various stages after confinement, ranging from one week to fifteen months.

All these patients, with the single exception of the patient in Case XXI, show marked and persistent depression from the twenty-eighth week of pregnancy till confinement. Moreover, in all those patients in whom subsequent estimations were carried out, with one exception again, namely, Case XVIII, the depression persists for varying periods after delivery. Consequently, the percentage of subnormal values in Group II is much higher than in Group I; out of forty-one tests carried out from the twenty-eighth week to delivery, only two gave normal results. Only three tests were carried out before the twenty-eighth week, in Cases XIX and XXIII. These appear in the table, and returned normal values.

As already observed in Group I, some of the patients of Group II tend to have a specially lowered *ante partum* excretion in the thirty-second to thirty-fourth week period (Cases XX, XXII and XXIII). In other respects the different cases show appreciable individual variations, and some will be described in detail.

Case XXIII was that of a *primipara*, aged seventeen, suffering from secondary syphilis and gonorrhœa, for which she received treatment from the seventh month onwards, receiving injections of "Novarsenobenzol" and "Mutbanol." Pregnancy, labour and the puerperium were uneventful, and her offspring was a healthy male. The behaviour of the thiosulphate excretion is depicted in Chart II. She received injections of 2·6 grammes. From a normal value at the twenty-seventh week of pregnancy the thiosulphate excretion rapidly fell in the course of a week to the low level of 9%, and to 7% a week later. After a temporary recovery, which, however, fell short of normality, a minimum excretion of only 6% was reached at the thirty-fourth week. From this period till the third week after confinement, when the last estimation was made, the excretion fluctuated, but showed some slight tendency towards improvement, but the last test yielded only 13%, exactly half the value of the minimum normal excretion.

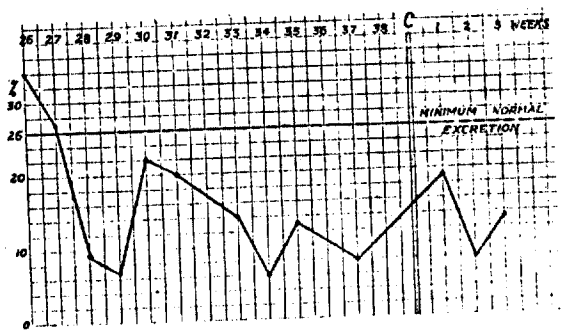

CHART II.
S.E., Case XXIII. Thiosulphate excretion from twenty-sixth week of pregnancy till third week after confinement.

The patient in Case XXII, depicted in Chart III, also received injections of 2·6 grammes. Both mother, aged twenty-two, and child had congenital syphilis, the child dying about thirty hours after birth from intracranial injuries received during birth. Pregnancy and puerperium were uneventful. The mother had previously been under antisyphilitic treatment for a period of two years. At the twenty-eighth week of pregnancy the first estimation of the thiosulphate excretion returned a sub-

injection of the solution and in the collection of the specimens of urine. The injections which resulted in the present series of figures were all purely intravenous. In the occasional case where some solution escaped into the subcutaneous tissues, the injection was stopped and the performance of the test deferred. As regards the second source of error, that of incomplete emptying of the bladder, the thiosulphate remaining in the bladder after collection of the first hour's specimen would certainly result in a comparatively high apparent second hour's output, probably of about equal value to that of the first hour. This, however, did not take place, since even with the lowest excretions it was unusual to have a second hour's excretion greater than one-fourth of that of the first hour, and in only one instance did they approximate, in Case VI at the thirty-second week, which estimation alone we therefore regard as possibly not reliable.

Type of Patient Used in the Present Investigation.

At the Royal Prince Alfred Hospital the only group of pregnant patients available for study are those suffering from venereal disease. They are mostly admitted about two months before the expected date of confinement, after which they remain in hospital for another month. To these patients, by the courtesy of the staff, we were given free access.

It may be presumed that the changes observed in the thiosulphate excretion are primarily due in some way to the venereal disease. That this is not the case, however, is suggested by the following facts. A large group of non-pregnant venereal patients completely reduplicating any of the clinical syndromes seen in the pregnant series returned results within normal limits. A second much smaller group did return subnormal values—these were all suffering from complications of their original infection, and, as already mentioned, will be dealt with on another occasion. Furthermore, the only common factor in the present study to account for the depression was the presence of pregnancy. Again, in a group of non-venereal patients being studied elsewhere in connexion with toxæmias of pregnancy, similar depressions have been noted from time to time. Lastly, a series of pregnant bitches previously reported on showed consistent depressions. For these reasons we consider the depressions reported in the present series of cases as being primarily due to pregnancy.

Experimental Results.

We arbitrarily divided our cases of pregnancy into two groups according to the magnitude and duration in the depression of the thiosulphate excretion. The division is not hard and fast as all grades of depression occur from none at all, in Case VII, to prolonged and extreme depression. In Group I, diminished excretions occurred from time to time during the latter months of pregnancy, and transient low values were again met with in the puerperium. But in general, so far as can be made out from the few cases in which estimations were done, the excretion of sodium thiosulphate shows little tendency to persist at a subnormal level after the puerperium.

Cases XVII and XXI of Group II show about the least depression in this group during pregnancy, but they are outspoken enough to justify their inclusion in the group. Similar but more outspoken depressions are found in the other cases. During the puerperium there are distinct differences between the cases of Group II. For instance, while the patient in Case XX, who had shown well-marked and prolonged depression during pregnancy, quickly returned to normal after confinement, the patient in Case XXII showed a definite further impairment of thiosulphate excretion, which reached its lowest level three weeks after confinement, and the patient in Case XIX, after a slight immediate improvement after confinement, also reached a lower level than previously and was still subnormal as long as twelve months later.

The reasons for the depression of the thiosulphate excretion, for the varying degrees of depression among different patients, and for the widely varying periods of time necessary for the complete reversion to normal of the thiosulphate excretion, are alike obscure. In this respect the phenomenon reminds us of the benign glycosuria of pregnancy.

In none of the cases of either of the two groups was there any evidence of renal insufficiency or toxæmia, the pregnancy in every instance being quite uneventful and the venereal disease being well controlled by appropriate treatment. We consider that a temporary depression of the thiosulphate excretion in the last three months of pregnancy is frequently a physiological process with no pathological significance. At this stage we are inclined to regard patients with marked and prolonged depression of the thiosulphate excretion as exhibiting to a more marked degree than occurs in the average case, evidence of the general metabolic disturbances normally associated with pregnancy. These disturbances are, of course, evident in other ways. For example, a subnormal blood urea content is a normal finding during pregnancy, and Gardner and Gainsborough[10] have shown that there occurs a marked disturbance of the cholesterol metabolism. Again it may be quite rational to assume that thiosulphate in view of its recognized detoxicating capabilities may partly be expended in carrying out this function. Some connexion between the cause of the onset of labour and the depression of thiosulphate excretion was looked for, but the results in our series do not support it. In some cases (III, VIII, XIX and XXII) there was a specially marked depression a few days before delivery, but in other instances where tests were done during the week preceding this event the results either remained stationary or showed some improvement as compared with the findings in previous weeks.

With regard to the findings after confinement, it may be pointed out that in Case XIX, nearly a year after lactation had ceased and menstruation had become re-established, there was still a distinctly subnormal output. Also the patient in Case XXII, whose infant died very soon after birth and who resumed menstruation almost immediately, continued to show a definitely subnormal thiosulphate excretion, while the patient in Case XX, who was nursing her baby,

after a marked depression during pregnancy, showed after confinement only some fluctuation similar to that seen in Group I, that is, a single depressed value at the fourth week after a previous return to normal. Again, the patient in Case XXIII, who also nursed her baby, showed as far as examined persistently low values, with a slight tendency for improvement one week after confinement. Again we shall confine ourselves to stating the findings in the hope that subsequent experiments may throw some light on the ætiology of this behaviour of the thiosulphate excretion after confinement.

It may be mentioned here that we were unable to obtain any assistance from the literature in explaining these phenomena. As far as we can determine, only one worker, Clauser,[11] has reported on the thiosulphate test during pregnancy. This original article is not obtainable, but it is reported by Stander[12] that:

> He concludes that a definite decrease in renal function, which in normal pregnancy is very slight, becomes marked during labour; and that during the puerperium the kidney usually regains its function in less than five days. This test, he claims, is of value in nephritis complicating pregnancy.

Our findings apparently differ markedly from those obtained by Clauser, and moreover, unlike him, we do not regard the depressed thiosulphate output during pregnancy as necessarily being evidence of a disordered renal function. Neither do we consider that it can be explained in terms of hepatic insufficiency. At this stage of the investigation we are forced simply to draw attention to the fact that in most cases of pregnancy the thiosulphate metabolism is disturbed to a varying degree.

Comparison with Findings in the Laboratory Animal.

Pregnancy in the bitch differs from human pregnancy in several respects. Its duration is about one-fourth of that in the human being, the pregnancy is usually multiple, and the rate at which fœtal tissue is laid down is relatively very much greater than that which obtains in the human subject. These considerations may help to explain the fact that while the thiosulphate depression during pregnancy in the bitch was consistently found (in a small series) and always well-marked, that which occurs in human pregnancy is less consistent and frequently of minor degree.

In view of these differences, the possibility of a diagnostic application of the test, which was suggested by the experimental findings, has not been realized, since a markedly low thiosulphate excretion, which we still consider in the absence of renal deficiency to be strongly suggestive of pregnancy, is not encountered till the stage when pregnancy can be more simply and more positively diagnosed by clinical examination.

As already mentioned, human pregnancy complicated by toxæmia or nephritis will be dealt with elsewhere.

Summary.

1. The excretion of intravenously injected sodium thiosulphate solutions containing approximately 0·95 gramme, 1·8 grammes and 2·6 grammes of the crystalline salt has been studied in a series of non-pregnant and pregnant patients suffering from venereal disease.

2. With the exception of a small group of complicated cases the thiosulphate excretion of the non-pregnant group of patients fell within normal limits. The percentage excretion was found to increase with increasing amounts of sodium thiosulphate injected.

3. In almost all cases the percentage thiosulphate excretion was found to be depressed to a varying extent below the normal value during the latter part of pregnancy and also in many cases during and after the puerperium, the patients falling into two main groups according to the extent of the depression.

4. In the first group the depression was not extensive and was of short duration, while in the second group the depression was marked and prolonged, persisting at times up till twelve months after confinement.

5. None of the patients studied showed any clinical evidence either of renal insufficiency or of toxæmia of pregnancy.

Acknowledgements.

We wish to express our sincere thanks to Dr. Charles A. Frew, Honorary Assistant Physician for the treatment of Female Venereal Diseases, Royal Prince Alfred Hospital, for affording us free access to the patients under his care, and to Dr. F. N. Chenall and Dr. C. H. McDermott, who during the period of this investigation successively occupied the position of Venereal Diseases Registrar, and the hospital staff, for their sympathetic co-operation.

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THE EXCRETION OF INTRAVENOUSLY INJECTED SODIUM THIOSULPHATE DURING PREGNANCY: AN EXPERIMENTAL STUDY IN THE DOG.

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IN a study on the thiosulphate renal function test in three pregnant but otherwise normal dogs it was found that after the injection of one gramme of sodium thiosulphate the two-hour thiosulphate excretion as compared with that of normal dogs was depressed.[1]

In the meantime further studies on pregnant dogs were made confirming the previous observation. In addition to the usual amount of thiosulphate given, namely, one gramme, injections of 0·2 gramme, 0·5 gramme and 2·0 grammes were administered. The following points were then investigated: (*a*) The percentage of excretion for the varying amounts was established on normal non-pregnant animals, (*b*) varying amounts of thiosulphate were injected into pregnant dogs, both normal and nephritic and the thiosulphate output determined through the entire period of normal gestation or a part thereof, (*c*) the thiosulphate output was studied through the course of and subsequent to pregnancy terminating in abortion with or without eclamptic symptoms.

Technique.

Solutions containing 2%, 5%, 10% and 20% sodium thiosulphate (British Pharmacopœia) were made up. A female dog was then catheterized and ten cubic centimetres of one of the thiosulphate solutions were injected intravenously. Two hours after injection the animal was catheterized again and the bladder was irrigated in order to obtain the complete output. The two-hour sample of urine, including the wash-out, was then made up to a known volume and examined for its thiosulphate content by titration against a $^{n}/_{100}$ iodine solution. The details of the procedure are the same as described in a previous paper,[1] with the exception that instead of Lloyd's reagent, Fuller's earth was used, Lloyd's reagent not being available at the time.

In brief, the thiosulphate determination was as follows: A few grammes of Fuller's earth were added to the urine diluted to a convenient volume. Then the mixture was shaken, filtered and acidified and a known fraction of the filtrate was titrated against $^{n}/_{100}$ iodine solution.

The dogs used were female mongrels of all kinds, sizes and ages. Some of them came pregnant to the laboratory, others conceived while kept together with males. The period of normal gestation in the dog, the actual date of conception usually being unknown, was considered as nine weeks (sixty-three days).

The renal insufficiency in the cases under discussion was either spontaneous or was produced by deep X ray irradiation[2] of one kidney followed by opposite nephrectomy.

Percentage Excretion of Sodium Thiosulphate After Administration of Varying Amounts.

Table I gives the results of the two-hour excretion of sodium thiosulphate after the injection of 0·2, 0·5, 1·0 and 2·0 grammes of the salt. As far as could be made out, the animals used were normal animals and not pregnant.

TABLE I.

Percentage excretion of sodium thiosulphate after injection of 0·2, 0·5, 1·0 and 2·0 grammes of sodium thiosulphate. The figures in parentheses were obtained from animals on heat.

Bitch No.	Body Weight Kilo-grams.	Injected 0·2 gramme Excretion %	Injected 0·5 gramme Excretion %	Injected 1·0 gramme Excretion %	Injected 2·0 gramme Excretion %
1	13·0	44	50, 59, 55	61	66, 68
2	7·0	34, 42, 32	52	(74), 51, 52, 51	
3	6·0		48, 42, 43	(66)	62
4	14·0	(93), 54, 70, 56		58, 62, 59, 56	
5	14·0	35		56, 53, 48, 45	62
6	6·5	(71), 37, 27	46	60	68
7	8·0	(50), 33, 34, 42	43, 45, 45, 44	59, 56	62, 62
8	17·0	48		54	
9	5·5	36, 45, 40	55, 57, 59	64	
10	6·5	(46), 31			
11	7·5	39		64	
12	7·5	36, 39		57, 53	

After injection of two grammes of sodium thiosulphate the percentage of excretion varied between 61 and 68. The administration of one gramme brought a recovery in the urine of from 51% to 64%, while the injection of half a gramme gave a yield of from 42% to 59%. Much more irregular results were obtained after the injection of 0·2 gramme. The bulk of the findings lay between 31% and 45%. In the case of dog 4 all the findings after the injection of 0·2 gramme are above 50%, while the excretion after the injection of one gramme was found to be within normal limits. The occasional high figures (shown in parentheses in Table I) refer to animals which were on heat on arrival at the laboratory.

The Excretion of Sodium Thiosulphate in Normal and Nephritic Dogs During Normal Gestation.

The bitches in this group (six normals, two nephritics) were considered as having a normal pregnancy because they bore apparently full term puppies which were in some instances stillborn.

Chart I demonstrates the findings in a small female fox terrier, body weight six kilograms, which received for the purpose of measuring the two-hour excretion injections of 0·5 gramme of sodium thiosulphate at various times from the period of heat, through gestation, till after the puerperium. Further, for comparison, a few tests were done with 2·0 grammes and 0·2 gramme of sodium thiosulphate. The results with one gramme of sodium thiosulphate obtained in another animal were also added. Twenty-nine days after conception, calculated from the date of delivery, the thiosulphate output after the injection of 0·5 gramme was found to be depressed below normal level, namely 39%. Forty-eight days after conception, fifteen days before

delivery, the maximum depression was observed, namely 32%. Five days before delivery the excretion rose to 39% and two days after delivery it was found to be normal again and it remained within normal limits for the period of observation. The injection of two grammes of sodium thiosulphate resulted in a depression from a 68% two-hour output shortly after conception down to 55% eighteen days before delivery. Five days before delivery the excretion was 60% and ten days after delivery it was 67%. The excretion of sodium thiosulphate after the injection of 0·2 gramme of the salt was also found to be depressed, but only about 2% below the lowest normal level as established in Chart I. This bitch gave birth during the night to three full-term puppies which appeared to be very large compared with the size of the mother. When found in the morning, they were dead.

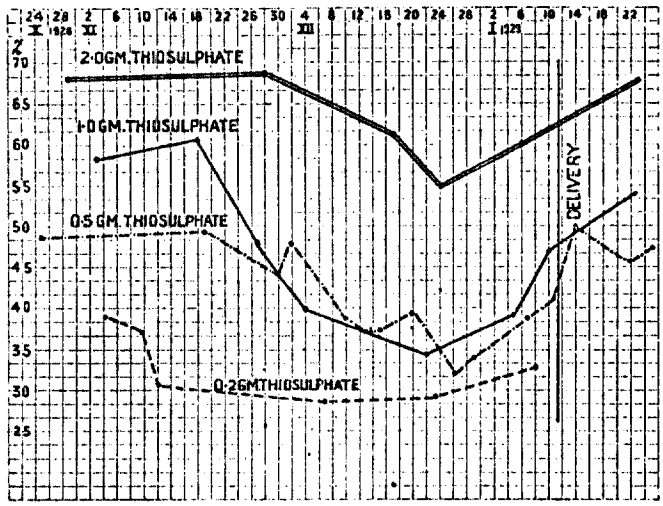

Chart I.
Results of 0·2, 0·5, 2·0 grammes thiosulphate injections in a normal bitch during normal pregnancy. The 1·0 gramme injections were done on another normal pregnant animal and are incorporated in the chart for comparison.

The animal mentioned in Chart I, which received one gramme injections, bore living pups which grew up to be healthy dogs. The curve is quite similar in character to that of the other animal after 0·5 gramme injection, but the depression is somewhat earlier and more pronounced, namely, from 60% before conception to 36% fourteen days before delivery.

In another animal which was brought to the laboratory on heat, the sodium thiosulphate excretion fell into the normal range with the exception of one extremely high reading. One week after menstruation had ceased the one gramme test showed a depression below normal, namely 44%. Eighteen days before delivery the lowest excretion in the one gramme series was observed, namely 34%. The depression of the excretion after 0·2 gramme was less, being 30% twenty days before delivery. No tests were done shortly before delivery, but a week after the birth of six healthy puppies normal figures were again encountered.

Three less complete test series on normal bitches with normal pregnancies gave similar results with the injections of 1·0 gramme and 0·2 gramme.

Chart II records the excretion in an animal which was not studied extensively before it was visibly pregnant. The test with one gramme of sodium thiosulphate twenty-eight days before delivery revealed a considerable depression, 38%, about coincident with the degree of pregnancy. In all other respects the animal appeared to be normal. Twenty-two days before delivery the left kidney was taken up surgically and exposed to X rays. Two days later the right kidney was removed. This procedure brought on a nephritis, as shown by the large albumin content of the urine and urea retention in the blood. Nine days before delivery the urea content of the blood was 86 milligrammes. The sodium thiosulphate excretion was 21%. Five days later the urea content of the blood was 63 milligrammes and the carbon dioxide combining power of the blood 38%.

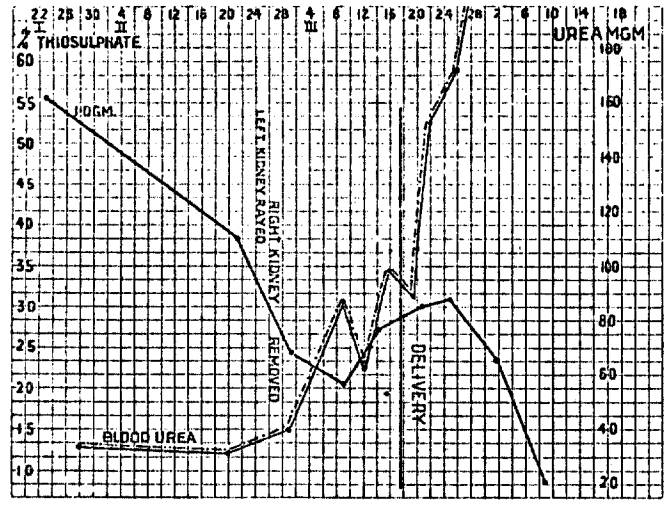

Chart II.
Results of thiosulphate injections during pregnancy complicated by experimental nephritis.

Clinically the animal was in good condition. It was eating well and the wounds had healed by first intention. Four days before delivery the phenolsulphonephthalein excretion was found to be 47% (60% normal minimum). The sodium thiosulphate excretion was 27%. Two days before delivery the urea content of the blood was 98 milligrammes and there was a moderate acidosis present, as evidenced by the diminished carbon dioxide combining power of the blood, namely 29%. The animal gave birth to six living and two dead puppies. Three days after delivery the sodium thiosulphate excretion was 30%. The blood urea was 107 milligrammes, creatinin 1·9 milligrammes and the carbon dioxide combining power 38%, which may be considered normal for a dog. The animal was in good condition and nursed her offspring. Fifteen days after delivery the sodium thiosulphate output was 22%. The urea content of the blood was 306 milligrammes. Twenty-two days after delivery the animal excreted only 9·5% of thiosulphate. The blood urea was 360, acidosis was present, the carbon dioxide combining power being 20%. The following day the animal died. On autopsy a definite enteritis was found, as is seen occasionally in nephritic animals. On microscopic examination of the remaining kidney a severe subacute nephritis was found to be present somewhat similar to the subacute stage of X ray nephritis as described in previous papers.

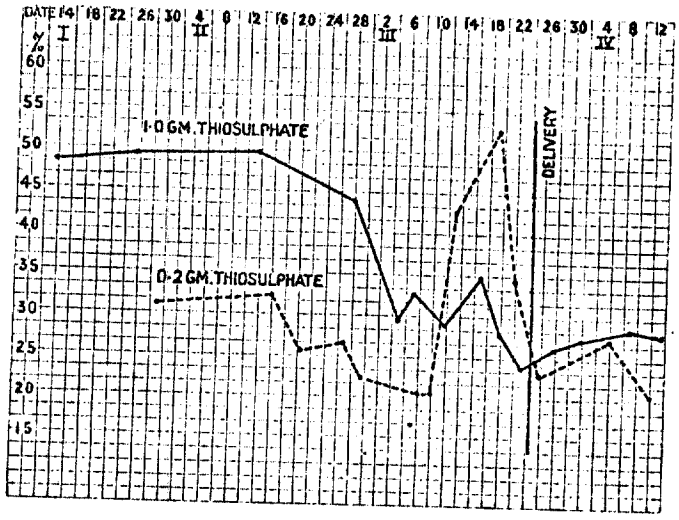

Chart III.
Results of thiosulphate injections during pregnancy complicated by a low grade nephritis.

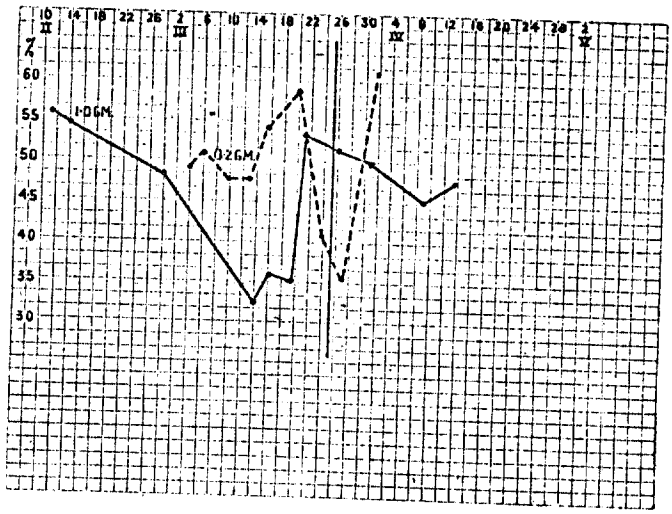

Chart IV.
Results of thiosulphate injections during pregnancy terminating in abortion. Heavy vertical line indicates the beginning of the abortion.

Chart VI reveals the excretions in an animal, weighing fourteen kilograms, which had a moderate renal insufficiency of long standing before impregnation. Before the bitch came on heat the blood urea in several determinations was found to be from 45 to 55 milligrammes per hundred cubic centimetres. The urine persistently contained large amounts of albumin. The thiosulphate excretion (one gramme) was found to be slightly below normal, 49%.

During gestation up to delivery the thiosulphate excretion after the injection of one gramme of sodium thiosulphate shows about the same curve as in normal bitches. After delivery there was no recovery towards normal as in non-nephritic animals. The thiosulphate excretion remained subnormal for a further period of observation of one month. The urea content of the blood ranged from 40 to 55 milligrammes per hundred cubic centimetres during this period.

The thiosulphate excretion after injection of 0·2 gramme also underwent some depression similar to the one gramme curve, although less distinct, but eleven days before delivery a rise above the pre-pregnancy level was noted. The excretion became subnormal again after delivery and remained so for the period of observation.

The bitch gave birth to five dead pups and one living pup which also soon died. All the pups appeared to be slightly premature. For this reason this experiment is considered to be of a transitional

nature, leading over to the next group in which undoubted abortion is dealt with.

The Excretion of Sodium Thiosulphate Through the Course of Pregnancy Terminating in Abortion.

In the group of animals whose pregnancy terminated in abortion, belong six of the animals studied. These six may be subdivided into two subgroups; one group which aborted apparently spontaneously, and another group which aborted secondarily to the experimental production of renal insufficiency. Chart IV depicts the excretion of a normal bitch which expelled its fœtuses about six to seven weeks after conception. It was a big, multiparous, elderly animal, weighing eighteen kilograms. The thiosulphate excretion, as well as other renal function tests, did not indicate any renal insufficiency, but about twenty days after conception the thiosulphate excretion (one gramme) was already found to be subnormal. A fortnight later the excretion was only 33% and remained in the neighbourhood of this low level for another week. Four days before the animal started to deliver, the excretion rose to a normal level, 53%. Then two premature dead fœtuses were expelled. The next day the thiosulphate excretion was still at normal level and after the test the animal gave birth to four more fœtuses. In the *post partum* period the sodium thiosulphate excretion fell a little below the normal level.

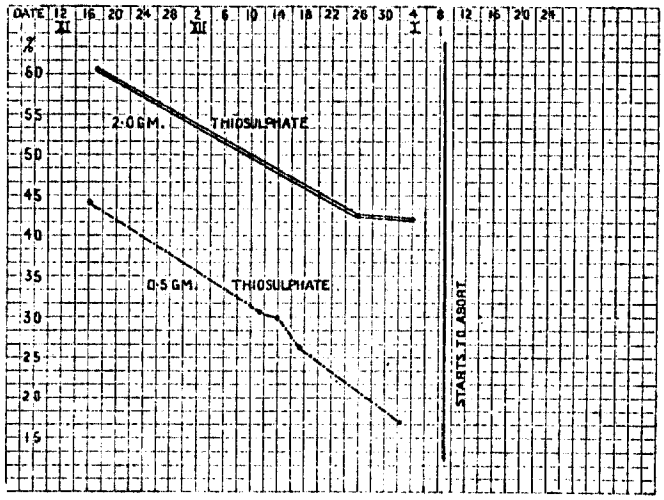

Chart V.
Results of thiosulphate injections during pregnancy complicated by toxæmia and eclampsia.

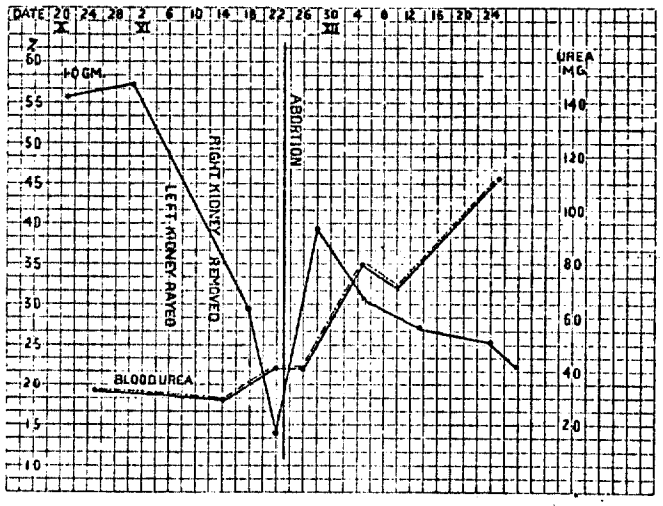

Chart VI.
Results of thiosulphate injections in a bitch which was made nephritic during pregnancy. After the thiosulphate excretion had reached the low value of 14%, abortion took place.

The study of the sodium thiosulphate excretion after injection of 0·2 gramme was begun only after the one gramme excretion had been already found to be depressed. In several instances the excretion was found to be normal or above normal. A few days previous to the rise of the one gramme excretion the 0·2 gramme results were already rising, but there was a distinct fall the day before abortion started and this fall became still more pronounced the day after the uterus became empty. After this the 0·2 gramme excretion rose again to levels well above normal.

In another instance a small dog, weighing six kilograms, aborting about three to four weeks after conception, was studied. The one gramme excretion showed nothing abnormal compared with other pregnant animals until about three weeks after menstruation, when it fell from normal level to 32%. A further rapid decline of 16% was noted four days later. The next day bloody slime was observed round the vaginal orifice and although it was assumed that abortion was taking place, no fœtuses could be found before or after this observation. The urea content of the blood remained normal, but the animal appeared to be depressed. About a fortnight after the assumed abortion the thiosulphate excretion was found to be 49%, nearly the pre-pregnancy level. The 0·2 gramme excretion revealed nothing of great interest except a rise of about 10% before abortion started; it fell to the pre-pregnancy level about three weeks after the

termination of pregnancy. After this the animal was killed and autopsy made. No evidence of renal insufficiency could be made out and according to the condition of the uterus pregnancy must have been terminated at a very early stage, not later than about four weeks after conception.

Chart V depicts the findings in a large young bull terrier bitch, weighing sixteen kilograms at the time of its first pregnancy. This animal had injections of 0·5 gramme and a few of 2·0 grammes of sodium thiosulphate. Menstruation was heavy and of long duration. It ceased thirty-six days before abortion began. The thiosulphate excretion was slightly in the lower normal range before impregnation. A week after menstruation had ceased the 0·5 gramme excretion was only 31% and declined to 26% in another week. A week before abortion started the thiosulphate excretion was only 17%. The two grammes excretion was examined thirteen days and four days before the fœtuses were expelled. The result of the two determinations was about the same, namely 42%. During the entire period of gestation the animal showed great lassitude. It also vomited almost regularly after the injections as well as at other times. After two days' bleeding from the vagina eight very macerated and immature fœtuses were expelled. The mother gave the impression of being very ill. She had convulsive tremors and was in an extreme state of shock and almost unconscious. The gums and tongue were practically white and no blood could be obtained from the smaller peripheral vessels. Blood was obtained by heart puncture from the dying animal and the urea content was twenty-two milligrammes. At autopsy the kidneys were found to be swollen and the liver appeared enlarged and was pale.

On microscopical examination the liver showed large and numerous areas of focal necrosis. Hæmorrhages and inflammatory changes were also present. In general the changes were comparable to those seen in human eclampsia. The kidneys presented œdema and areas of tubular necrosis.

The next three animals belong to the subgroup which aborted secondarily to the production of an experimental renal insufficiency during pregnancy. The renal insufficiency was produced by exposure of one kidney to X rays and the subsequent removal of the non-exposed kidney.

Chart VI is the excretion record of a good sized fox terrier, weighing eight and a half kilograms, which was operated on for the purpose of exposing the left kidney to X rays before any pregnancy was demonstrable. Seven days later the right kidney was removed. Five days after the second operation the thiosulphate output (one gramme) was found to be just below 30%. Four days later the very low level of 14% was reached. At this date the urea nitrogen content of the blood was only slightly elevated (41 milligrammes). The next day abortion started. The animal appeared to be ill for the next forty-eight hours, until all the fœtuses were expelled. Four days after the beginning of the abortion the thiosulphate excretion again was nearly 40%. It did not increase further, but declined with the progress of renal insufficiency, as shown by the increasing urea nitrogen retention of the blood.

Only a very few determinations of the thiosulphate output (one gramme) were done on the other two animals of this subgroup.

A bull terrier bitch, weighing eight kilograms, was found to be in early pregnancy at the second operation for the removal of the unexposed kidney. Two days after the operation the thiosulphate output (one gramme) was only 15%, but the blood urea content was of normal value, 29 milligrammes. Two days later it was noticed that abortion was taking place. Five days after the last determination and after the uterine discharge had stopped the thiosulphate excretion was 37%. Ten days later it was 35% and after this the thiosulphate excretion was diminishing constantly coincident with the increasing urea nitrogen retention as in the previous experiment.

The last animal in this group, a spaniel, weighing six and a half kilograms, had a thiosulphate excretion of only 8% (one gramme) one day before abortion. The blood urea content was high, 276 milligrammes. Two days later the animal died. The dilated uterine horns did not contain any more fœtuses.

Comment.

McNider in his studies on the acid base equilibrium during gestation in the dog has pointed out that few attempts have been made to determine whether there may exist during pregnancy some generalized disturbance of the organism which may manifest itself through different organs, such as the kidney, and which may in particular appear responsible for the changes found sometimes in the tissues. As a result of his researches he frequently found a tendency of the reserve alkalis of the blood to become diminished during gestation.[3]

The depression of the thiosulphate excretion may be considered as the expression of a generalized disturbance of the organism due to gestation, but primarily of distinct physiological rather than pathological nature, because it had been found in contrast to the diminution of the alkali reserve in all pregnant animals so far examined. On the other hand, it may be considered as a purely renal phenomenon of pregnancy. In fact the thiosulphate test is known as a clinical renal function test which I found also to be of distinct value in the case of experimental renal disease produced in the dog by X ray application.[1] The maximum depressions as seen consistently in normal pregnant bitches would indicate severe renal insufficiency in a non-pregnant animal. On the other hand, it may be mentioned that other renal function tests were not able to disclose a renal insufficiency constantly associated with pregnancy. For example no distinct changes in the phenolsulphonephthalein excretion could be found by McNider in a large series and I was able to confirm his findings. Neither did I find any

depression in the phosphate excretion after diagnostic injections of phosphates.[4]

Although the depressed liver function of pregnancy is well known, I could not compare the dye tests of liver function with the sodium thiosulphate test. In the liver function tests the insufficiency is measured by the inability of the organ to destroy the dye which is recovered in excess compared with the normal function. The diminished recovery of the injected sodium thiosulphate during pregnancy indicates retention or increased destruction in the body.

I thought that I might be able to throw some light on this question by injecting different amounts of sodium thiosulphate. Anticipating a demand for thiosulphate or possible derivatives of thiosulphate during the period of pregnancy I expected to find more characteristic curves after injection of smaller amounts. This assumption was found to be untrue. After the injection of one gramme of thiosulphate in dogs the most distinct curve is obtained. After the injection of 0·5 gramme of thiosulphate the curve becomes slightly less distinct and after the injection of 0·2 gramme of thiosulphate quite frequently values were found during the period of greatest deviation which were hardly below the lower normal levels. Also after injecting on a few occasions two grammes of thiosulphate a less interesting curve was found than after the injection of one gramme of thiosulphate. I cannot explain the reason for the difference in the curves after the injection of different amounts, but in applying this test to pregnant human beings one would have to keep in mind that it would be advisable to use an amount of sodium thiosulphate which would be the equivalent of 0·5 to 1·0 gramme in a fair sized dog. In other words, one would have to inject at least about two grammes of sodium thiosulphate in order to expect favourable results. It must not be forgotten, of course, that the non-pregnant animal shows interesting differences in regard to the thiosulphate excretion after injection of different amounts. In normal dogs the highest percentage output was found after the administration of two grammes of thiosulphate and the lowest output after the administration of 0·2 gramme. But while the excretion values appeared to be quite fixed after a large amount, such as two grammes, they showed sometimes considerable fluctuation in different animals or even on the same subjects after an injection of 0·2 gramme. The excretions after the injection of one gramme and half a gramme could be considered as quite consistent, although less than in the case of two grammes.

With this experimental evidence in hand I do not yet feel prepared to attribute the changes in the thiosulphate excretion to any definite organ.

The high values obtained in animals which came to the laboratory while on heat cannot yet be interpreted, since I have not studied a sufficient number of controls to decide whether these elevated values are typical of some stage of the œstrous cycle or whether they are due to the change of environment combined with a minor operation, namely, removal of their vocal cords.

In animals with experimentally produced nephritis the sodium thiosulphate excretion became diminished according to the degree of renal damage and a depression due to pregnancy was superadded. This is shown by the fact that improvement in the thiosulphate excretion occurred after delivery. The improvement, of course, was only of a temporary nature, because of the progression of the experimental disease (Charts II and VI). It was found that if during the course of pregnancy this depression falls below 20% in the case of the one gramme or a half of a gramme injections, abortion is most likely to occur, such depression being observed in all aborting animals which had an experimental renal lesion. A sudden fall below 20% was also seen in an apparently normal animal a few days before early abortion occurred. A similarly low excretion of thiosulphate was further observed in an animal (Chart V) in which fatal eclamptic symptoms combined with abortion developed spontaneously after toxæmic symptoms of several weeks' standing.

In the case of the aborting animal of Chart IV a thiosulphate excretion below 20% (one gramme) was not noted. On the contrary, there was an increase right up to the pre-pregnancy level a few days before premature birth. As will be remembered (see Chart I) a similar but not so considerable pre-labour rise is seen in bitches with full term delivery. Although I prefer not to put too much weight on this single observation I should like to emphasize the fact that the fœtuses were more developed than those of the other animals with low pre-abortion thiosulphate excretion; this animal showed no ill effects before or during labour.

In this connexion I should like to call attention also to the curve after 0·2 gramme of thiosulphate as seen in Charts III and IV. The 0·2 gramme excretion rose to pre-pregnancy level about two weeks before delivery and fell very abruptly just before and after delivery. On account of the small number of these observations it cannot be decided whether we are dealing only with a coincidence demonstrating the irregularities after the injection of 0·2 gramme or whether this curve is typical for late abortion and premature births.

The number of bitches in my series which aborted, is unquestionably larger than would be expected from veterinary experience and I thought that it would be quite justifiable to assume that in the case of uncomplicated abortion the miscarriage was due to the large amount of handling involving numerous catheterizations, particularly when I attempted to obtain as complete a curve as possible. Another possibility also might be that frequently repeated injections of sodium thiosulphate were responsible for some of the miscarriages, but since there is nothing known about such an action of sodium thiosulphate I am rather inclined to ascribe the premature birth to the first mentioned cause. The animal (Chart V) in which eclamptic

symptoms occurred, most probably presents a condition corresponding to human eclampsia in its symptoms and pathological findings.

This report is preliminary in nature and, as can be seen, many more experiments are needed to solve several questions which came up during the investigations. The experiments are published now for technical reasons which caused a temporary interruption of the work at this stage. I consider them advanced enough to be applied to the pregnant woman for further studies and think that it would be possible to gain from such a study further information regarding the physiology of pregnancy and that some practical information regarding the diagnosis and prognosis of pregnancy may be obtained. Diagnosis of pregnancy in the bitch by means of the thiosulphate test was in some instances quite satisfactory. At a period of ten days to three weeks after conception a distinct depression of the thiosulphate excretion after the injection of one gramme could already be observed, which indicated two possibilities, renal damage or pregnancy. After excluding renal damage the existence of pregnancy was regarded as established and up to the present no wrong diagnosis has been made at the early stage in which it is not yet possible to diagnose pregnancy with certainty by physical examination. On the other hand, a thiosulphate excretion below 20% after injection of one gramme or a half of a gramme indicated abortion with or without eclamptic symptoms. Similar evidence in human beings is still required and should in doubtful cases be of service to the practitioner. As pointed out by Harding, Allin and van Wyck[5] the information gained by urine and blood chemistry examinations alone is often incomplete and misleading in the investigation of the toxæmias of later pregnancy. However, as has already been mentioned, the proper amount of thiosulphate to be injected into human beings will have to be established by experiment.

The anatomical changes in the kidneys of the animals with experimental renal insufficiency will not be discussed on this occasion. A report on the histological features of X ray nephritis will form the basis of subsequent articles.

Conclusions.

1. The excretion of injected sodium thiosulphate solutions containing 0·2 gramme, 0·5 gramme, 1·0 gramme and 2·0 grammes was studied in the bitch. The percentage excretion and the constancy of results increase with increasing amounts of sodium thiosulphate injected.

2. The thiosulphate excretion in pregnant but otherwise normal bitches was found to be depressed during a period lasting from one to three weeks after conception up till delivery, the maximum depression being found from three weeks to one week before delivery. All the concentrations studied showed this depression, but it was usually best observed after the injection of one gramme of sodium thiosulphate and was least distinct after the injection of 0·2 gramme.

3. By determining the thiosulphate excretion it is possible to diagnose pregnancy in bitches with comparative certainty.

4. In bitches with diminished thiosulphate excretion due to renal insufficiency, pregnancy aggravates the diminution.

5. If in cases of pregnancy complicated by nephritis or toxæmia, the excretion of sodium thiosulphate falls below 20%, abortion occurs.

I wish to record my grateful appreciation of the courtesies extended to me by the staff of the pathological and biochemical laboratories, Royal Prince Alfred Hospital. I am particularly indebted to Dr. A. H. Tebbutt and Dr. F. S. Hansman, honorary directors of the departments.

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EXPERIENCES WITH ENCEPHALOGRAPHY.[1]

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I PROPOSE to deal with the subject of encephalography in the following order: Definition of terms, discussion of necessity for better localization, brief indication of other methods available, dangers and limitations and indications for method, with technique, what practical results can be expected, with a series of films in which good results can be shown as a direct consequence, and general conclusions.

I ask your indulgence, from the personal standpoint because I feel very keenly that many here have had much wider experience and may have tried and discarded methods that seem to give fair promise. But as far as facts and treatment are concerned, only the severest criticism will winnow the wheat from the chaff and insure progress on right lines; I am hoping tonight for much information and help, particularly in distinguishing abnormal or normal detail on the films.

Definitions.

Ventriculography[1][2] is direct injection of air or other substances into ventricles through a trephine hole in the skull, followed by X ray examination.

Ventricular estimation[3] is the aspiration of fluid from both lateral ventricles with measure-

[1] Read at a meeting of the New South Wales Branch of the British Medical Association on August 8, 1929.

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Protection studies with sodium thiosulfate against methyl bis (β-chloroethyl)amine hydrochloride (HN2) and its ethylenimonium derivative

37

Methyl bis (β-chloroethyl)amine hydrochloride (HN2) and chlorimine picrylsulfonate derivative (CPS) were injected alone and with sodium thiosulfate (TS) in laboratory animals and in patients with widespread metastatic carcinoma in order to study the protective effect of TS on the bone marrow depression produced by HN2 and CPS. In rats and rabbits 1.0 Gm. per kilogram of TS gave complete protection against supralethal doses of CPS, whereas it provided little protection against lethal doses of HN2. In man, however, 200 mg. per kilogram of TS protected against daily doses of HN2 and CPS (2 to 3 times the usual daily dose) administered for several days to a total of 2 to 9 times the amount given in the usual course of treatment. Nausea and vomiting usually followed each injection of HN2 and CPS, and thus was not prevented by TS. With one exception, tumor regression in these metastatic carcinomas was not observed after the administration of either HN2 or CPS preceded by protective doses of TS. TS gives much better protection against CPS than against HN2 in rodents, but this difference, while suggested by our data, was not clearly defined in man.

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Since methyl bis (β-chloroethyl)amine hydrochloride (nitrogen mustard, HN2), a polyfunctional alkylating agent, was introduced into clinical use in 1946, many techniques as well as protective agents have been investigated in order to prevent its toxic effects in animals[1, 3, 7, 10-12, 18-20, 29-32, 36] and in humans.[4, 23-25, 27, 34]

Earlier studies[33] demonstrated that the reactions of HN2 in vivo are completed within 2 to 3 minutes after intravenous injection, and, as in the case of total body irradiation, the toxic effects of HN2 cannot be prevented if HN2 is injected before the protective agent.

In recent years, polyfunctional alkylating agents have been used widely in regional perfusion and infusion procedures.[23-25, 27, 34]

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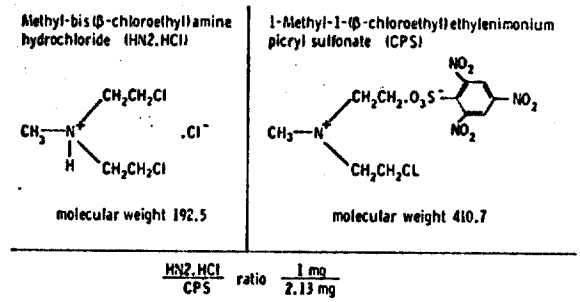

Fig. 1. Structural formulas of HN2 and CPS with their molecular weight and ratio.

In developing techniques for the local administration of an alkylating agent, it appeared desirable to develop both a more reactive form of nitrogen mustard, which would tend to react promptly in the local areas, and drugs which could protect against the systemic toxicity of nitrogen mustard entering the systemic circulation from the treated area.

The first transformation product of methyl bis (β-chloroethyl)amine, 1-methyl-1-(β-chloroethyl)ethylenimonium, or chlorimine compound, is a water-soluble compound, more reactive under physiologic conditions than HN2. A more stable picrylsulfonate salt of the HN2 chlorimine (chlorimine picrylsulfonate or CPS) was prepared by Merck Sharp & Dohme according to the procedure of Columbic and co-workers.[15] The molecular weight of CPS is approximately twice that of HN2; the ratio between the two compounds in equivalent amounts of alkylating agent in milligrams is 1:2.13 (Fig. 1). Sodium thiosulfate (TS) was used as the protective agent, since it is known to inactivate HN2 in vitro,[26, 28] the thiosulfate ion reacting with the ethylenimonium ion (Fig. 2). The reaction with thiosulfate was measured by Columbic and co-workers[15] as an index of the formation of the ethylenimonium compound. In this investigation the toxic and bone marrow depressant effects of HN2 and CPS have been compared in laboratory animals and man when both agents were

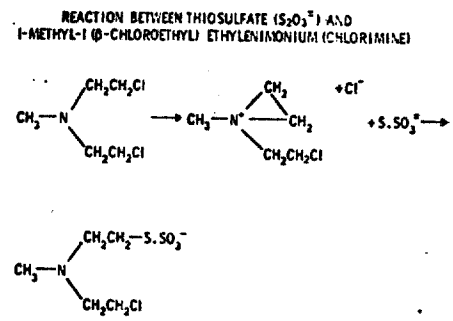

Fig. 2. Methyl bis (β-chloroethyl)amine is converted in water to 1-methyl-1-(β-chloroethyl)-ethylenimonium ion (chlorimine) which reacts with thiosulfate ion.

injected intravenously alone and following the intravenous injection of sodium thiosulfate.

Materials and methods

The animals used were female albino rats weighing 220 to 240 grams from the Charles River Farms, and male albino New Zealand rabbits weighing between 2.0 and 2.5 kilograms from the Carworth Farms. Water and food ad libitum were given.

The patients studied were hospitalized on the Adult Chemotherapy Research Floor of the James Ewing Hospital. They had advanced metastatic cancer and were not candidates for surgery and or local radiation therapy. None of the patients had received bone marrow depressant anticancer

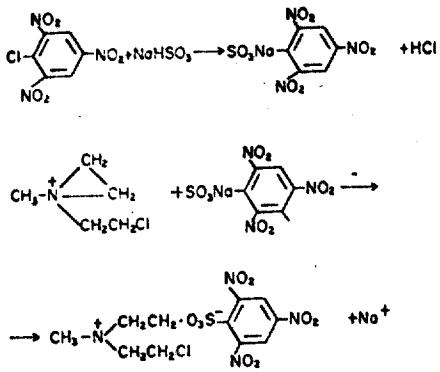

Fig. 3. Reaction between picrylchloride with sodium bisulfite to form sodium picrylsulfonate which reacts with the 1-methyl-1-(β-chloroethyl)-ethylenimonium ion to form 1-methyl-1-(β-chloroethyl)ethylenimonium picrylsulfonate.

drugs prior to this study, and their peripheral blood picture was within the expected range for patients with advanced cancer.

Methyl bis (β-chloroethyl)amine hydrochloride (HN2) was dissolved in NaCl 0.9 Gm. per cent to make a concentration of 1 mg. per milliliter, and was injected within one hour.

1-Methyl-1-(β-chloroethyl)ethylenimonium picrylsulfonate or chlorimine picrylsulfonate (CPS), was supplied as a yellow-red bulk powder (Fig. 3). CPS was stored over a desiccant at −20° C. in tightly stoppered containers to minimize loss of activity.[15] The containers were warmed to room temperature for 30 to 40 minutes before opening to prevent condensation of water on the anterior walls. The solutions were prepared at the concentration of 1 mg. per milliliter in 5 per cent dextrose in water. Solutions with NaCl 0.9 Gm. per cent can produce a back reaction of the ethylenimonium ion with Cl to form HN2. Since CPS is not stable in solution,[15] the solution was kept chilled in ice and injected within 30 minutes.

Sodium thiosulfate (TS), when injected in the experimental animals, was prepared as a 10 per cent solution from the analytical reagent of Mallinkrodt Chemical Works ($Na_2S_2O_3 \cdot 5H_2O$); when injected in patients the Lilly preparation of sodium thiosulfate was used (10 c.c. ampules, 10 per cent solution).

The injections into rats were given under general anesthesia produced by the inhalation of methoxyflurane. No anesthesia was necessary for the rabbits. Intravenous injections in rats were given into the femoral vein, through a small skin incision; the ear veins were used in rabbits. In patients, HN2 and CPS were administered by injecting the compounds through the tubing of a running intravenous infusion of 5 per cent dextrose in water.

In both animals and patients TS was given 5 to 8 minutes before the injection of either HN2 or CPS. TS had to be injected slowly into the rats over a 3 to 5 minute period in order to avoid death from respiratory arrest. No toxic effects were noted in patients who received TS (200 or 400 mg. per kilogram, about 10 to 20 Gm. in the average patient) within 10 or 15 minute periods. Control animals received 0.10 c.c. of a 0.9 per cent solution of NaCl per 100 Gm. of body weight.

All animals were checked daily for diarrhea, weight loss, and neurological signs for a period of 2 to 3 weeks. In rabbits white blood counts were obtained daily on blood from the ear vein, until the blood picture had returned to normal. The criteria used in evaluating toxicity in patients were: white blood cells, platelet counts and hemoglobin levels, and the occurrence of central nervous system disturbances, nausea and vomiting, and diarrhea. Therapeutic response was evaluated by criteria appropriate to the disease being treated.

Results

Toxicity in rats and rabbits. The lethal effects of HN2 and CPS in rats and rabbits are summarized in Tables I and II. In the dose range used, the initial effect of HN2 and CPS in rats and rabbits was a significant weight loss within 24 hours after the injection, and then the appearance of listlessness shortly afterward. At the higher

doses of both drugs, conjunctival and nasal hemorrhages occurred, and the rats showed ataxia and tonic-clonic contractions and sometimes walked in circles. In rats surviving in the range of the LD_{50}, the weight recovery began between the fifth and sixth day and there was complete recovery at approximately 2 weeks. In rabbits, those which survived regained their initial weight in approximately 3 weeks. Maximal leukopenia was noted between the third and fifth day, sometimes preceded by early leukocytosis (Figs. 4 and 5). The signs of drug toxicity in this study were essentially similar to those described in detail by other authors.[2, 3, 13, 16, 17, 21, 28] In rats the LD_{50} of CPS was approximately twice as high as that of HN2 in millimolars per kilogram; whereas in rabbits the reverse took place, CPS being about twice as toxic as HN2 in

Table I. *Toxicity of single intravenous doses of HN2 and CPS in rats (female albino rats, 220 to 240 grams)*

Agent	Dose (mg./Kg.)	Dose (mM./Kg.)	Death	Day of death after treatment	Average weight loss (%)	Estimated LD_{50} (mg./Kg.)	Estimated LD_{50} (mM./Kg.)
HN2	1.2 to 2.0	0.0062 to 0.0104	18/18	3 to 6	13		
	0.6 to 0.8	0.0031 to 0.0042	10/12	6 to 7	24		
	0.5	0.0026	4/6	4 to 6	20	0.4 to 0.5	0.0021 to 0.0026
	0.4	0.0021	0/6		16		
CPS	2.7 to 3.0	0.0073 to 0.0066	11/12	4 to 5	19		
	2.0	0.0049	4/6	4 to 6	14	1.8 to 2.0	0.0044 to 0.0049
	1.8	0.0044	0/6		16		
	0.8 to 1.2	0.0019 to 0.0029	0/6		4		

Table II. *Toxicity of single intravenous doses of HN2 and CPS in rabbits (male albino rabbits, 2.0 to 2.5 kilograms) (Figs. 4 and 5)*

Agent	Dose (mg./Kg.)	Dose (mM./Kg.)	Death	Day of death after treatment	Average weight loss (%)	Estimated LD_{50} (mg./Kg.)	Estimated LD_{50} (mM./Kg.)
HN2	3.0	0.0156	4/4	4,4,4,4	13	2 to 3	0.0104
			4/5	4,6,6,7	5		0.0156
	2.0	0.0104	1/4	11	12		
CPS	5.0	0.0122	2/2	2,2	10		
	3.0	0.0073	3/4	4,5,8	13		
	2.0	0.0049	4/4	3,3,4,5	9	< 2	< 0.0049
			2/3	3,7	9		

Table III. *Per cent protection by TS against single intravenous injections of HN2 and CPS in rats*

Agent	Dose (mg./Kg.)	Dose (mM./Kg.)	Without TS			With TS protection				Estimated LD_{50} (mg./Kg.)	Estimated LD_{50} (mM./Kg.)
			Death	Day of death after injection	Average weight loss (%)	Death	Day of death after injection	Average weight loss (%)	Survivors (%)		
HN2	1.35 to 1.50	0.0070 to 0.0078	12/12	3 to 4	18.5	12/12	3 to 6	16	0		
	0.9	0.0047	6/6	3 to 5	13	4/6	4 to 5	10	33		
	0.6	0.0031	6/6	4 to 6	16	3/6	4 to 6	14	50	0.6	0.0031
	0.4	0.0020	3/6	4	16	0/6		14	100		
CPS	12.0	0.0292	6/6	2 to 3	24	6/6	4 to 5	18	0		
	10.0	0.0243	6/6	2 to 4	24	5/6	4 to 5	18	17		
	7.5	0.0183	6/6	4	22	4/6	4 to 5	21	33	7.0 to 7.5	0.0170 to 0.0183
	7.0	0.0170	6/6	3 to 4	22	2/6	5 to 9	19	67		
	6.5	0.0158	6/6	3 to 4	20	0/6		19	100		
	6.0	0.0146	6/6	4	17	0/6		11	100		
	5.0	0.0122	6/6	3 to 5	18	0/6		15	100		
	3.0	0.0073	6/6	4 to 5	23	0/6		14	100		

Female albino rats, 220 to 240 grams.

millimolars per kilogram (Tables I and II).

Protection in rats and rabbits. Tables III and IV summarize the per cent of protection obtained with TS against HN2 and CPS; Table V compares the LD_{50} of HN2 and CPS with and without TS. Complete protection with TS was achieved against a dose of CPS up to 6.8 mg. per kilogram in rats and up to 7.0 mg. per kilogram in rabbits. No significant protection was obtained with TS against HN2 in rats and rabbits. HN2 was more toxic to rats than rabbits, whereas the LD_{50}'s of CPS in rats and rabbits are in the same range of 7.0 to 7.5 and 8 to 10.0 mg. per kilogram, respectively.

Figs. 6 and 7 show the white blood cell

Table IV. *Per cent protection by TS against single intravenous injection of HN2 and CPS in rabbits*

Agents and doses										
TS (Gm./Kg.)	*HN2 (mg./Kg.)*	*HN2 (mM./Kg.)*	*CPS (mg./Kg.)*	*CPS (mM./Kg.)*	*Death*	*Day of death*	*Average weight loss (%)*	*Protection*	*Estimated LD_{50} (mg./Kg.)*	*Estimated LD_{50} (mM./Kg.)*
1.0	3.0	0.0156			3/4	4, 5, 6	11	25	< 3	< 0.0156
1.0			5.0	0.0122	0/3		3	100		
1.0			7.0	0.0170	0/4		13	100	8 to 10	0.0195 0.0243
1.0			10.0	0.0243	3/4	2, 4, 9	12	25		

Table V. *Summary of LD_{50} data on HN2 and CPS in rats and rabbits, with and without TS protection*

	HN2	*HN2 + TS*	*CPS*	*CPS + TS*
Rats*	0.4 to 0.5 mg./Kg. (0.0021 to 0.0026 mM./Kg.)	0.6 mg./Kg. (0.0031 mM./Kg.)	1.8 to 2.0 mg. per kilogram (0.0044 to 0.0049 mM. Kg.)	7.0 to 7.5 mg. Kg. (0.0170 to 0.0183 mM. Kg.)
Rabbits†	2 to 3 mg./Kg. (0.0104 to 0.0156 mM./Kg.)	< 3.0 mg./Kg. < (0.0156 mM./Kg.)	< 2.0 mg. Kg. < (0.0049 mM. Kg.)	8.10 mg. Kg. (0.0195 to 0.0243 mM. Kg.)

*Female rats, Charles River, 220 to 240 grams.

†Male albino New Zealand rabbits, 2.0 to 2.5 kilograms.

Table VI. *The effect of lowering doses of TS on its protective action against CPS*

Agent (single intravenous doses)					
TS (Gm./Kg.)	*CPS (mg./Kg.)*	*Death*	*Day of death after treatment*	*Average weight loss (%)*	*Protection (%)*
	6.0	6/6	4 to 5	18	0
1.0	6.0	0/6		9	100
0.5	6.0	4/6	4	17	33
0.1	6.0	5/6	4	15	17

Female rats, 220 to 240 grams.

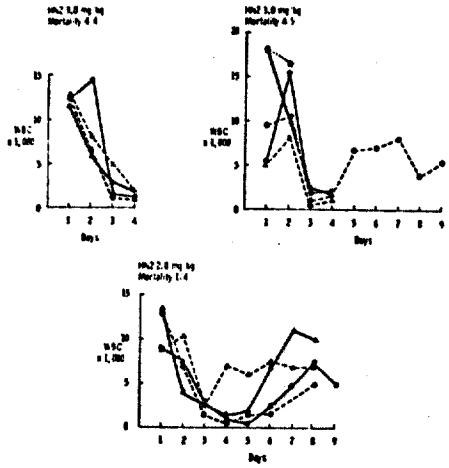

Fig. 4. Leukopenia after single intravenous injections of HN2 in rabbits (male albino New Zealand rabbits, 2.0 to 2.5 kilograms).

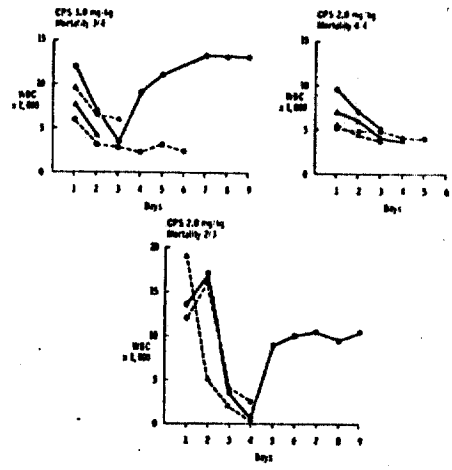

Fig. 5. Leukopenia after single intravenous injections of different doses of CPS in rabbits (male albino New Zealand rabbits, 2.0 to 2.5 kilograms).

counts in rabbits injected with HN2 and CPS following a protective dose of TS. With HN2 (Fig. 6) three fourths of the animals died, all with severe leukopenia; the survivors developed modest leukopenia with prompt recovery. Among the protected animals CPS produced only mild leukopenia (Fig. 7), while those unprotected died with a marked fall in white blood cells (Fig. 5). In both groups leukopenia was often preceded by an initial leukocytosis as seen in irradiated animals.[22] At lower doses of TS (0.1 to 0.5 mg. per kilogram) in rats, there was a progressive decrease in protection against a supralethal dose of CPS (6.0 mg. per kilogram) (Table VI).

Clinical studies

Toxicity of HN2 and CPS. HN2, when injected intravenously at the conventional dose of 0.4 mg. per kilogram, causes nausea and vomiting within a period of 2 to 6 hours and produces definite leukopenia and thrombocytopenia, the maximal depression occurring usually between the twelfth and the eighteenth day, followed by a gradual recovery.[8] When given intravenously in equivalent doses (0.8 mg. per kilogram), CPS causes a similar picture of nausea and vomiting and depression in leukocytes and platelets (Fig. 8).

Protection against HN2 and CPS. The protective effect of TS against HN2 and CPS was studied in 7 patients with widespread metastatic carcinomas. Five patients received 0.4 mg. per kilogram of body weight of HN2 preceded by TS, 200 mg. per kilogram, given at 2 to 7 day intervals until leukopenia and thrombocytopenia developed. After recovery of the peripheral blood picture, a second course with equivalent doses of CPS, preceded by the same dose of TS, was administered and continued until the development of toxicity. Nausea and vomiting within a period of 2 to 6 hours almost invariably followed the administration of either HN2 or CPS, although these symptoms were minor in most patients because of premedication with oral phenobarbital 100 mg. and intramuscular Thorazine 25 mg., given one hour and 30 minutes, respectively, before drug administration.

Case 1. A 49-year-old Negro male with carcinoma of the urethra, metastatic to inguinal nodes, pelvis, and lungs (Fig. 9) received 1 dose of CPS 0.4 mg. per kilogram and 9 doses of CPS 0.8 mg. per kilogram for a total dose of 7.6 mg. per kilogram (0.0153 mM. per kilogram) with TS without depressing the peripheral blood picture. Subsequently, a second course with HN2 0.4 mg. per kilogram (0.0021 mM. per kilogram) × 5 was administered with TS for a total dose of 2.9 mg. per kilogram (0.0104 mM. per kilogram) with a fall in white blood cells and platelets after the fourth dose of HN2. The patient complained of severe nausea and vomiting with both courses of treatment, symptoms probably enhanced by the presence of hypercalcemia. The treatment had no effect on the serum calcium levels or the measurable metastases.

Case 2. The patient was a 48-year-old white woman with carcinoma of the breast metastatic to bones, central nervous system, abdomen, inguinal and femoral nodes (Fig. 10). During the first part of the study she received 4 doses of CPS 1.6 mg. per kilogram with TS for a total dose of 6.4 mg. per kilogram (0.0156 mM. per kilogram) with leukopenia and thrombocytopenia developing after the fourth dose. During the second part of the study HN2 was administered with TS at the dose of 0.8 mg. per kilogram (0.0042 mM. per kilogram). After only one dose the white blood cells dropped to 1,000 and platelets from 400,000 to 200,000. There was significant shrinkage of the abdominal mass and enlarged inguinal nodes after the course of CPS.

Case 3. A 66-year-old white woman with carcinoma of the endometrium locally recurrent and with bilateral pulmonary metastases (Fig. 11) received at the beginning of the study 2 doses of HN2 0.8 mg. per kilogram with TS for a total dose of 1.6 mg. per kilogram (0.0053 mM. per kilogram) with the development of severe leu-

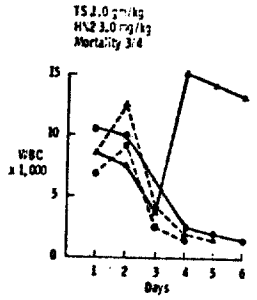

Fig. 6. Leukopenia after single intravenous dose of HN2 preceded by TS (male albino New Zealand rabbits, 2.0 to 2.5 kilograms).

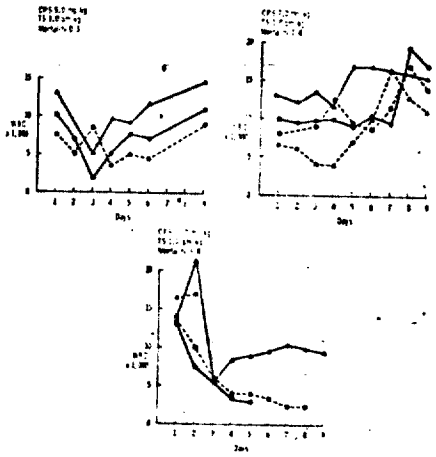

Fig. 7. Leukopenia after single intravenous dose of CPS preceded by TS (male albino New Zealand rabbits, 2.0 to 2.5 kilograms).

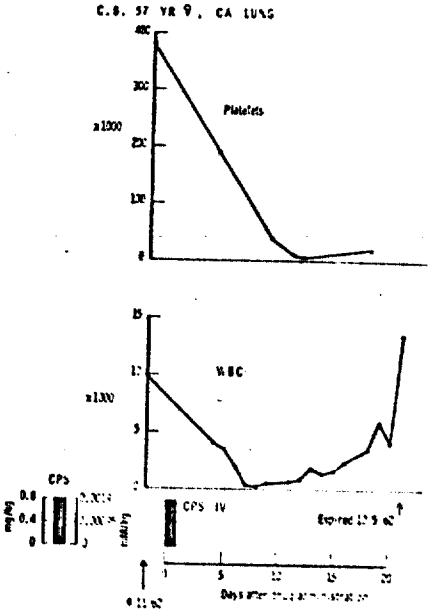

Fig. 8. Leukopenia and thrombocytopenia in man after a single intravenous dose of CPS, 0.8 mg. per kilogram (0.0010 mM. per kilogram). There was no therapeutic response.

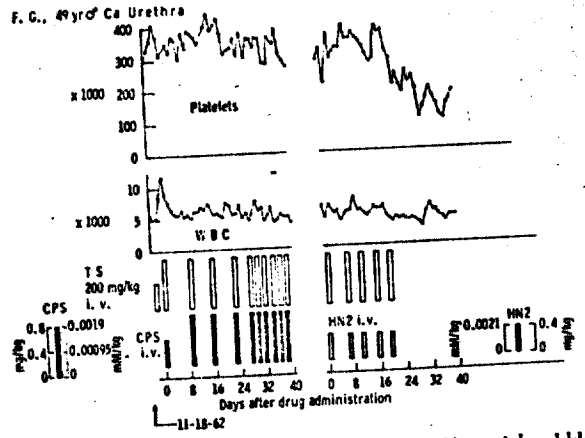

Fig. 9. Protection study in man with TS against CPS and HN2. No peripheral blood depression was noted after a total dose of 7.6 mg. per kilogram (0.0185 mM. per kilogram) of CPS; thrombocytopenia and mild leukopenia developed after a total dose of 2.0 mg. per kilogram (0.0104 mM. per kilogram) of HN2. There was no therapeutic response.

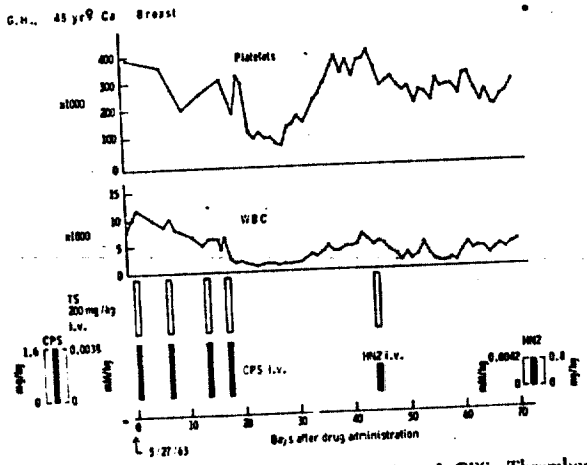

Fig. 10. Protection study in man with TS against HN2 and CPS. Thrombocytopenia and leukopenia developed after a total dose of 6.4 mg. per kilogram (0.0156 mM. per kilogram) of CPS and 0.8 mg. per kilogram (0.0042 mM. per kilogram) of HN2. A metastatic mass in the abdomen as well as right inguinal nodes shrunk after the course with CPS.

kopenia and thrombocytopenia. After recovery of the peripheral blood picture, the patient received 3 doses of CPS 1.6 mg. per kilogram for a total dose of 4.8 mg. per kilogram (0.0117 mM. per kilogram). After the third dose leukopenia and thrombocytopenia developed; the metastatic lesions were unchanged.

Case 4. A 74-year-old white woman with carcinoma of the breast and widespread bone metastases received HN2 administered during the first part of the study at the dose of 0.4 mg. per kilogram × 6 for a total dose of 2.4 mg. per kilogram (0.0125 mM. per kilogram), followed by leukopenia (2,100 white blood cells) and thrombocy-

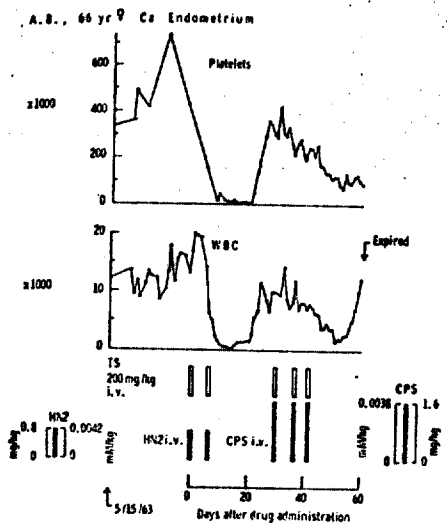

Fig. 11. Protection study in man with TS against HN2 and CPS. Thrombocytopenia and leukopenia developed after a total dose of 1.6 mg. per kilogram (0.0083 mM. per kilogram) of HN2 and 4.8 mg. per kilogram (0.117 mM. per kilogram) of CPS. There was no therapeutic response.

topenia (46,000 platelets). When the peripheral blood count returned to normal, CPS, 0.8 mg. per kilogram, was injected at 3 to 4 day intervals. After the fourth dose (total 0.0078 mM. per kilogram) mild leukopenia (3,500 to 3,800 per cubic millimeter) developed. This was probably a delayed biphasic effect of the previous course with HN2. Thirteen days later, CPS was restarted at 0.8 mg. per kilogram, and the patient tolerated 4 more doses without toxicity. When dose was then raised to 1.6 mg. per kilogram, definite leukopenia (1,500 per cubic millimeter) and severe thrombocytopenia (20,000 per cubic millimeter) were noted almost immediately. The total dose of CPS was 8.0 mg. per kilogram (0.0195 mM. per kilogram). No therapeutic response was noted.

Case 5. A 48-year-old white woman with carcinoma of the breast metastatic to bones and a cauda equina syndrome received CPS administered at the dose of 1.6 mg. per kilogram × 3 for a total dose of 4.8 mg. per kilogram (0.0117 mM. per kilogram), and HN2 at the dose of 0.8 mg. per kilogram × 3 for a total dose of 2.4 mg. per kilogram (0.0125 mM. per kilogram). The patient developed thrombocytopenia and leukopenia after equivalent doses of CPS and HN2 with TS; TS thus did not protect more selectively against CPS. No therapeutic response was obtained with either course.

Case 6. A 54-year-old white male with inoperable epidermoid carcinoma of the lung received only 2 doses of HN2 0.8 mg. per kilogram for a total dose of 1.6 mg. per kilogram (0.0083 mM. per kilogram) with fall in platelets to 110,000 which started between the first and the second injection. Three days after the administration of the second dose the patient died, probably because of pulmonary embolus.

Case 7. This patient was a 34-year-old white male with adenocarcinoma of jejunum and intrapelvic metastases. In the first part of the study he received CPS 1.6 mg. per kilogram × 3 for a total dose of 4.8 mg. per kilogram (0.0117 mM. per kilogram), each preceded by TS 400 mg. per kilogram. No significant leukopenia developed, but the platelet level dropped from 400,000 to 150,000 per cubic millimeter. In the second part of the study the patient received 2 doses of CPS 1.6 mg. per kilogram for a total dose of 3.2 mg. per kilogram (0.0078 mM. per kilogram) at 15 day intervals, each dose preceded by TS, 200 mg. per kilogram. A definite leukopenia (2,300) developed after the second dose. Four hundred milligrams per kilogram of TS seemed to give better protection than 200 mg. per kilogram against CPS. No therapeutic response was obtained after either course.

Discussion

Comparison of HN2 and CPS toxicity in rats and rabbits. In rats the approximate LD_{50} of HN2 on intravenous injection was 0.4 to 0.5 mg. per kilogram, which is less than that reported by others, 1.1 mg. per kilogram[2] and 1 to 2 mg. per kilogram.[21] The approximate LD_{50} of HN2 given intravenously in rabbits (2 to 3 mg. per kilogram) was slightly higher than the reported figures, 1.6 mg. per kilogram[2] and 1 to 2 mg. per kilogram.[25] The LD_{50} of CPS in rats and rabbits was similar (1.8 to 2.0 mg. per kilogram) (Tables I and II) and equivalent to 0.85 to 0.94 mg. per kilogram of HN2. Thus, on a millimolar per kilogram of body weight basis, CPS is approximately half as toxic as HN2 in the rat, and twice as toxic as HN2 in the rabbit.

The LD_{50} of the chlorimine derivative of HN2 injected intravenously into rats was found by Hunt and Philips[21] to be between 0.004 and 0.008 mM. per kilogram as com-

pared to an approximate LD_{50} of 0.0049 mM. per kilogram obtained in this study.

Philips and associates[28a] studied the toxicity of CPS in mice, rats, cats, and dogs. They concluded that CPS, when given intraperitoneally, is more toxic than HN2 in mice (1 to 2 times when dosage is calculated in milligrams per kilogram, and about 2 to 4 times when dosage is calculated in millimolars per kilogram). In dogs, however, the LD_{50} of HN2 was 0.2 to 0.4 mg. per kilogram and that of CPS was 0.4 to 0.8 mg. per kilogram, which is approximately equal on a millimolars per kilogram basis.

Protective effects of TS in rats and rabbits. When TS preceded the administration of HN2 in rats and rabbits, no significant change in the LD_{50} of HN2 was found (Table V). On the contrary, when TS preceded the administration of CPS, the LD_{50} rose from 1.8 to 2.0 mg. per kilogram to 7.0 to 7.5 mg. per kilogram in rats, and from less than 2.0 mg. to 8 to 10 mg. per kilogram in rabbits. TS, when injected intravenously in high doses, such as 1.0 Gm. per kilogram, prior to CPS, thus gave a significant degree of protection against CPS. TS prevented drug-induced leukopenia also in most of the rabbits when CPS was injected at the supralethal doses of 5.0 and 7.0 mg. per kilogram.

Protective effects of TS against HN2 and CPS in man. Our data indicate (Figs. 9, 10, and 11) that 200 mg. per kilogram of TS gave protection against multiple doses of both HN2 and CPS, with apparently somewhat greater protection against CPS. In only one case (Case 5) did 200 mg. per kilogram of TS give the same degree of protection against equivalent doses of CPS and HN2. In one case (Case 7) the same dose of CPS, 1.6 mg. per kilogram, was administered in two courses. During the first course CPS was preceded by 400 mg. per kilogram of TS without any significant depression of white blood cells or platelets; during the second part of the course 200 mg. per kilogram of TS was given and leukopenia developed after the second injection of CPS. The larger dose of TS appeared to have a greater protective effect.

Mechanism of protective action of TS. The reaction in vitro between TS and HN2 has been demonstrated by Golumbic and co-workers[13] and Ogston and associates.[26] Thompson and colleagues[38] investigated the kinetics of the action of TS and the piperazinium dimer of HN2 and showed analytically that for this reaction the formation of Cl^- agrees with $S_2O_3^=$ disappearance.

TS has been used[5, 14] to measure extracellular fluid volume,[5, 14] since this drug after intravenous injection is rapidly distributed in the extracellular space. It has been shown to be an essentially innocuous substance and has been injected in 10 per cent solution in humans and up to 3.0 Gm. per kilogram in mice[19] without adverse effects.

Hatiboglu and co-workers[18, 19] were able to protect 70 and 100 per cent of the mice against the lethal effects of doses of 25.0 and 12.5 mg. per kilogram of HN2 injected intraperitoneally when TS was given at the dose of 3.0 Gm. per kilogram 15 minutes before by the same route. Protection was also observed when TS was injected intraperitoneally before either intravenous or subcutaneous administration of 25.0 mg. per kilogram of HN2. In dogs complete protection was obtained by an intravenous injection of 3.0 Gm. per kilogram of TS given 5 minutes before the administration of a single lethal dose of HN2, 2.0 mg. per kilogram. Callaway and Pearce,[7] injecting intraperitoneally 3.0 Gm. of a mixture of TS and trisodium citrate in the ratio of 10 to 1, obtained complete protection in albino rats against greater than the median lethal dose of mustard gas (di[2-chloroethyl]sulfide) injected subcutaneously; the LD_{50} of mustard gas was raised approximately threefold. Schultz and associates[32] reported in dogs that HN2, when injected intraarterially or intravenously into a perfusion tube carrying a TS solution, was completely neutralized in vitro. HN2, injected intraarterially at dose levels up to 4.0 mg. per

kilogram over a period of 45 minutes, was effectively neutralized intravascularly by the simultaneous intravenous administration of 200 mg. per kilogram of TS. TS protection against the toxic effects of HN2 was studied in liver perfusion in dogs. Nitrogen mustard at the dose of 2.0, 2.5, and 3.0 mg. per kilogram was given via portal vein or hepatic artery while TS was injected simultaneously via femoral vein.[12] An isolated arterial system of a canine extremity has also been perfused with HN2 while TS was given systemically.[29] Recently, Anderson[1] confirmed the results of the abovementioned studies, showing that when HN2 was infused in dogs intra-arterially, while TS 10^{3}M per molar of nitrogen mustard was infused into the ipsilateral femoral vein, complete protection was obtained from the local and systemic effects of HN2. A successful protection of bone marrow by TS has been achieved also in 12 patients with cerebral gliomas when HN2 was infused intra-arterially and intravenous TS administered simultaneously in amounts of 250 mg. per milligram of HN2.[27]

The ratio of TS to nitrogen mustard is important. In the study of Hatiboglu and co-workers,[19] no protection was seen when 1.5 Gm. per kilogram of TS was administered instead of 3.0 Gm. per kilogram; in that of Anderson,[1] the dose of TS was 10^{3}M per molar of HN2. Most of the other investigators[12, 29, 32] found the dose of 200 mg. per kilogram adequate. Our animal data (Table VI) clearly demonstrate that by decreasing the dose of TS to 100 to 200 mg. per kilogram, the protection against CPS is reduced.

In the work of others, TS protected against HN2 in many situations, whereas in our studies TS gave negligible protection against HN2; in rodents, however, TS was highly effective against CPS. Besides the importance of the dose of TS used, the route of administration of the alkylating agents is an important factor in these protection studies. Following intravenous injection, HN2 leaves the circulation rapidly and enters cells to produce its characteristic cytotoxic action on proliferating cells. Under physiologic conditions HN2 is rapidly converted to its reactive ethylenimonium form, which can react with many substances, including TS. When introduced by the intravenous route, HN2 may be able to penetrate into cells before it is converted to a reactive form, and it is thus not neutralized effectively by TS. When HN2 is injected subcutaneously, intraperitoneally, or regionally, however, it may have sufficient time to be converted to its reactive form before it is absorbed into the systemic bloodstream; thus, TS may protect effectively against HN2, locally administered. CPS, on the other hand, is in a reactive form immediately on injection and it can react with, and be inactivated by circulating TS before the CPS reaches the cell.

A second factor may be the size of the animal. Protection against intravenously injected HN2 appears to be poorer in small rodents than in larger animals, such as dogs and man. One factor may be the rate of distribution of HN2. In the small animal HN2 may reach and penetrate cells more rapidly in relation to the formation of the ethylenimonium derivative, and it is thus not inactivated. In larger animals, the circulation time and diffusion of the drug through the tissues may be slower and there will be more time for the conversion of HN2 to its reactive form.

In man, TS protected almost as well against HN2 as compared to CPS following intravenous injection, in contrast to the results in rats and rabbits. Besides the factor of species size, drug dosages were different in the human and rodent studies. The rodents received single supralethal doses with TS, whereas in man a supralethal dose was fractionated into 2 to 9 daily injections. While the cumulative dose was high in man, the circulating concentrations of HN2 and CPS achieved in patients was considerably lower than that in animals. The dosage of the alkylating agents was fractionated in man for reasons of safety; but, if single supralethal doses of HN2 and CPS could have been given, there might

have been a more distinct difference in the protective effect of TS on HN2 and CPS.

The intent of this study was to find a more reactive form of HN2 for clinical use in regional infusion and perfusion, and to determine if a selective local effect of CPS could be further enhanced by protecting the patient against the systemic leak of CPS with TS. The availability of CPS and of TS, as a protective agent against HN2 and CPS, did not appear to be of substantial therapeutic interest. In most situations where an alkylating agent is used for perfusion, local toxicity in the perfused areas is the limiting factor rather than systemic toxicity due to the systemic absorption of the drug. TS did not appear to provide substantially greater protection against CPS as compared to HN2 in man. Furthermore, a protective agent, injected systemically, may leak into an area under isolation perfusion, and does enter an area under continuous infusion. These occurrences complicate the delivery of optimal local doses of an alkylating agent. When TS protects against the toxicity of these alkylating agents, it would appear to neutralize its therapeutic properties as well.

Summary and conclusions

Nitrogen mustard (HN2) is converted to its ethylenimonium form under physiologic conditions. This form of nitrogen mustard, available as a stable picrylsulfonate salt (CPS), reacts with thiosulfate (TS) in vitro, and its specific biologic activity is neutralized. The ability of TS injected intravenously to prevent the toxicity by the intravenous route of HN2 and CPS in vivo in rats, rabbits, and man has been investigated.

In rats and rabbits 1.0 Gm. per kilogram of TS injected before the alkylating agents protected against supralethal doses of CPS, whereas it gave minimal protection against lethal doses of HN2.

In humans 200 mg. per kilogram of TS protected against multiple doses of CPS as well as HN2: the total dose of the alkylating agents administered ranged from 2 to 9 times the usual total therapeutic dose. From the limited data available, TS appeared to give somewhat better protection against CPS than HN2. With a single exception, no tumor regression was observed in 7 patients with metastatic carcinomas following the administration of HN2 and or CPS following TS. The toxicologic effects of HN2 and CPS were prevented or diminished by TS, and the therapeutic benefit from the combination was negligible.

Laboratory studies on the ethylenimonium derivative of HN2 and protection against its effects with thiosulfate were begun by Dr. Gilmour Harris and Dr. Leo H. Buchner in our laboratories in 1959-1960.

We wish to acknowledge the advice and cooperation of Drs. Frederick S. Philips and Irwin H. Krakoff, and the technical assistance of Miss Karen Artzt.

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result from hydrolysis is negligible and would have little effect on the ratio (*1*), whereas the liberation of pantoate would have a considerable effect.

These observations do not detract from the possible usefulness of pantoyltaurine and other analogues of pantothenic acid against Group A streptococci and presumably other organisms which are incapable of utilizing, or cannot readily utilize, pantolactone or pantoic acid for pantothenic acid synthesis. As already indicated, reduction of analogue concentration (by hydrolysis) would be inconsequential. Furthermore, the inhibition of growth of Group A streptococci, for instance, with pantothenic acid analogues is an established fact.

Summary

Like pantothenic acid, pantolactone, and the pantoate ion, pantoyltaurine and other pantothenic acid analogues of this type antagonize the inhibitory effect of salicylate on *E. coli*.

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Interference With the Antibacterial Action of Streptomycin by Reducing Agents[1]

Amedeo Bondi, Jr., Catherine C. Dietz, and Earle H. Spaulding

Temple University School of Medicine, Philadelphia

The authors have repeatedly observed that the antibacterial action of streptomycin is significantly reduced by incubation in an anaerobic jar. When filter-paper discs are saturated with streptomycin and placed on the surface of infusion blood agar plates inoculated with *E. coli*, the resulting zones of inhibition on plates incubated anaerobically are much smaller than those on the comparable aerobic plates. The reduced activity of streptomycin anaerobically is not due to better growth conditions, since *E. coli* produces its maximum growth when incubated aerobically. Similar results may be obtained with staphylococci and other species of bacteria whose growth is favored by aerobic incubation.

These observations seemed to us to be significant, and further studies were carried out, the results of which suggest that they may have some bearing on the antibacterial action of streptomycin.

Method and results. Duplicate sets of infusion agar plates containing 2-fold concentrations of streptomycin were inoculated with various species of bacteria. One set of plates was incubated aerobically, the other in the anaerobic jar by the palladium-hydrogen method as modified by Spaulding and Goode (*3*). Plates were read for evidence of growth inhibition at the end of 24 and 48 hours incubation at 37° C. Table 1 shows the results of these tests.

TABLE 1

Inhibition of Bacterial Growth by Streptomycin on Infusion Agar

Organism	Incubation	Growth* in presence of streptomycin; units/ml. of agar							
		None	1.0	2.0	4.0	8.0	16.0	32.0	64.0
E. coli-S	aerobic	4	4	1	–	–	–	–	–
	anaerobic	3	3	3	3	3	3	–	–
E. coli-M	aerobic	4	4	2	–	–	–	–	–
	anaerobic	3	3	3	2	–	–	–	–
S. aureus-SM	aerobic	4	4	1	1	1	–	–	–
	anaerobic	4	4	3	3	2	2	–	–
S. aureus #7739	aerobic	4	1	–	–	–	–	–	–
	anaerobic	3	3	3	3	2	1	–	–
S. paratyphi B	aerobic	4	4	4	3	–	–	–	–
	anaerobic	3	3	3	3	2	2	2	1
S. typhimurium	aerobic	4	4	4	3	2	–	–	–
	anaerobic	2	2	2	2	2	2	1	–
Pneumo. Type I	aerobic	4	4	4	4	4	1	–	–
	anaerobic	4	4	4	4	2	1	–	–
Str. Hemolyticus-C-203	aerobic	4	4	4	4	4	3	–	–
	anaerobic	4	4	4	4	3	1	–	–

* Readings made at end of 24 hours incubation; 48-hour readings were essentially the same.

Under anaerobic conditions the amount of streptomycin necessary to produce bacteriostasis was from 2 to 16 times greater than the comparable aerobic concentration. With one exception (*S. aureus*-SM), these results were observed only with organisms which produced their maximum growth on aerobic plates.

A similar experiment was carried out with extract agar. Although there was some evidence that the antibacterial action of streptomycin was greater on the aerobic plates, the difference was not large. It appeared that various reducing agents in the infusion medium played a role in inhibiting streptomycin anaerobically. The greater reducing power of infusion agar was evident from the observation that methylene blue in a concentration of 1:100,000 in this medium

[1] Streptomycin used in this study was generously supplied through the courtesy of Dr. D. F. Robertson, Merck and Company, Inc., Rahway, New Jersey.

was reduced during anaerobic incubation, while methylene blue extract agar plates similarly incubated were not reduced. The presence or absence of streptomycin had no apparent effect.

The anti-*E. coli* action of streptomycin was subsequently determined in tryptone and infusion broth containing cysteine or sodium thioglycollate (Table 2).

TABLE 2

THE EFFECT OF CYSTEINE AND THIOGLYCOLLATE ON THE ANTIBACTERIAL ACTION OF STREPTOMYCIN AGAINST *E. coli*

Medium	Inhibiting concentration of streptomycin Units/ml.
Tryptone*	1.0
Tryptone plus 0.1 per cent thioglycollate	2.0
Tryptone plus 0.1 per cent cysteine	64.0
Infusion†	16.0
Infusion plus 0.1 per cent thioglycollate	64.0
Infusion plus 0.1 per cent cysteine	> 128.0

* Difco tryptone 1.0 per cent.
† Veal infusion, 1.0 per cent tryptose, 0.03 per cent glucose.

Aside from the added SH compounds, infusion broth itself contains interfering substances, since 16 times as much streptomycin was necessary for bacteriostasis in the infusion control as in the tryptone control. The presence of cysteine increased the concentration required for bacteriostasis 64-fold in tryptone and at least 8-fold in infusion broth; the increase with thioglycollate was only 2- to 4-fold.

TABLE 3

EFFECT OF INORGANIC REDUCING AGENTS ON THE ANTIBACTERIAL ACTION OF STREPTOMYCIN AGAINST *E. coli*

Reducing agent*	Inhibiting concentration of streptomycin Units/ml.
Tryptone control	2.0
0.5 per cent sodium formate	32.0
0.05 per cent stannous chloride	32.0
0.05 per cent sodium bisulfite	32.0
0.05 per cent sodium hydrosulfite	16.0
0.5 per cent sodium thiosulfate	32.0

* Final concentration in tryptone broth.

The effect of cysteine on streptomycin could be due either to a reducing action or to some specific chemical reaction, especially with the sulfhydryl groups. The inactivation of penicillin (*2*) and other antibiotic agents (*1*) by cysteine is well known. The action of cysteine on streptomycin appears, however, to be different from that on penicillin, as was demonstrated in the recent report of Denkelwater, Cook, and Tishler (*4*), published while this manuscript was being prepared. These workers state that the inactivation of streptomycin by cysteine is reversible. Similar interference by sulfhydryl compounds with the antibacterial properties of mercurials (*6*) and quinones (*3*) has been reported.

If interference with the activity of streptomycin by sulfhydryl compounds is due to reducing powers, other reducing agents should likewise interfere with its action. Consequently, various reducing agents have been tested. Each of the substances studied definitely antagonized the antibacterial action of streptomycin against *E. coli*, as shown in Table 3. It is evident that the antibacterial action of streptomycin is greatly diminished by a variety of reducing agents other than sulfhydryl compounds.

Discussion. Reducing agents definitely antagonize the antibacterial activity of streptomycin. Further work will be required, however, to determine the true nature of this interference. Whether it is due to a lowering of oxygen tension or to some specific chemical reaction or both acting simultaneously, is still unknown. The SH compounds and the inorganic reducing agents could function in either capacity. The reduction in activity of streptomycin in the presence of glucose, as reported by Waksman and co-workers (*9*), could be attributed to the reducing action of this carbohydrate.

It is entirely possible that this phenomenon is related to the mode of action of streptomycin. This antibiotic displays its greatest activity against bacteria which grow better aerobically than anaerobically, e. g. *E. coli*, *S. aureus*, and *Myco. tuberculosis*. None of the obligate anaerobes is susceptible (*7*). The antibacterial action of streptomycin may be due to its ability to block some enzyme system, oxidative in nature, which is essential only to the growth of susceptible aerobic bacteria, an enzyme system which anaerobes do not possess. If this is true, it is possible that the antagonism by the reducing agent is a specific effect upon this streptomycin-enzyme relationship rather than a direct effect on the streptomycin itself. In fact, addition of the SH compounds and the inorganic reducing agents to a concentrated solution of streptomycin (1,000 units/ml.) does not result in material loss of activity, although Denkelwater and co-workers (*4*) recently reported that this antibiotic in a lower concentration was inactivated by cysteine.

Certain other practical implications are worthy of note. It is conceivable that in certain parts of the body where organic reducing agents are present or where a low oxygen tension exists, larger concentrations of streptomycin may be necessary to inhibit the growth of bacteria. Elias and Durso (*5*) recently reported that typhoid bacilli were isolated from stools in spite of the presence of large concentrations of streptomycin. These investigators suggested the presence in the body of a substance inhibitory to strepto-

mycin. It remains to be seen whether these two phenomena are related.

It is evident that the choice of a medium is important in assaying streptomycin and in determining the susceptibility of bacteria to its antibacterial activity. Because of its antagonism for streptomycin, an infusion medium would be an unwise choice for this purpose. Eventually one of these reducing agents may prove helpful in testing the sterility of streptomycin solutions or in the culturing of fluids from patients under treatment. Cysteine may prove to be such an agent.

Summary. The antibacterial activity of streptomycin in infusion agar plate cultures of *E. coli* and other bacteria is diminished by anaerobic incubation. The bacteriostatic activity of this antibiotic for *E. coli* is reduced in the presence of cysteine, sodium thioglycollate, stannous chloride, sodium bisulfite, sodium hydrosulfite, sodium formate, and sodium thiosulfate. Cysteine was the most active of the agents tested. Further investigation is necessary to determine the nature of this interference. It is possible that this phenomenon is related to the mode of action of streptomycin.

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Water-level Regulator for a Series of High-temperature Water Baths[1]

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It was necessary in this laboratory to conduct a series of tests at 70° C., the nature of which required the use of water as a bath liquid. The tests were carried out in two water baths, and, as efficient covers for the baths were not feasible, the diagrammed system (Fig. 1), set up in less than an hour, served to maintain the levels in both baths to within ¼ in.

In operation, the reservoir bottle, located on a shelf above the water baths, is filled from a tap, J, by closing screw-clamps, I and I′, and opening clamp I″. After the reservoir bottle is filled, clamp I″ is closed and I and I′ are opened. Line D extends below the surface of bath B and serves as a delivery tube, while line E is the regulator and cuts off the supply of air to the reservoir, F, when it is reached by the water level in the bath. Siphon C keeps the level in bath A at the same level as that in B.

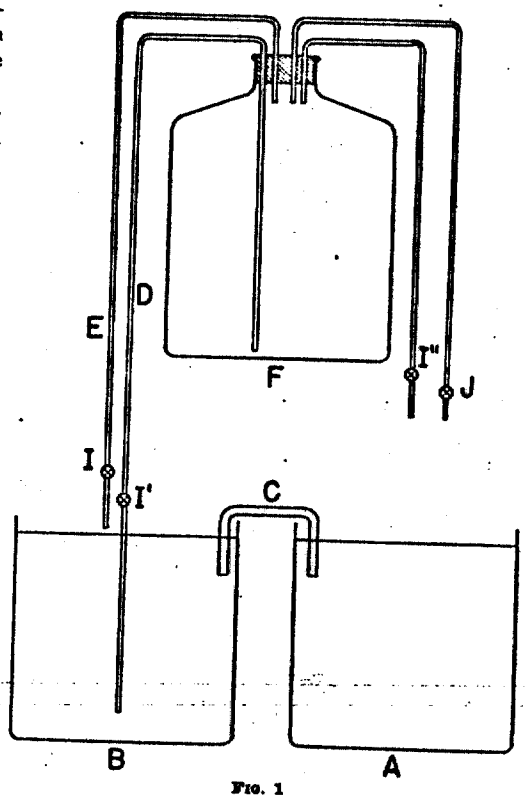

Fig. 1

The baths are of 6-gallon capacity, and the reservoir bottle holds 5 gallons. If the reservoir is filled in the late afternoon, this capacity is sufficient to make up for evaporation overnight. The tubes leading out of the reservoir are of glass, and the cork holding them is sealed in with DeKhotinsky cement. The siphon is of 15-mm. glass tubing to preclude stoppage by air bubbles.

With a sufficiently large reservoir, the system can be used to control a large number of baths. If the cost of the water is neglected, as it can be in most cases, this arrangement has a definite advantage over mineral oil baths in that cleaning of any glassware placed in the baths is much less troublesome.

[1] Contribution from the Industrial Fellowship on Optical Cements, Mellon Institute. Published by permission of the donor, Ordnance Department, U. S. Army.

2) La transformation en ammoniums quaternaires, des corps (I) et II), augmente leur action antinicotinique, ainsi que leur pouvoir inhibiteur vis-à-vis de l'excitabilité du bout périphérique du vague, d'une part, et de l'excitabilité du splanchnique, d'autre part. Cette augmentation d'activité est à peu près du même ordre si l'on compare entre eux les divers ammoniums quaternaires vis-à-vis de l'excitabilité [illegible] la phase parasympathique de l'action vasopressive de la nicotine. Par contre, elle revêt une particulière ampleur dans le seul cas des bromobenzylates si l'on considère l'excitabilité splanchnique [illegible] rtensive de l'action de la nicotine.

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[illegible]ire de Pharmacologie, Faculté de Médecine).

Thiosulfate

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Action anticurarimimétique de certains hyposulfites.

par J. Cheymol, F. Bourillet et H. Balestrieri.

Dans un travail récent, nous avons montré l'action anticurarimimétique de deux dithio-hydantoïnes (1*). Poursuivant la prospection de substances antagonistes des curarimimétiques parmi les molécules soufrées, nous apportons aujourd'hui les résultats d'expériences obtenus avec des hyposulfites.

Ces sels ont été essayés sur la préparation nerf-sciatique-jambier postérieur du Lapin anesthésié au somnifène (0,5 cm³/kg, voie i. v.), soumis à la respiration artificielle ; curarisants et antagonistes étant injectés dans la veine jugulaire (2*).

Nous avons recherché l'action antagoniste éventuelle de ces substances sur deux curarimimétiques : la D-tubocurarine et le 336 H. C. (3*).

L'hyposulfite de sodium à doses relativement élevées (100 à 150 mg/kg) a une action antagoniste nette sur la D-tubocurarine (fig. 1, tracé 1) et le 336 H. C. Le sulfate de sodium n'ayant pas d'action anticurarimimétique, on est en droit d'admettre que ces effets sont dus à l'anion $S_2O_3^{--}$. Si l'on remplace le cathion Na^+ par K^+, NH_4^+, Ca^{++}, Mg^{++}, on obtient des actions différentes selon l'élément métallique envisagé.

Antagonisme progressivement croissant vis-à-vis des curarimimétiques pour $S_2O_3Na_2$, S_2O_3Ca, $S_2O_3K_2$ (v. figure 1) ; prolongation de l'action curarisante pour $S_2O_3(NH_4)_2$ et S_2O_3Mg (v. figure 2).

(1*) J. Cheymol, P. Chabrier, F. Bourillet et C. Levassort, *J. de Physiol.*, 1954, t. 46, p. 294.

(2*) Préparation nerf-muscle excitée 10 fois par minute à l'aide d'une valve électronique au néon.

(3*) 336 H. C. = di-(iodoéthylate de bis pipéridyléthyle) pipérazine. R. Hazard, J. Cheymol, P. Chabrier, E. Corteggiani et F. Nicolas, *Arch. int. Pharmacodyn.*, 1950, t. 84, p. 237 ; 1952, t. 90, p. 78 ; *Thérapie*, 1950, t. 5, p. 129.

L'ion K^{+} a des propriétés anti-curares bien connues. Il augmente nettement les effets semblables de $S_2O_3^{--}$. On obtient une action com-

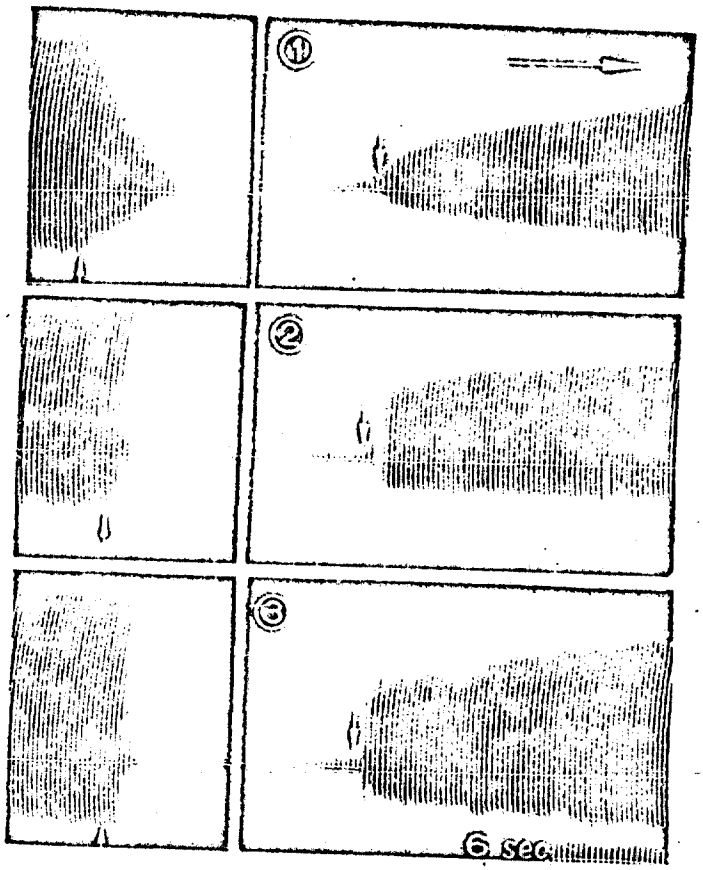

Fig. 1. — Action sur la préparation nerf sciatique-jambier postérieur de Lapin.
Tracés de gauche (↑), curarisation par D-tubocurarine 120 à 200 µg/kg.
Tracés de droite (↓).
1. — Hyposulfite de sodium : 150 mg/kg ;
2. — Hyposulfite de calcium : 150 mg/kg ;
3. — Hyposulfite de potassium : 50 mg/kg.

parable à celle de $S_2O_3Na_2$ avec des doses 3 à 5 fois moins grandes pour $S_2O_3K_2$ (v. fig. 1, tracés 1 et 3).

Nous avons vérifié que l'action anticurarimimétique ne peut être rattachée à un pouvoir anticholinestérasique de ces molécules (4*).

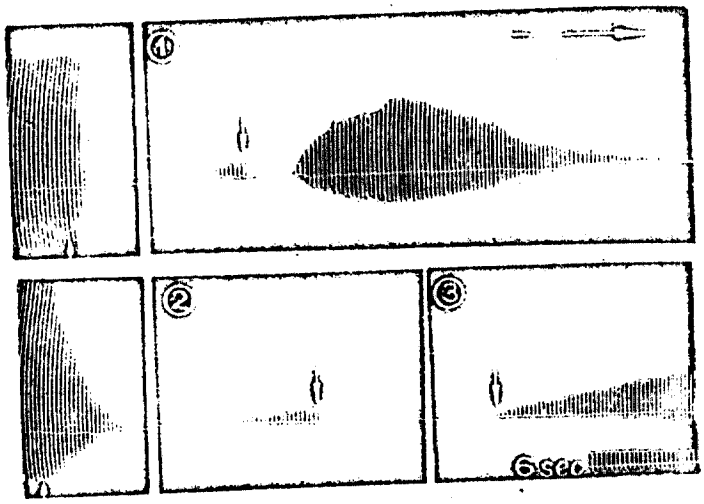

Fig. 2. — Action sur la préparation nerf sciatique-jambier postérieur de Lapin.

Tracés de gauche (↑), curarisation par D-tubocurarine 120 à 200 µg/kg.
Tracés de droite (↓).
1. — Hyposulfite d'ammonium : 100 mg/kg ;
2. — Hyposulfite de magnésium : 100 mg/kg ;
3. — Néostigmine : 200 µg/kg (cliché réduit aux 10/13).

Les cathions NH_4^+ et Mg^{++} sont curarisants, ce pouvoir masque l'action anticurarimimétique de $S_2O_3^{--}$ et impose le sens de l'activité de la molécule dans $S_2O_3(NH_4)_2$ et S_2O_3Mg (v. figure 2, tracés 1 et 2). Cette action paralysante est particulièrement nette si on injecte la solution lorsque les mouvements reprennent légèrement. Le $S_2O_3(NH_4)_2$ donne de curieuses figures avec arrêt, reprise très brève puis à nouveau curarisation. Le S_2O_3Mg fait réapparaître la curarisation, curarisation que l'on peut lever par la néostigmine (v. fig. 2, tracé 3).

Ces faits peuvent être résumés dans le schéma suivant :

$S_2O_3^{--}$ anticurarimimétique				
$S_2O_3K_2$	S_2O_3Ca	$S_2O_3Na_2$	$S_2O_3(NH_4)_2$	S_2O_3Mg
Action anticurarimimétique			Action curarisante	
+++	++	+	+	++

(4*) Essais réalisés sur la pseudocholinestérase sérique humaine et sur la cholinestérase vraie du cerveau de Cobaye.

Nous y voyons l'action curarisante des ions Mg et NH_4 s'imposer dans la molécule de l'hyposulfite correspondant. L'action anticurare de l'ion K augmente, comme il est logique, la tendance du même type de l'anion $S_2O_3^{--}$ (5*). Plus inattendu est l'effet anticurare de S_2O_3Ca, intermédiaire entre ceux de $S_2O_3K_2$ et $S_2O_3Na_2$, étant donné l'antagonisme habituel des ions K et Ca.

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Modifications chimiques du muscle au cours de la sénescence chez le Rat.

par JEAN-CLAUDE DREYFUS, GEORGES SCHAPIRA et FRANÇOIS BOURLIÈRE.

Les modifications biochimiques du muscle strié imputables aux processus de sénescence sont encore peu connues. Ayant eu l'occasion d'étudier 5 rats mâles, âgés de 1.050 jours, présentant des signes cliniques évidents de sénilité, nous avons pratiqué sur leurs muscles un certain nombre de dosages, ainsi que sur un nombre égal ou supérieur de témoins de moins de un an et sur trois animaux en parfait état âgés de 900 jours.

Les composés suivants ont été dosés : collagène [méthode de Lowry et coll. (1)] ; glycogène ; fractions phosphorées ; enzymes de la glycolyse : glycogénolyse totale [quotient lactique, mesuré d'après Utter et coll. (2) et Meyerhof et Wilson (3)], phosphorylase [d'après Cori et Cori (4)], phosphoglucomutase [d'après Najjar (5)], aldolase [d'après Sibley et Lehninger (6)], hexokinase [d'après Long (7)]. Le tableau suivant résume nos résultats.

Il est impossible, sur un nombre aussi restreint d'animaux, de prétendre tirer des conclusions définitives. Néanmoins un certain nombre de données se dégagent du tableau :

(5*) Bacq et coll. ont montré l'action sensibilisante au K de l'hyposulfite de sodium sur le *rectus abdominis* de Grenouille. Z. M. Bacq, *Arch. int. pharmacodyn.*, 1942, t. 67, p. 323 ; E. Vanremoortere, M. Goffart et Z. M. Bacq, *Arch. exp. Path. Pharmakol.*, 1950, t. *212*, p. 31 ; M. Goffart, Z. M. Bacq, *Ergebn. der Physiol.*, 1952, t. 47, p. 556 ; E. Vanremoortere, J. Lecomte, Colloque sur la physiologie du potassium, C.N.R.S., Paris, 1954, p. 1.

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Comptes Rendus Soc. de Biologie 148: 1062 (1954).

thiosulfate

ANTICURARIMIMETIC ACTION OF CERTAIN HYPOSULFITES

by J. Cheymol, F. Bourillet and H. Balestrieri

In a recent study we reported the anticurarimimetic action of two dithio-hydantoins (1*). Continuing our study of the sulfur molecules that act as antagonists of the curarimimetics, we shall present in this report our results obtained with hyposulfites.

These salts were tested on the sciatic nerve preparation from the hind limb of the rabbit anesthetized with a soporific (0.5 cm^3/kg, intravenous route) and subjected to artificial respiration. The curare agents and their antagonists were injected into the jugular vein (2*).

We studied the eventual antagonistic action of these substances against two curarimimetic agents: d-tubocurarine and H.C. 336 (3*).

In relatively high doses (100 to 150 mg/kg) sodium hyposulfite has a definite antagonistic action against D-tubocurarine (Figure 1, tracing 1) and against H.C. 336. Sodium sulfate has no anti-curarimimetic action and this is to be expected if its effects are due to the presence of the anion $S_2O_3^{=}$. If the cation Na+ is replaced by K^+, NH_4^+, Ca++, Mg++, different actions are obtained, depending on the element used.

(1*). J. Cheymol, P. Chabrier, F. Bourillet and C. Levassort, J. de Physiol., 1954, t. 46, p. 294.

(2*). A nerve-muscle preparation stimulated ten times per minute by means of an electronic neon valve.

(3*). H.C. 336 = di(iodoethylate of bis-piperidylethyl) piperazine. R. Hazard, J. Cheymol, P. Chabrier, E. Corteggiani, and F. Nicolas, Arch. Int. Pharmacodyn., 1950, t. 84, p. 237; 1952, t. 96, p. 78; Therapie, 1950, t. 5, p. 129.

The antagonism increases progressively against the curarimimetics for $S_2O_3Na_2$, S_2O_3Ca, $S_2O_3K_2$ (see Figure 1); with $S_2O_3(NH_4)_2$ and S_2O_3Mg (see Figure 2) there is prolongation of the curare action.

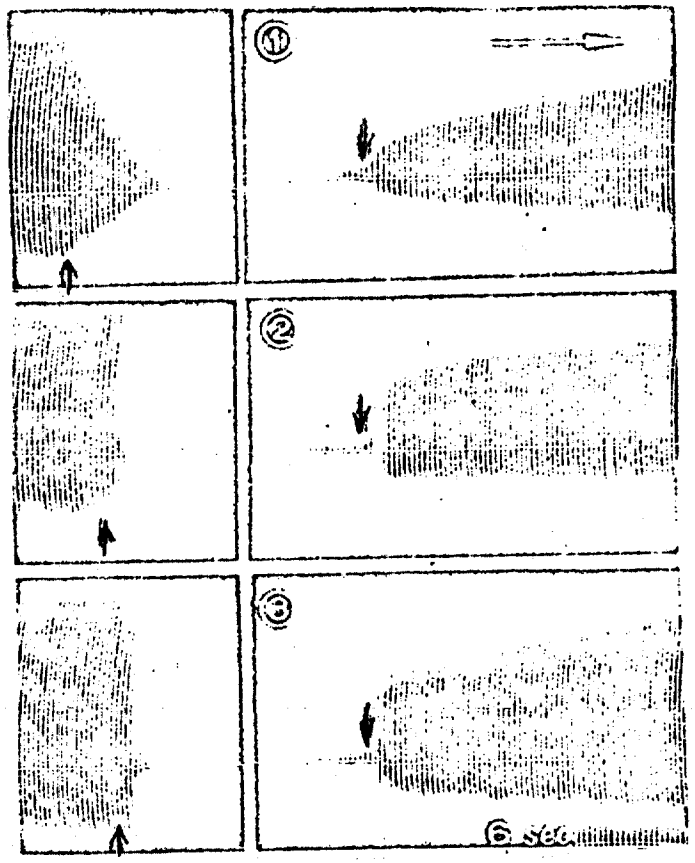

Figure 1. Effects on a sciatic nerve preparation from the hind leg of a rabbit.

The left tracings (↑); curarization with D-tubocurarine in doses of 120 to 200 micrograms/kilogram.

Right tracings (↓).

1.- Sodium hyposulfite: 150 mg/kg;

2.- Calcium hyposulfite: 150 mg/kg;

3.- Potassium hyposulfite: 50 mg/kg.

The K^+ ion possesses well known anti-curare properties. It definitely augments the effects resembling $S_2O_3^{--}$. Doses three to five times lower than $S_2O_3K_2$ (see Figure 1, tracings 1 and 3) produce an action comparable to that of $S_2O_3Na_2$.

We have confirmed the fact that the anticurarimimetic action cannot be connected to the anticholinesterase activity of these molecules (4*).

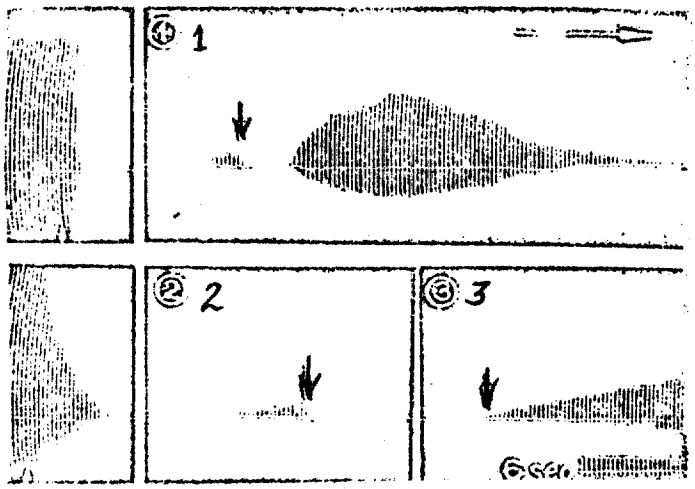

Figure 2. Action on the sciatic nerve preparation of the hind leg of a rabbit.

Left tracings (↑); curarization obtained with D-tubocurarine in doses of 120 to 200 micrograms/kilograms.

Right tracings (↓).

1.- Ammonium hyposulfite: 100 mg/kg;

2.- Magnesium hyposulfite: 100 mg/kg;

3.- Neostigmine: 200 micrograms/kg (the photo is reduced to 10/13).

(4*). The tests were performed on human serum pseudo-cholinesterase and on the true cholinesterase obtained from guinea pig brain.

The cations, NH_4^+ and Mg^{++} are curarizing agents; this ability is masked by the anticurarimimetic activity of $S_2O_3^{--}$ and is responsible for the activity of the molecule in $S_2O_3(NH_4)_2$ and S_2O_3Mg (see Figure 2, tracings 1 and 2). This action is particularly marked if the solution is injected when the movement is slightly regained. $S_2O_3(NH_4)_2$ produces a curious tracing with cessation, then there is a brief recovery and then again a new curare effect. S_2O_3Mg produces the reappearance of curarization, a curarization that may be removed with neostigmine (see Figure 2, tracing 3).

These facts may be summarized in the following manner:

$S_2O_3^{--}$ anticurarimimetic				
S_2O_3K	S_2O_3Ca	$S_2O_3Na_2$	$S_2O_3(NH_4)_2$	S_2O_3Mg
anticurarimimetic action			curare action	
+++	++	+	+	++

Thus we observe that Mg^{++} and NH_4^+ ions have a curare effect within the corresponding hyposulfite molecules. The anticurare effects of the K^+ ion increase, as it would logically be expected, the same type of tendency in the anion $S_2O_3^=$ (5*). More unexpected is the anticurare effect of S_2O_3Ca, acting intermediately between $S_2O_3K_2$ and $S_2O_3Na_2$, and which is normally regarded as the antagonist of the K^+ and Ca^{++} ions.

(Faculty of Medicine - Pharmacology Laboratory)

(5*). Bacq et al. have shown the sensitivity of sodium hyposulfite to potassium on the rectus abdominis of the frog Z.M. Bacq, Arch. int. pharmacodyn., 1942, t. 67, p. 323; E. Vanremoortere, M. Goffart and Z.M. Bacq, Arch. exp. Path. Pharmakol., 1950, t. 242, p. 31; M. Goffart, Z.M. Bacq, Ergebn. der Physiol., 1952, t. 47, p. 556; E. Vanremoortere, J. Lecomte, Colloque sur la physiologie du potassium, C.N.R.S., Paris, 1954, p. 1.

Arch. int. pharmacodyn., 1951, LXXXVI, No 2. 86 : 223 - 226 (1951)

INSTITUT J. F. HEYMANS DE PHARMACODYNAMIE ET DE THÉRAPEUTIQUE.
UNIVERSITÉ DE GAND. DIR. : PROF. DR. C. HEYMANS

INFLUENCES DU MALONITRILE ET DU THIOSULFATE DE SOUDE SUR LES ÉCHANGES RESPIRATOIRES (1)

With Summary

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PAR

Thiosulfate

A. L. DELAUNOIS ET M. KREULA (2)

(Travail reçu le 13-1-1951).

L'introduction, en 1948, du nitrile malonique en thérapeutique par HYDEN et HARTELIUS (1), ainsi que l'utilisation en clinique de nitriles plus stables tel que le dinitrile succinique (2), a attiré à nouveau l'attention sur ces substances.

Déjà en 1896 J. F. HEYMANS et P. MASOIN (3) montrèrent que le thiosulfate de soude est un antidote très efficace du nitrile malonique. RENTZ (4) étudia un grand nombre d'antidotes de l'intoxication par les dérivés cyanogénés et examina particulièrement la désintoxication du nitrile malonique in vivo par la dihydroxyacetone chez le lapin et la grenouille.

Plus récemment, NASH, DOUCET, EWING et EMERSON (5) ont repris le même problème en étudiant l'action antidote vis à vis du nitrile malonique, du $NaNO_2$, du $Na_2S_2O_3$, du dimercaptopropanol, du glutathion, de la dl-méthionine, du bleu de méthylène, du formaldéhydesulfoxylate de soude et de la dextrose. Ils observèrent, chez la souris, que l'action antidote la plus efficace appartient au thiosulfate de soude.

Nous avons examiné les actions du nitrile malonique et du thiosulfate de soude sur les échanges respiratoires.

MÉTHODE

Les expériences ont été effectuées chez le chien anesthésié à la chloralosane-morphine et maintenu sous respiration artificielle. Les échanges

(1) Communication à la réunion du 13 janvier 1951 de la Société Belge de Physiologie.
(2) Assistant à l'Institut Biochimique, Helsinki.

TABLEAU I

Expériences Dates	Métabolisme Normal		Métabolisme après Malonitrile						Valeur maximale du métabolisme atteinte après $Na_2S_2O_3$		
Chiens Poids	CO_2/min/kg	O_2/min/kg	3″ après l'injection		3″ avant l'injection $Na_2S_2O_3$		Diminution %		CO_2/min/kg	O_2/min/kg	
			CO_2/min/kg	O_2/min/kg	CO_2/min/kg	O_2/min/kg	CO_2	O_2			
Chien 10,5 kg 5-6-50	3,95 cm³	6,00 cm³	5,17 cm³	6,82 cm³	2,99 cm³	1,08 cm³	24,3	81,9	5,64 cm³	7,49 cm³	
Chien 15,5 kg 6-6-50	5,51 cm³	6,85 cm³	6,23 cm³	7,82 cm³	4,39 cm³	3,18 cm³	20,3	53,6	—	—	⊞ après malonitrile
Chien 9,5 kg 8-6-50	3,90 cm³	4,77 cm³	4,81 cm³	5,62 cm³	2,79 cm³	2,49 cm³	28,5	47,8	—	—	⊞ après malonitrile
Chien 17 kg 10-6-50	4,31 cm³	4,96 cm³	5,63 cm³	5,98 cm³	3,40 cm³	2,35 cm³	21,1	60,6	5,87 cm³	5,91 cm³	
Chien 19 kg 14-6-50	3,83 cm³	4,47 cm³	4,86 cm³	5,72 cm³	3,32 cm³	2,16 cm³	13,3	51,7	6,32 cm³	8,13 cm³	

respiratoires furent déterminés en utilisant la méthode diaférométrique de NOYONS (6), suivant la technique décrite par C. HEYMANS et A. L. DELAUNOIS (7).

RÉSULTATS EXPÉRIMENTAUX

Le tableau 1 résume les résultats enregistrés au cours des expériences. On observe que l'injection intraveineuse de malonitrile à la dose de 20 mg/kg provoque d'abord une augmentation suivie d'une diminution marquée et prolongée des échanges respiratoires, allant pour le CO_2 de 13,3 à 28,5 % et pour l'O_2 de 47,8 à 81,9 %.

L'injection intraveineuse, au cours de l'intoxication par le malonitrile, de 400 mg/kg de thiosulfate de soude, en solution à 10 %, détermine une augmentation rapide et considérable des échanges respiratoires.

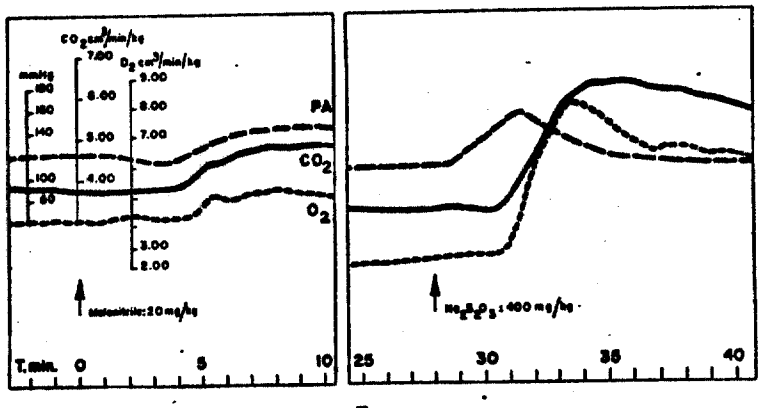

FIG. 1

Courbes d'un enregistrement.
De haut en bas : PA pression artérielle (— — —).
CO_2 Production de CO_2 en cm³/min/kg (———).
O_2 Absorption d'O_2 en cm³/min/kg (- - - - -).
T temps en min.

Les échanges respiratoires dépassent d'abord notablement le niveau normal et diminuent ensuite progressivement jusqu'au niveau normal.

La figure 1 reproduit les courbes du CO_2 éliminé et du O_2 absorbé, ainsi que la courbe de la pression artérielle enregistrées au cours d'une des expériences.

Conclusions

L'intoxication par le nitrile malonique détermine d'abord une augmentation, suivie d'une diminution très marquée des échanges respiratoires, qui est supprimée rapidement par l'injection intraveineuse de thiosulfate de soude.

Summary

Intoxication by malonitril induces a primary increase of metabolism, followed by a marked and prolonged depression. Intravenous injection of sodium thiosulfate restores rapidly the metabolism to normal levels.

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Arch. int. pharmacodyn., 19

LABORATOIRE DE PH
DE L'

A PROPOS DES
ET ANALGÉSIQUES

Jean LA

Une première substanc
appelée par ces auteurs
Ledrut et Combes (2), ce
nous avons pu nous en re
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Dans la suite, Bodendor
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l'objet de la première parti

(1) Communication à la réunion

Arch. Int. Pharmacodyn. 86: 223-226 (1951).

thiosulfate

EFFECT OF MALONONITRILE AND SODIUM THIOSULFATE ON RESPIRATORY EXCHANGE

by A. L. Delaunois and M. Kreula

The introduction of malononitrile into therapeutics in 1948 by HYDEN and HARTELIUS (1) and the application of the more stable nitriles such as succinic dinitrile in clinical practice (2) have again drawn attention to these substances.

In 1896 J. F. HEYMANS and P. MASOIN (3) showed that sodium thiosulfate is a very effective antidote for malononitrile. RENTZ (4) studied a very large number of antidotes to reverse the intoxication caused by cyanogenic derivatives and he also studied in great detail the detoxification of malononitrile in vivo by dihydroxyacetone in rabbits and in frogs.

More recently, NASH, DOUCET, EWING and EMERSON (5) have reviewed the same problem by studying the antidotal action of $NaNO_2$ and $Na_2S_2O_3$ dimercaptopropanol, gluthatione, di-methionine, methylene blue, sodium formaldehydesulfoxylate and dextrose against malononitrile. These authors observed that in mice sodium thiosulfate has the most effective antidotal action.

We have studied the effects of malononitrile and sodium thiosulfate on respiratory exchange.

METHOD

The experiments were carried out on dogs anesthetized with chloralosane-morphine and maintained with artificial respiration. The respiratory exchange was determined by using the diaferometric

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(1) Presented at the January 13, 1951 meeting of the Belgium Society of Physiology.

(2) Assistant at the Biochemical Institute at Helsinki.

TABLE I

Date of Experiment	Normal Metabolism		Metabolism after malononitrile						Maximal value of metabolism after $Na_2S_2O_3$		
Dogs Weight	CO_2/ min/ kg*	O_2/ min/ kg*	3" after injection		3" before injection of $Na_2S_2O_3$		% decrease		CO_2/ min/ kg*	O_2/ min/ kg*	
			CO_2/ min/ kg*	O_2/ min/ kg*	CO_2/ min/ kg*	O_2/ min/ kg*	CO_2	O_2			
Dog 10.5 kg 6/5/50	3.95	6.00	5.17	6.82	2.99	1.08	24.3	81.9	5.64	7.49	
Dog 15.5 kg 6/6/50	5.51	6.85	6.23	7.82	4.39	3.18	20.3	53.6	-	-	after malononitrile
Dog 9.5 kg 6/8/50	3.90	4.77	4.81	5.62	2.79	2.49	28.5	47.8	-	-	after malononitrile
Dog 17 kg 6/10/50	4.31	4.96	5.63	5.98	3.40	2.35	21.1	60.6	5.87	5.91	
Dog 19 kg 6/14/50	3.83	4.47	4.86	5.72	3.32	2.16	13.3	51.7	6.32	8.13	

*All figures given in cm^3

method devised by NOYONS (6), according to the technique described by C. HEYMANS and A. L. DELAUNOIS (7).

Table I summarizes the results recorded during the course of the experiment. An intravenous injection of malononitrile in a dose of 20 mg/kg produced at first an increase followed by a marked and prolonged decrease in respiratory exchange; the carbon dioxide metabolism changed from 13.3 to 28.5 per cent, while the oxygen metabolism was altered from 47.8 to 81.9 per cent.

During malononitrile intoxication an intravenous injection of sodium thiosulfate in a dose of 400 mg/kg as a 10 per cent solution, produced a rapid and considerably increased respiratory exchange.

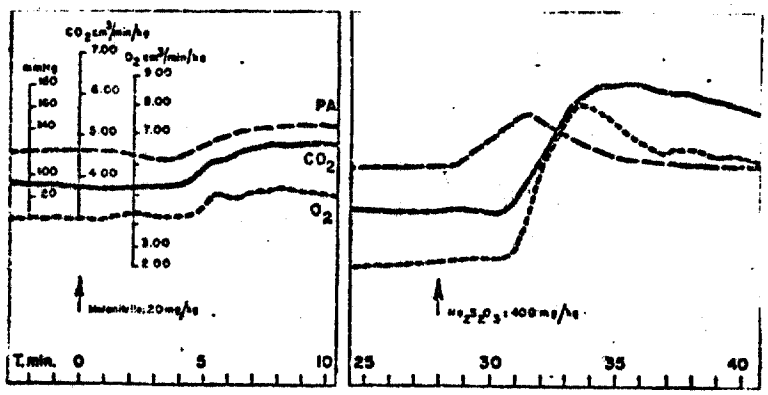

Figure 1

Recording curve.

From top to bottom: PA Arterial pressure (_ _ _)

CO_2 production of carbon dioxide in cm^3/min/kg (——)

O_2 absorption of oxygen in cm^3/min/kg (---)

T time in minutes

The respiratory exchange at first appreciably surpassed the normal level and then progressively decreased until it again

reached a normal level.

Figure 1 reproduces the curves representing the elimination of carbon dioxide and the absorption of oxygen, as well as the curve representing the arterial pressure recording during the course of the experiment.

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TOXICITY OF SODIUM THIOSULFATE (NSC-45624), A NITROGEN MUSTARD ANTAGONIST, IN THE DOG[1,2,3]

Daniel L. Dennis[4] and William S. Fletcher[5]

80

SUMMARY

When dogs were given 3000 mg/kg of sodium thiosulfate pentahydrate intravenously, the following effects developed rapidly: metabolic acidosis, hypoxemia, hypernatremia, changes in the electrocardiogram, and changes in arterial and venous pressure. Only 2 of the 5 dogs survived 24 hours. Doses of sodium thiosulfate pentahydrate as high as 3000 mg/kg could not be used safely in humans.

The possibility of cancer chemotherapy by regional infusion with neutralization of the excess drug by a specific antagonist is a theoretically attractive concept which has been of benefit to patients (1). Most familiar is the regional infusion of methotrexate (NSC-740)[6] with the simultaneous systemic administration of citrovorum factor. Regional administration of 3000 mg/kg of sodium thiosulfate to neutralize lethal doses of nitrogen mustard (NSC-762)[7] has also been described (2). In attempting to treat cancer of the liver by hepatic artery and portal vein infusion (3), we found that 3000 mg/kg of sodium thiosulfate pentahydrate, infused into a leg vein over 15 minutes, resulted in the death of 4 of 5 dogs in less than 24 hours. Three of the dogs died during the injection or immediately afterwards. This prompted the authors to study some of the metabolic and hemodynamic effects of sodium thiosulfate pentahydrate given intravenously (iv).

METHODS AND MATERIALS

Five mongrel dogs weighing 13–21 kg were anesthetized with pentobarbital (NSC-10816)[8] given iv. Cannulas were placed in a femoral artery and vein and connected through Sanborn pressure transducers to a Sanborn recorder, to measure arterial and central venous pressures. Lead II of the electrocardiogram was continuously monitored with a Sanborn recorder. Blood pH, carbon dioxide pressure (pCO_2), and oxygen pressure (pO_2) were determined with an Epsco blood gas analyzer. Serum sodium and serum potassium were determined with the flame attachment to the Beckman DU spectrophotometer. Serum calcium was determined according to the Clark and Collip modification of the Kramer-Tisdall method (4). Sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$), 3000 mg in 10 ml of water per kg of body weight, was injected iv over a period of 20 minutes. The injections were made through a cannula in a forelimb vein. Thereafter, the injection cannula was kept open with a very slow drip of 5 percent dextrose and water.

RESULTS

Of the 5 dogs treated as described, one died a few minutes after the injection of sodium thiosulfate pentahydrate. The remaining 4 survived the period of sampling and 2 were alive

[1] Received Dec 6, 1965; revised Feb 10, 1966.

[2] Supported by grant CA-07208-01 from the National Cancer Inst, Public Health Service.

[3] NSC-45624; sodium thiosulfate pentahydrate.

[4] Instructor in Surgery (Advanced Clinical Fellow, American Cancer Society), Univ of Oregon Medical School, Portland.

[5] Associate Professor of Surgery (Markle Scholar in the Medical Sciences), Univ of Oregon Medical School, Portland.

[6] NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl][methylamino]benzoyl]-.

[7] NSC-762; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride.

[8] NSC-10816; barbituric acid, 5-ethyl-5-(1-methylbutyl)-, sodium deriv.

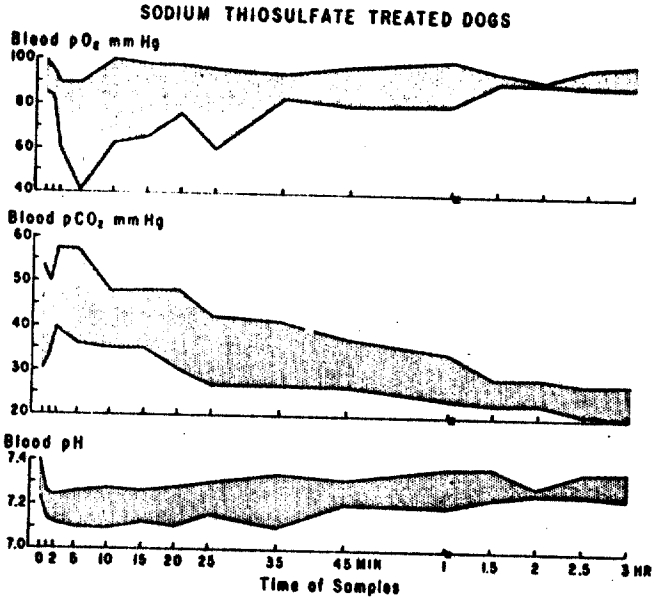

FIGURE 1.—Range of change in blood pH, pCO_2, and pO_2 in 5 dogs after iv injection of sodium thiosulfate pentahydrate, 3000 mg/kg.

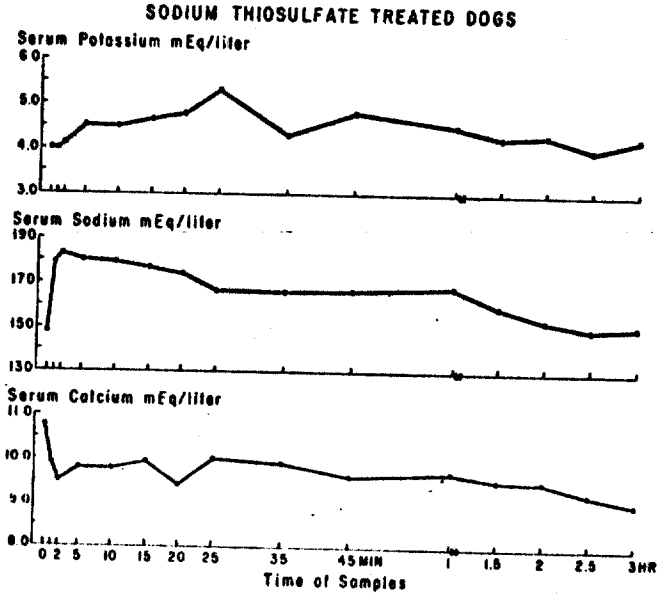

FIGURE 2.—Mean serum electrolyte changes occurring in 5 dogs after iv injection of sodium thiosulfate pentahydrate, 3000 mg/kg.

24 hours after the experiment. During injection of sodium thiosulfate there was a rapid rise in central venous pressure, an increase in the arterial pulse pressure, tachycardia, tachypnea, flattening of T waves, and frequent T-wave inversion. The QRS voltage decreased without a change in the QT interval. There was considerable oozing of blood from operative incisions and gross muscular twitching.

The arterial pH and pO_2 decreased and the pCO_2 increased in the first few minutes after the injection (fig 1). The most remarkable electrolyte change was a rapid rise in the serum sodium concentration (fig 2). Serum potassium increased and serum calcium decreased. In 2 dogs, observed for 3 hours, the arterial pH, pO_2, serum sodium, and serum potassium concentration returned to preinjection levels. Arterial pCO_2 decreased to below preinjection levels and the serum calcium concentration remained depressed.

DISCUSSION

Sodium thiosulfate is distributed primarily through the extracellular fluid space and is rapidly excreted by the kidneys (5). In these experiments the immediate and rapid rise in the serum sodium concentration would be expected from an injection of 3000 mg/kg of sodium thiosulfate pentahydrate, since the sodium ion in sodium thiosulfate pentahydrate is about 24 mEq/3000 mg. (In a subsequent experiment, one dog was given 24 mEq of sodium chloride per kg buffered to pH 7.4 intravenously in a concentration of 24 mEq/10 ml of water. The metabolic and hemodynamic effects were the same as those after injections of sodium thiosulfate pentahydrate.)

Rapid metabolic acidosis and hypoxemia developed in these dogs. One dog died immediately after the thiosulfate injection and showed pronounced pulmonary edema. The osmotic load given to these dogs in 20 minutes would produce a pronounced, acute water shift. The large water shift and change in pulmonary compliance might account for the decrease in arterial pO_2.

Although urine output was not measured in each instance, the bladder filled to overflowing within minutes of the injection, and dogs surviving the injection had a marked diuresis. This would be expected from the large osmotic load given. The muscular twitching, recorded cardiovascular effects, metabolic acidosis, and decrease in arterial oxygen tension are probably due to the hyperosmolality and hypernatremia. Hypernatremia in humans is associated with a similar clinical and metabolic picture. The use of sodium thiosulfate given systematically to neutralize the ethylenimonium ion of nitrogen mustard during local infusion is theoretically sound. However the concentration of the compound needed to achieve neutralization threatens survival. Subsequent work in this laboratory suggests that 1500 mg/kg of sodium thiosulfate pentahydrate given iv at a constant rate over a 30-minute period is well tolerated and will also neutralize lethal doses of nitrogen mustard given 15 minutes after the sodium thiosulfate is started (Fletcher, W. S., and Awe, W. C., unpublished data).

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rend obligatoire pour les coiffeurs, même instruits des dangers des teintures, la touche d'essai, la lecture de cette touche et l'appréciation de la capacité réactogène du sujet.

M. Tara (3), dans une communication présentée ici-même, traitant des relations qui pouvaient exister entre la pratique des épidermo-réactions et la responsabilité professionnelle médicale, a rappelé avec raison non seulement les difficultés d'appréciation des résultats, mais encore les accidents d'ordre général qui pouvaient se manifester à l'occasion des tests cutanés. Il rapportait deux observations dramatiques de réaction à la quinine où la responsabilité médicale aurait pu être engagée.

Pour notre part nous estimons que le décret du 16 février 1951 donne aux coiffeurs des possibilités d'action qu'ils ne peuvent avoir et qu'ils ne doivent pas avoir. Ce décret rend obligatoire, pour des personnes non médecins, la pratique d'actes médicaux ; il se trouve être en contradiction formelle avec l'ordonnance du 24 septembre 1945 sur l'exercice de la médecine.

Une telle orientation législative nous paraît dangereuse, d'autant plus qu'une tendance générale veut faire exécuter par des personnes non compétentes des actes auxquels leur profession ne les destinait pas.

Certains n'ont-ils pas été quelque peu étonnés d'entendre dire, au Conseil Supérieur d'Hygiène Publique de France, que certains médicaments, appelés « médicaments de commandement », pouvaient être ordonnés par le commandement militaire, d'office, sans contrôle ni intervention médicale possible, lorsque certaines conditions de la guerre l'exigeaient, afin de mettre les hommes, en les dopant, si l'on peut dire « en état » ! Des membres du Conseil ont dit que c'était inexact : nous l'espérons !

; tenu à mettre la Société de Médecine légale au courant de pour le moins, peuvent être considérés comme curieux et

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Thiosulfate

Mort subite au cours d'une injection intra-veineuse d'hyposulfite de soude

PAR MM. L. DÉROBERT et R. MARTIN

Les accidents thérapeutiques peuvent avoir de telles répercussions que nous avons pensé intéressant de rapporter devant vous l'observation suivante d'autant plus qu'il s'agissait d'une thérapeutique habituellement considérée comme étant anti-choc et qu'un examen anatomo-pathologique complet mettait justement en évidence les lésions non habituelles des chocs.

Le nommé X..., âgé de 28 ans, est atteint depuis plusieurs années d'eczéma chronique, récidivant et d'asthme. Son médecin traitant habituel a, à de nombreuses reprises, suspendu l'évolution des phénomènes cutanés par une thérapeutique hyposulfitée, considérée comme active et non dangereuse.

(3) Annales de Médecine légale N° 1, Janvier-Février 1950, p. 24-26.

31: 233-235 (1951)

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Depuis deux mois une poussée eczémateuse évolue et il est instaur une thérapeutique intraveineuse de 10 cm3 d'une solution à 20 °/₀ d'hypo sulfite de soude et de 10 cm3 d'une solution à 10 °/₀ de gluconate d calcium suivie d'injection intramusculaire de 20 cm3 de sang prélevé immédiatement après l'injection intraveineuse précédente.

Deux fois par semaine, depuis le début du traitement, ces diverses injections n'ont entraîné aucun trouble.

Le 7 décembre 1948, le nommé X..., se rend, après la cessation de son travail, au cabinet de son médecin qui pratique, comme d'habitude, l'injection intraveineuse au cours de laquelle le malade ne se plaint d'aucun malaise. Avec la même aiguille et la même seringue restées en place il prélève par voie veineuse 20 cm3 de sang lorsque subitement, avant que l'aiguille ait été retirée du vaisseau ponctionné, le malade se plaint d'angoisse intense avec sensation de constriction thoracique, d'étouffements, de soif d'air.

Le médecin fait immédiatement allonger le patient, se rend compte qu'un trouble respiratoire profond se manifeste, pratique des manœuvres de respiration artificielle qui n'améliorent nullement la gêne respiratoire, la cyanose du visage. Avec l'aide de Police-Secours le malade est transporté très rapidement à l'Hôtel-Dieu où l'on constate, à son arrivée, le décès.

L'examen anatomique montrait effectivement des lésions d'eczéma des membres supérieurs, du cou et de la face. Il était en outre à remarquer que les muscles, ainsi que le sang des cavités cardiaques, présentaient une coloration brunâtre foncée.

Macroscopiquement les poumons étaient emphysémateux, non œdémateux et les vaisseaux semblaient vides de sang. Au microscope ces seules lésions consistaient en un emphysème généralisé, avec quelques ruptures pariétales, une certaine vaso-dilatation avec de fines infiltrations d'hématies intrapariétales qui venaient encore souligner la dilatation alvéolaire et surtout existait un bronchospasme intense et généralisé.

Le foie d'apparence congestif présentait des lésions très importantes de fibrose périlobulaire prédominant autour des espaces fortes réunis d'ailleurs par des travées de sclérose aux espaces de Kiernan, une sclérose moins accentuée autour des vaisseaux sushépatiques, une infiltration très importante plasmo-lymphocytaire périforto-biliaire.

Existait également un œdème péri trabéculaire mais aucune vasodilatation des capillaires radiés, aucune hémorragie.

La cellule hépatique offre un cytoplasme pâle mais très fortement granuleux aux noyaux acidophiles, peu chargés en chromaxine, certains éléments sont en nucléolyse et cette dernière lésion est prédominante dans les espaces porto-biliaires.

Si le parenchyme hépatique offrait des lésions anciennes de fibrose on retrouvait également au niveau du rein des lésions de sclérose peritubulaire et des lésions de néphrite chronique.

Certains glomérules apparaissaient scléreux, d'autres œdématiés, d'autres encore hémorragiques. L'espace glomérulo capsulaire se trouvait être le siège d'un magma hyalin dans lequel existait quelques débris cellulaires.

Les éléments des tubes contournés offraient des lésions de nécrose, de coagulation avec nucléolyse et des cylindres abondants. On retrouvait des

lésions identiques au niveau des pièces intermédiaires. Par contre au niveau de la médullaire, en dehors d'une vaso-dilatation importante et d'hémorragies, les tubes collecteurs et les portions descendantes des tubes de Henlé, en dehors de nombreux cylindres, n'offraient pas de lésions caractéristiques.

Macroscopiquement le cœur, la rate, le pancréas un peu volumineux cependant, n'offraient pas de lésions visibles.

La surrénale microscopiquement présentait une glomérulaire peu modifiée, par contre les éléments de la zone fasciculée offraient une surcharge lipoïdique intense. Les cellules de la zone réticulée étaient au contraire en dégénérescence cytoplasmique et en nucléolyse.

La glande thyroïde offrait des vésicules très chargées en thyrocolloïde, tous les vaisseaux étaient fortement dilatés et les hémorragies abondantes.

Du point de vue macroscopique cérébral on notait un œdème méningé intense avec piqueté hémorragique de la matière cérébrale, piqueté prédominant dans la région bulbaire, mais il n'existait aucune hémorragie.

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SUDDEN DEATH OCCURRING DURING AN INTRAVENOUS INJECTION OF SODIUM THIOSULFATE

by Messrs. L. Dérobert and R. Martin

Since therapeutic accidents can have so many repercussions, we thought it would be interesting to report the following case to you, particularly since it involves a therapy which is usually considered to combat shock and since a complete necroscopic examination did show unusual lesions of shock.

The patient X..., aged 28 yr, had had chronic, recurrent eczema and asthma for several years. Using conventional therapy, his physician had stopped the development of skin symptoms by using a thiosulfate preparation repeatedly; this was considered to be active and safe.

An episode of eczema had been developing for two months, and intravenous therapy was instituted with 10 ml of a 29% sodium thiosulfate solution and 10 ml of a 10% calcium gluconate solution followed by intramuscular injection of 20 ml blood taken immediately after the above intravenous injection.

These various injections had not caused any difficulties when administered twice a week since institution of therapy.

On December 7, 1948, patient X... went after work to the office of his physician who gave, as usual, the intravenous injection during which the patient did not complain of any malaise. With the same needle and the same syringe remaining in place, he took 20 ml of blood from the vein when suddenly, before the needle

had been removed from the punctured blood vessel, the patient complained of intense distress with the feeling of chest constriction, smothering, and lack of air.

The physician immediately stretched the patient out, became aware of the severe respiratory disorder, performed artificial respiration which did not improve the respiratory distress at all or the facial cyanosis. With the help of the Police Rescue Squad the patient was taken very rapidly to the Hotel-Dieu where he was found to be dead on arrival.

The necroscopic examination did show eczema lesions on the upper extremities, neck, and face. It was also noted that the muscles and blood in the chambers of the heart were dark brown in color.

On gross examination the lungs were emphysematous, not edematous, and the blood vessels appeared to be empty of blood. Under the microscope the only lesions consisted of generalized emphysema with some parietal ruptures, some vasodilation with small infiltrations of intraparietal blood cells which emphasized alveolar dilatation, and severe and generalized bronchospasm.

The liver, which appeared congested, had very pronounced lesions of perilobular fibrosis which predominated around large spaces connected with sclerotic bands to Kiernan's spaces; a less pronounced sclerosis around the subhepatic vessels; and very marked periforto [?]-biliary infiltration of plasmocytes and lymphocytes.

There was also peritrabecular edema, but without vasodilation of the radial capillaries and bleeding.

The hepatocytes had a pale but very granular cytoplasm with acidophilic nuclei containing little chromaxine, some elements undergoing nucleolysis, and this latter lesion was found primarily in portobiliary spaces.

While the hepatic parenchyma showed old fibrotic lesions, lesions of peritubular sclerosis and chronic nephritis were also found in the kidney.

Some glomeruli appeared to be sclerotic, others were edematous, and still others were hemorrhagic. The glomerulocapsulary space was found to be the site of a hyalin magma in which some cell debris was present.

Elements of the convoluted tubules showed lesions of necrosis, coagulation with nucleolysis, and many cylinders. Identical lesions were found in some intermediate parts. However, no characteristic lesions were found in the medulla with the exception of many cylinders in collecting tubules and descending parts of Henle's loops, and pronounced vasodilation and hemorrhages.

On gross examination no lesions were found in the heart, spleen, or pancreas although the latter was somewhat enlarged.

Microscopically the adrenals had a slightly changed zona glomerulosa, while cells in the zona fasciculata contained abnormally large amounts of lipid-like material. On the other hand, cells in the zona reticulata were undergoing cytoplasmic degeneration and nucleolysis.

The thyroid gland contained vesicles with large amounts of thyrocolloid, all of the blood vessels were markedly dilated,

and there was abundant bleeding.

Gross examination of the brain showed marked meningeal edema with hemorrhagic spots in the cerebral material and spots predominating in the bulbar region. However, there was no bleeding.

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SPECTROPHOTOMETRIC DETERMINATION OF SODIUM THIOSULPHATE IN BODY FLUIDS BY USE OF AN IODINE–AMYLOSE COMPLEX

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(Received January 22nd, 1962)

Sodium thiosulphate is used in the determination of the body extracellular water[1] and for the assessment of renal glomerular filtration rate. These methods give plasma sodium thiosulphate (anhydrous) concentrations up to 400 μg/ml which have usually been determined by the direct iodimetric titration method of BRUN[2], or by the indirect iodimetric titration method of NEWMAN *et al.*[3]. The direct method gives a smaller plasma blank value and is more specific; in particular the determination may be carried out on plasma samples containing *p*-aminohippurate. The indirect method is eight times more sensitive than the direct, but plasma blank values may be as high as 30 μg/ml. It has been modified to use plasma ultrafiltrate containing sodium thiosulphate in the range 0–200 μg/ml[4], but cannot be considered a true micro method, since at least 50 μl of plasma ultrafiltrate are required for analysis. The method to be described is simpler and quicker to carry out than the techniques described above, and requires 20 μl of plasma ultrafiltrate, or 50 μl of plasma, for each estimation.

EXPERIMENTAL

Reagents

Iodine solution. Potassium iodide (1 g) and iodine (60 mg) are dissolved in a small quantity of distilled water, and 1 l of distilled water added. This solution should be stored in a dark bottle. *Amylose solution.* Amylose* (0.5 g) is added to 100 ml of water and the mixture boiled for 10 min. Hyflo supercel is added, and after cooling the mixture is centrifuged at 3000 × *g* for 5 min. The amylose solution is then decanted and filtered through Whatman No. 1 filter paper. 10 ml of acetic acid are then added and the solution stored at 4°. The solution should be discarded with the appearance of opalescence. *Amylose–iodine reagent A.* The following are mixed in the order given:

Acetic acid	100 ml	Iodine solution	60 ml
Distilled water	450 ml	Amylose solution	50 ml

Iodine solution or distilled water is used to adjust the absorbance of the solution at 590 mμ to 0.75. The solution is then filtered through a No. 4 glass sinter. The reagent is stable for at least two weeks at room temperature and for two months at 4°, although the absorbance has occasionally to be readjusted. *Amylose–iodine*

* Obtained from B.D.H., Poole, Dorset, Eng.

reagent B. This is made up in a similar manner to amylose–iodine solution A, with the addition of sufficient iodine solution to bring the absorbance at 590 mμ to 0.90. *Sodium sulphate*. (1.34%) 1.34 g of $Na_2SO_4 \cdot 10H_2O$ are made up to 100 ml with distilled water. *Sodium tungstate*. (2.5%) 2.50 g of $Na_2WO_4 \cdot 2H_2O$ are dissolved in distilled water and made up to 100 ml. *Sulphuric acid*, 1/6 *N*. *Sodium thiosulphate standard*. Working standards containing 0–200 μg/ml must be prepared freshly each week and stored at 4°.

Method A

This procedure makes use of plasma ultrafiltrate, obtained using the procedure described by DIXON AND MAKELA[4]. 20 μl of ultrafiltrate samples, distilled water, and of standards, are placed in test tubes using a Lang-Levy constriction pipette. A blow-out pipette is then used to add 1.000 ml of amylose–iodine reagent A to each tube. Immediately after each addition a piece of parafilm is placed over the mouth of the test tube, and the contents mixed by shaking. The solution is then transferred to a cuvette of 1-cm light path and the absorbance at 590 mμ determined in a Unicam SP 600 visible spectrophotometer, using a distilled water blank. The sample may be read immediately since air bubbles clear rapidly.

Method B

50 μl of plasma are washed into 200 μl of sodium tungstate (2.5%) and 200 μl of sulphuric acid (1/6 *N*) added. The mixture is stirred with a glass rod. After 10 min the protein precipitate is spun down for 1 min at 7000 × *g*. A plasma blank is similarly prepared. 200 μl of supernatant are then transferred to a dry test tube. The standards are treated in a similar way, but sodium sulphate (1.34%) is substituted for the sodium tungstate (2.5%) and sulphuric acid (1/6 *N*). 50 μl of distilled water provide a blank for the standards. 1.000 ml of amylose–iodine reagent B is then added to each tube and the absorbance at 590 mμ determined immediately, against a distilled water blank.

EXPERIMENTAL RESULTS

Sodium thiosulphate was added to plasma and to water to give concentrations of 0–1/600 *N*, *i.e.* 0–264 μg/ml. Analysis by each method gave the values shown in Table I. The plasma blank values were zero.

For method A the gradients indicate a recovery from plasma ultrafiltrate of 107.5%. The theoretical recovery $\frac{100\%}{1-\text{protein fraction}}$ was $\frac{100}{1-0.07} = 107.5\%$. Absorbancies below 0.15 were however unreliable. Method B gave a recovery of 97.8%. This result was probably significantly different from the theoretical recovery of 100% ($p = 0.1$). Previous workers have also noted recoveries less than the theoretical when protein precipitation was used. When distilled water replaced sodium sulphate (1.34%) recovery was 95% and the absorbance at 590 mμ obtained from plasma samples was higher than that from corresponding standards.

The effect of some naturally occurring substances and laboratory chemicals of interest is shown in Table II. Groups (i) and (ii) are non-interfering substances.

TABLE I

Sodium thiosulphate (µg/ml)	*Method A*		*Method B*	
	water	*plasma ultrafiltrate*	*water*	*plasma*
0.00	741	743	742	738
26.4	667	672	682	670
52.8	601	598	620	617
79.2	536	527	554	566
105.6	462	450	507	501
132.0	397	370	438	436
158.4	328	296	363	379
184.8	263	232	314	318
211.2	189	160	257	258
237.6	135*	100*	—	—
264.0	74*	45*	136	142
Gradient	2.59	2.875	2.303	2.26
Recovery	$\frac{2.785 \times 100}{2.59} = 107.5\%$		$\frac{2.26 \times 100}{2.303} = 97.8\%$	

Absorbance × 1000 is shown in the Table. Method A consists of the addition of 1.000 ml of amylose–iodine reagent A to 20 µl of plasma ultrafiltrate. Method B consists of the addition of 1.000 ml of amylose–iodine reagent B to 200 µl of a 1 : 9 dilution of protein-free plasma.

* These values do not lie on the straight line joining the other nine points and they have not been used in the calculation of the gradient.

TABLE II

1 *Group*	*2* *Substance*	*3* *Quantity added (µg)*	*4* *Effect on blank*	*5* *Effect on standard thiosulphate soln.*
(i)	Sodium citrate	10		
	Sodium chloride	900		
	Creatinine	50		
	Uric acid	20		
	Bilirubin	2	No effect	No effect
	Urea	100		
	$FeSO_4 \cdot 7H_2O$	280		
	Glucose	100		
	Glycine	25		
	Sodium arsenate	0.4		
(ii)	Sodium glutamate	5	Low consumption of iodine	Iodine consumed slowly after reaching control level
	Tyrosine	5		
	Phenylalanine	5		
	Arginine	5		
	Antipyrine	4		
(iii)	Albumin	50		Less iodine apparently consumed
	$NH_4FeSO_4 \cdot 12H_2O$	480	No effect	
	$CuSO_4 \cdot 5H_2O$	250		
(iv)	Pyruvic acid	5		
	Ammonium sulphate	50	Rapid consumption of iodine	Excessive consumption of iodine
	Sodium arsenite	0.2		
	Sodium oleate	0.4		
	Glutathione	4		

The effect of the quantity given in Column 3 of the various substances on 1.000 ml of amylose–iodine reagent A is shown in Column 4. Column 5 describes the effect of half the quantity given in Column 3 on a thiosulphate standard containing 3 µg of sodium thiosulphate.

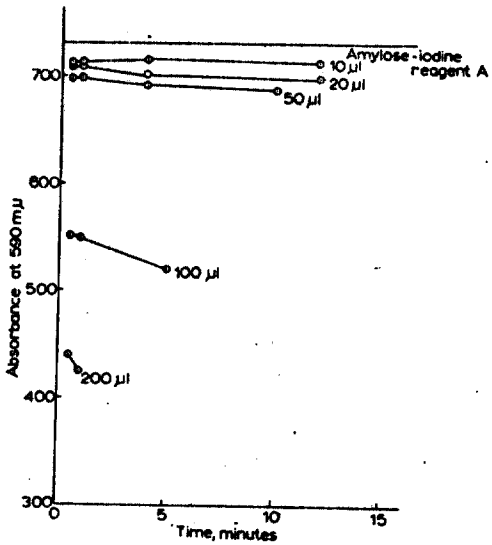

Fig. 1. The effect of interfering substances present in plasma ultrafiltrate. 1.000 ml of amylose–iodine reagent A was added to the quantity of plasma ultrafiltrate indicated.

Those in group (ii) gave a falling absorbance at 590 mμ of less than 0.005/min. Varying quantities of plasma ultrafiltrate were added to 1 ml of amylose–iodine solution A. The results (Fig. 1) show a falling absorbance with time related to amount of plasma. 20 μl of plasma ultrafiltrate was chosen as the maximum quantity taken for assay.

DISCUSSION

Starch was first used for the spectrophotometric determination of iodine by SENDROY AND ALVING[5], and SENDROY[6], who pointed out that the blue colour obtained was variable. When amylose was used in the estimation of oxidising agents and iodine[7, 8] it was necessary to wait 15 min for maximum colour development. PASCHALL AND FOSTER[9] using potentiometric titration methods showed that the reaction of iodine and amylose approached equilibrium slowly, but that back-titration of the blue complex with thiosulphate took place rapidly and was accompanied by low free iodine concentrations, in equilibrium with the iodine–amylose complex. To avoid the effect of this marked hysteresis on the spectrophotometric estimation of thiosulphate ion, it is necessary to formulate a stable amylose–iodine solution, and make use of the fall in absorbance on addition of thiosulphate solution. If thiosulphate is added to a standard iodine solution before the amylose, other substances present in biological fluids will react with the iodine during the necessary equilibration period.

LOEWUS AND BRIGGS[10] have show that 0.05% aqueous solutions of amylose were more stable than those of greater concentration, and that the rate of fall of

iodine-binding capacity accompanying retrogradation was least when the solution contained 0.1 *M* potassium iodide. Retrogradation was slowest at pH 4—5.5, but the binding capacity was reduced below pH 6.9. Similarly it has been shown that strong electrolytes, and in particular polyvalent cations, cause precipitation of the amylose-iodine complex[7, 11] and that acetic acid has a stabilising effect on the blue complex[12]. Acetic acid has however the effect of moving the absorption maximum from 620 mμ to 590 mμ; solutions containing acetic acid in strengths greater than 30% have low absorbancies, and have a reddish-blue colour. Similar effects have been observed in this laboratory, where it has also been found that the presence of 0.3 *N* hydrochloric or 0.3 *N* phosphoric acid results in the atmospheric oxidation of iodide ion and the precipitation of the blue complex within a few hours.

Sufficient potassium iodide must be present both for the initial formation of the blue complex[12] and for the reaction with thiosulphate[14]. When potassium iodide is present in quantities significantly greater than 100 mg/l, atmospheric oxidation becomes apparent even in solutions made acid with acetic acid.

The amylose-iodine reagent also suffers loss of iodine because of its volatility, and it is necessary to make up volumes of at least 500 ml and to keep the solutions in glass-stoppered bottles.

The formulation described above minimises the effect of atmospheric oxidation, volatility of iodine, and retrogradation; precipitation of the amylose-iodine complex has not been observed, but retrogradation occurs slowly with a fall in iodine-binding capacity. This may result in the absence of sufficient amylose activity, and it was found necessary to determine whether this had occurred with stored reagents. The addition of 5 ml of amylose solution to 500 ml of amylose-iodine reagent results in a slight fall in absorbance at 590 mμ if the solution contains sufficient amylose activity, whilst insufficient activity is shown by an increase in absorbance. In the latter event excess amylose solution may be added, and the reagent will give satisfactory results; it is however preferable to discard the reagent. The quantity of iodine present in the reagent, giving an initial absorbance of 0.75, represents the maximum range of 0.75–0.15 of the reagent. For smaller quantities of thiosulphate greater precision would be obtained using a reagent having an initial absorbance in the region of 0.5.

The methods described above are suitable for the estimation of the high concentrations of sodium thiosulphate occurring in urine during tests of kidney function. Since however the amylose-iodine reagent is not specific it is unlikely to be suitable for the estimation of sodium thiosulphate occurring in normal urine, since even the relatively specific method of SORBO[15] suffered from interference. The method could be used for the determination of physiological quantities of sodium thiosulphate however in conjunction with the use of triethylenediamine nickel nitrate, a precipitant for thiosulphate ion, as described by SPACU AND SPACU[16]. Such a method would be analogous to that of GAST *et al.*[17], in which the consumption of iodine was determined by measurement of the iodine absorption maximum at 355 mμ.

The amylose-iodine complex provides a reagent which may prove suitable for spectrophotometric estimation of other iodine-consuming substances in the range of 0–0.025 μequiv., though such possibilities were not explored in the present study.

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SUMMARY

The spectrophotometric determination of thiosulphate ion in body fluids by a micro method is described. 20 μl of plasma ultrafiltrate or 50 μl of plasma containing 0–250 μg/ml sodium thiosulphate are required. The reagents described are suitable for the determination of other iodine-consuming compounds in quantities of 0–0.025 μequiv.

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EXCRETION OF SODIUM THIOSULPHATE BY THE PERFUSED KIDNEY[1]

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Among tests commonly used for the determination of the function of the kidney, Nyiri's test occupies a special place. Sodium thiosulphate, unlike the injected dyes or any other substances which are secreted by the urine almost completely, is partly oxidized in the organism into the stable compound, sodium sulphate, and partly excreted by the kidneys unchanged. The rate of excretion of thiosulphate, as Nyiri claimed, is conditioned by the functional value of the renal parenchyma, that is, the more thiosulphate in the urine, the better the function of the kidney. Clinical experience, although not extensive, is not in contradiction with this claim.

Holbøll, Humbert and Finck, Eskelund, Iversen, Iversen and Fasting-Hansen, and Elaut have tried the thiosulphate test in a series of varied diseases of the kidney, both medical and surgical. They found the new method reliable; however, they did not show evidence of any superiority over the usual tests, such as the phenolsulphonephthalein test, determination of the blood urea or the tests of concentration and dilution. There is certainly no reason as yet to abandon the other tests in favor of the pretended electivity of thiosulphate as a particular test for vascular impairment of the kidney.

There is little experimental investigation on record concerning excretion of sodium thiosulphate by the kidney. Most such investigations have been done by Nyiri. The data may be summarized as follows: when sodium thiosulphate is injected in-

[1] Work done in the Division of Experimental Surgery and Pathology, The Mayo Foundation. Submitted for publication May 19, 1931.
[2] C. R. B. Fellow in Urology.

J. Urology, 26 1931

travenously, it is oxidized in the organism quickly; about 45 per cent of 1 gram of the salt injected into a normal dog is destroyed, the remaining 55 per cent passes unchanged into the urine, and after two hours no trace of thiosulphate can be detected in the urine. When it is injected subcutaneously or intra-abdominally, or when it is given by mouth, the excretion lasts longer and the amount of thiosulphate destroyed is greater. Data or suggestions are not available concerning the site of oxidation.

In the determination of the content of thiosulphate in the urine, an iodine solution is used after the urine has been cleared with charcoal and the iodine fixation power which exists in normal urine has been deducted. Among the methods devised for performing the test, the simplest seems to be that of Humbert and Finck in which 10 cc. of half-normal thiosulphate solution (1.24 grams) is injected intravenously; after two hours the amount of salt in the urine is determined volumetrically by means of twentieth-normal iodine solution; 100 cc. of the latter corresponds to 10 cc. of the thiosulphate solution used.

I shall present here observations on the comparison of the excretion of thiosulphate by the perfused kidney when sodium thiosulphate is added to the perfusing fluid as contrasted with the excretion of the same amount of thiosulphate by both kidneys of the intact dog on the day preceding the perfusion. I had hoped to obtain data concerning the relative amount of excretion of the substance and the place where the oxidation of the amount not excreted occurs. I shall disregard completely the clinical aspect of the problem, and also the many objections which have been formulated from the standpoint of clinicians and experimental observers (1, 3).

Verney and Starling showed convincingly that when an isolated kidney is perfused with defibrinated blood by a heart-lung circuit, after a preliminary period of anuria lasting fifteen to thirty minutes progressive polyuria develops which may be likened to the condition of diabetes insipidus. The quantity of urine secreted by the isolated kidney in their experiments was so much greater than previously had been reported as to establish their technic as the most desirable one for perfusing the isolated kidney.

I have had an opportunity to substantiate Verney and Starling's observations and wish to amplify them by detailing a few modifications which have led to the excretion of much larger quantities of urine than they observed. The technic is as follows: Blood is obtained from a large dog whose tissues have been filled with water by the intravenous injection of sodium chloride solution and by the forced administration of water. The blood is defibrinated and diluted after defibrination with a third of its volume of Locke's solution. This circulation fluid is placed in the reservoir of the heart-lung preparation. Ordinarily about 1,200 cc. of blood and 400 cc. of Locke's solution are employed. A small dog is employed for the heart-lung perfusion. After the circuit is established in the usual manner, a kidney is removed. Cannulas are inserted into the renal artery, renal vein, and ureter. The arterial side of the heart-lung preparation is connected to the cannula in the renal artery by rubber tubing, care being taken to avoid air embolism. We found, as did the originators of the method, that it was essential to prevent cooling the kidney. After about half an hour under these conditions the kidney secretes urine which at first is turbid from the contained salts but soon becomes crystal clear. The urine is free from protein and blood pigment. By means of this technic relatively enormous quantities of urine are excreted. The following protocol is illustrative of the output of thiosulphate by the perfused kidney:

The kidneys of a male dog weighing 11 kgm., one of which was to be perfused, were tested with phenolsulphonephthalein and thiosulphate; 65 per cent of phenolsulphonephthalein was recovered in seventy minutes, and 51 per cent of thiosulphate was recovered in two hours after intravenous injection.

The next day a typical heart-lung-kidney preparation was made. At 11 a.m. the kidney was connected with the blood stream; at 11:35 a.m., the flow of urine started. The first drops contained some blood which evidently came from the pelvis and was due to the manipulation. At 11:45 a.m. the urine was perfectly clear; 10 cc. of the half-normal solution of thiosulphate was added (1.24 grams of the salt) to the perfusing blood. A specimen of urine was collected every thirty minutes. Five specimens were collected. The last specimen contained traces of hemo-

globin. The preparation was in good condition at 2:15 p.m., and the experiment was discontinued.

Analysis of the specimens showed that the first specimen, 21 cc., contained 18 per cent of the unchanged thiosulphate; the second specimen, 26 cc., contained 25 per cent of the unchanged thiosulphate; the third specimen, 40 cc., contained 7.2 per cent of the unchanged thiosulpate; the fourth speciman, 61 cc., contained 6.1 per cent of the unchanged thiosulphate, and the fifth specimen, 49 cc., contained 2.9 per cent of the unchanged thiosulphate. The total volume of urine in two hours was 148 cc. The total amount of thiosulphate excreted in two hours was 55.2 per cent.

The striking feature in this experiment was the similarity in the excretion of the thiosulphate by the kidneys of the living animal (51 per cent) and the excretion by the isolated kidney (55 per cent). These results settle conclusively various discussions and presumptions of previous authors as to the pretended electivity of the kidney to excrete or to destroy the thiosulphate. It is evident that the kidney is among the organs that regulate the rate of output of the substance and possibly also among those which destroy it. The oxidation of the thiosulphate takes place within the lung-heart-kidney system and the blood which it contains. Although it is not contended that oxidation occurs in the lung, there is a probability that it does because of the instability of thiosulphate and its liability to be converted into sodium sulphate in the presence of oxygen. A more direct study by means of a heart-lung circuit was not made because I lacked a method of determining accurately the thiosulphate content of the blood, but the sulphate determination in blood and urine may presumably throw some light on the problem. The following protocol represents an experiment which was performed for that purpose:

The kidneys of a male dog weighing 8.5 kgm., one of which was to be perfused, were tested with phenolsulphonephthalein and thiosulphate; 65 per cent of phenolsulphonephthalein was recovered in seventy minutes, and 62.6 per cent of thiosulphate was recovered in two hours after intravenous injection.

The next day a typical heart-lung-kidney preparation was made. At

10:10 a.m. the kidney was connected with the blood stream. At 10:40 a.m. urine started to flow slowly; this urine was collected for sulphate determination. At 11:10 a.m. 10 cc. of the half-normal solution of thiosulphate (1.24 grams) was added to the perfusing blood. A specimen of urine was collected every thirty minutes. Four specimens were collected. At 1:15 p.m. function of the preparation began to decrease, and the experiment was discontinued.

Analysis of the specimens showed that the first specimen, 49 cc., contained 15.7 per cent of the unchanged thiosulphate and 85 mgm. of sulphate (as sulphate) in each 100 cc.; the second specimen, 58 cc., contained 15.6 per cent of unchanged thiosulphate and 74 mgm. of sulphate in each 100 cc.; the third specimen, 38 cc., contained 10.26 per cent of the unchanged thiosulphate and 67.5 mgm. of sulphate in each 100 cc., and the fourth specimen, 28 cc., contained 7.5 per cent of the unchanged thiosulphate and 66.5 mgm. of sulphate in each 100 cc.

The specimen of urine collected before the thiosulphate was added to the perfusing blood contained 43 mgm. of sulphate in each 100 cc. The blood taken before the addition of thiosulphate contained 12.2 mgm. of sulphate in each 100 cc. When the perfusion was finished there was found in the blood 9.85 mgm. of sulphate in each 100 cc. The total amount of urine in two hours was 173 cc. The total percentage of thiosulphate excreted in two hours was 49.06.

In this particular experiment the output of thiosulphate of the perfused kidney was lower than for both kidneys in the living animal. Both figures, however, are still within the ranges of normal renal function and I have often found that in the same animal the rate of the excretion may vary from 5 to 15 per cent.

The urine that comes from a perfused kidney has a low content of sulphate (45 mgm. in each 100 cc.) whereas the normal urine taken from the bladder of the dog previous to the perfusion contained 317 mgm. in each 100 cc. It is obvious that after adding the thiosulphate an increase of the sulphate content of urine would be noticeable. If a kidney is perfused with blood that does not contain thiosulphate, the concentration of sulphate decreases (8). The increase in my experiment is explained by the presence of the thiosulphate, which is constantly oxidized into sulphate. The total amount of the converted thiosulphate, however, cannot be recovered from the blood or urine as sulphate. One would expect

to find retention of the sulphates in the blood. This does not seem to be true since the concentration of sulphate in the blood actually decreased from 12.2 to 9.18 mgm. in each 100 cc., the diminution probably being due to dilution of the blood with about 150 cc. of Locke's solution during the experiment.

SUMMARY

The kidneys of normal dogs were perfused in a heart-lung-kidney preparation. When sodium thiosulphate was added to the perfusing blood the output of this substance in the urine obtained from the isolated kidney was found to be within the limit of the method of experimentation and was similar to the output of the animal on the preceding day.

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SULFITE OXIDATION IN RAT LIVER (*).

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(Mémoire reçu le 12 septembre 1958).

Previous studies in this laboratory demonstrated the presence in mammalian liver of enzyme systems capable of catalyzing the oxidation of inorganic sulfite to sulfate [1, 2, 3]. With dog liver preparations it was possible to observe the catalytic role of hypoxanthine [2] and to implicate lipoic acid [3] in the enzymatic oxidation of sulfite. Studies currently in progress are designed to ascertain the manner in which hypoxanthine participates in this system. Meanwhile, it appeared desirable to re-examine rat liver and to determine whether sulfite oxidation in this tissue is effected in a manner similar to that in dog liver.

EXPERIMENTAL.

Unless otherwise stated all reactions were performed in 0.050 M potassium phosphate buffer, pH 7.8, which contained 0.005 per cent of versene Fe-III to control autoxidation of sulfite. All reagents were prepared with demineralized water. Calcium phosphate gel was prepared according to KEILIN and HARTREE [4]. Crystalline horse liver catalase and xanthine oxidase were obtained from the Worthington Biochemical Corporation of Freehold, New Jersey. Versene Fe-III is a product of the Bersworth Chemical Company. Ammoniacal ammonium sulfate was prepared by mixing 94 parts of saturated ammonium sulfate with 6 parts of 28 per cent ammonia. Protein concentration was determined by the spectrophotometric method of WARBURG and CHRISTIAN [5].

I. *Purification of the Enzyme.*

Unless otherwise stated all manipulations were performed at 0° C. Fresh or frozen rat liver was acetone powdered at 15° C and then stored *in vacuo* over concentrated sulfuric acid until used. Forty grams of liver acetone powder was extracted for two hours by stirring with 400 ml. of cold buffer. Centrifugation of the resultant slurry yielded

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340 ml. of supernatant which was dialyzed against buffer and designated stage *A*. A saturated, neutralized solution of ammonium sulfate was added to stage *A* to 50 per cent saturation. The precipitate, collected by centrifugation and dissolved in buffer to a volume of 300 ml., was designated stage *B*. This was immersed in a hot water bath and stirred mechanically while the enzyme solution was brought to 56° and maintained at that temperature for 5 minutes after which it was rapidly transferred to an ice bath and chilled. The precipitate was removed by centrifugation and discarded and the supernatant solution, designated stage *C*, was placed in an ice-salt bath and fractionated with alcohol at −10°. The fraction which precipitated between 44 and 74 per cent alcohol was collected, dissolved in 250 ml. of buffer and designated stage *D*. This was fractionated with neutralized ammonium sulfate ; the 0 — 50 per cent fraction was dissolved in 125 ml. of buffer and was designated stage *E*. This material was dialyzed for 48 hours against changes of cold water and then was stirred for 20 minutes with 0.3 volumes of calcium phosphate gel. The gel, collected by centrifugation, was eluted with 110 ml. of buffer. The eluate, designated stage *F*, was fractionated with ammoniacal ammonium sulfate and the fraction collected between 42 and 63 per cent saturation was taken up in buffer to a volume of 65 ml. and was designated stage *G*. After thorough dialysis against buffer, this material was frozen in small aliquots until used. Stage *G* is a pale yellow solution which retains sulfite oxidase activity when kept at −15° for more than one year, if not subjected to repeated freezing and thawing. As shown in Table I, this procedure permitted a purification of fifty fold with an over-all yield of 15 per cent.

TABLE I.

PURIFICATION OF SULFITE OXIDASE.

Stage	Specific activity (*)	Per cent recovery
A	0.35	100
B	0.61	—
C	0.90	78
D	2.4	56
E	3.9	—
F	13.8	23
G	17.8	15

(*) A unit of enzyme is defined as that quantity catalyzing an optical density change of 0.01 per minute in the 2,6 dichlorobenzenone indophenol assay. Specific activity is units of enzyme per mg. of protein.

II. *Assays of Sulfite Oxidase.*

A. 2,6 Dichlorobenzenone indophenol is rapidly reduced by 0.001 M sulfite but is slowly reduced by the low concentrations of sulfite

maintained by dissociation of the formaldehyde-bisulfite addition compound. Rat liver sulfite oxidase catalyzes rapid reduction of this dye by the minute steady state concentration of sulfite present in solutions of formaldehyde-bisulfite. Since the leuco dye does not autoxidize appreciably, the assay may be performed in open tubes. In practice, each 18 × 150 mm. tube contained 0.4 μmoles of dye plus enzyme in a final volume of 7.0 ml. buffered as usual by 0.050 M potassium phosphate and containing 0.005 per cent versene Fe-III. The reaction was started by the addition of 1.0 ml. of a 0.010 M solution of formaldehyde-bisulfite and the decrease in optical density at 620 mμ was followed spectrophotometrically. A control from which enzyme was included in every assay. Because of its rapidity, this assay was used in monitoring purification studies.

B. Sulfite reduces methylene blue very slowly in the dark and somewhat more rapidly in bright light. Sulfite oxidase catalyzes rapid bleaching of methylene blue by sulfite. The reaction was performed in anaerobic Thunberg tubes which contained 0.10 μmole of dye plus enzyme in a final volume of 8.0 ml. in the main chamber and 0.5 ml. of 0.040 M sodium sulfite in the side arm. After tipping in the side arm contents, bleaching of the dye was followed at 650 mμ. A control from which enzyme was omitted was included in every assay.

C. The enzymatic oxidation of sulfite may be followed by oxygen uptake in conventional Warburg microrespirometers shaken at 37.5° with air as the gas phase. The main chamber of each Warburg vessel contained enzyme and buffer in a final volume of 2.0 ml. while the side arm contained 20 μmoles of sodium sulfite in 0.20 ml. of buffer.

D. Sulfite exhibits intense absorption in the short ultraviolet region while sulfate does not [6]. The oxidation of sulfite can be assayed by observing the decrease in optical density at 212.5 mμ. The reaction was started by the addition of enzyme to a quartz cuvette containing 1.0 μmole of sodium sulfite in 3.0 ml. of buffer. The blank cuvette contained no sulfite. Due to the intense absorption of radiant energy in this region of the spectrum exhibited by proteins and diverse organic compounds, this assay was useful only with the most purified fractions of sulfite oxidase.

E. Sulfite reacts reversibly with diphosphopyridine nucleotide yielding an adduct which absorbs strongly at 320 mμ [7]. When sulfite oxidase was added to a solution containing 1.0 μmole of sodium sulfite and 6.0 μmoles of the nucleotide in a final volume of 1.5 ml., oxidation of sulfite caused dissociation of the nucleotide-sulfite adduct and a consequent decrease in optical density at 320 mμ. This assay has proven useful in systems containing components which absorb strongly at shorter wavelengths.

In all of these assays of sulfite oxidase, the measured response is proportional to the concentration of enzyme employed and in no case was there any response to boiled enzyme.

III. *Michaelis Constant.*

The influence of substrate concentration on the activity of sulfite oxidase was followed by the direct spectrophotometric assay at 212.5 mμ. The results of these experiments, plotted as reciprocals according to LINEWEAVER and BURK [8], are shown in Figure 1. K_m derived from this data is approximately 5×10^{-5} M. In addition to indicating a high affinity of sulfite oxidase for its substrate, these results establish the absence of sulfite oxidation by free radical chains, in this system, since the rate of sulfite oxidation by free radical chains continues to increase with increasing sulfite concentration [9].

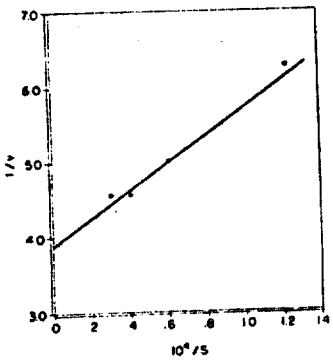

FIG. 1. — Reciprocal plot of the influence of substrate concentration on the rate of oxidation of sulfite catalyzed by rat liver sulfite oxidase. Velocity is expressed as the decrease in optical density at 212.5 mμ catalyzed by the enzyme in 40 minutes at 25° C.

IV. *Production of Hydrogen Peroxide.*

Since hydrogen peroxide reacts rapidly with sulfite, its generation in a sulfite containing system is difficult to establish directly. However, two lines of evidence favor the hypothesis that hydrogen peroxide is produced as a consequence of the enzymatic oxidation of sulfite. Addition of catalase plus ethanol, as a peroxide trap [10], to a Warburg microrespirometer containing 20 μmoles of sulfite and sulfite oxidase, caused a doubling of the total oxygen consumption. Ethanol or catalase alone had no effect on total oxygen consumption although either served to decrease the initial rate of gas uptake. These results are illustrated in Figure 2. Prolonged aerobic incubation of sulfite oxi-

dase with sulfite led to a partial loss of enzymatic activity. Catalase prevented this loss.

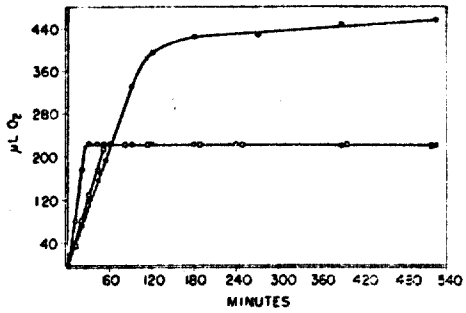

FIG. 2. — Influence of catalase and ethanol on the oxygen consumed during the enzymatic oxidation of 20 μmoles of sodium sulfite. All Warburg flasks contained sulfite plus enzyme. In addition, (— —) contained 40 μmoles of ethanol *or* 150 units of crystalline catalase, while (◑◑) contained 150 units of catalase *plus* 40 μmoles of ethanol.

V. *Inhibition Studies.*

Rat liver sulfite oxidase was inhibited 50 per cent by 10^{-3} M sodium thiosulfate or sodium arsenite. Cyanide up to 10^{-2} M was entirely without effect. Incubation of the sulfite oxidase for 15 minutes at 37.5° with 0.2 volumes of a dialyzed solution of xanthine oxidase, which contained 8.3 mg. of protein per ml. and which catalyzed the oxidation of 0.25 μmoles of hypoxanthine per ml. per minute when acting on 10^{-4} M hypoxanthine at 25°, caused 97 percent inhibition. Similar incubation with a heat inactivated preparation of crude xanthine oxidase caused only 40 per cent inhibition.

VI. *Effects of Hypoxanthine.*

Although rat liver sulfite oxidase could be extensively dialyzed without loss of activity in any assay, the inhibitory effect of xanthine oxidase suggested that hypoxanthine may participate in this system as it was previously observed to do in the dog liver system [2]. Since xanthine oxidase acting on its purine substrates has been found capable of initiating sulfite oxidation by a free radical chain mechanism and since very low levels of the enzyme suffice to cause readily measureable oxygen uptake by this mechanism [11], the effect of hypoxanthine on sulfite oxidation by the rat liver sulfite oxidase was investigated in the presence of 10^{-3} M mannitol. This level of mannitol has been observed to cause complete inhibition of sulfite oxidation by xanthine oxidase plus hypoxanthine. Hypoxanthine at a final concentration of 10^{-3} M was observed to cause a

50 per cent increase in the initial rate of oxygen consumption in the manometric assay of rat liver sulfite oxidase. In the absence of sulfite, hypoxanthine caused no oxygen uptake when incubated with sulfite oxidase nor could uric acid production be detected spectrophotometrically.

Discussion.

The rat liver sulfite oxidase appears to be similar in some respects to the dog liver system previously studied [2, 3]. Thus, both exhibit a high affinity for sulfite, both are inhibited by arsenite, thiosulfate and by incubation with xanthine oxidase and both are accelerated by hypoxanthine when assayed manometrically. However, there are differences which remain, for the present, unexplained. The dog liver enzyme has been separated into two fractions both of which are required to effect sulfite oxidation while the rat liver enzyme is resistant to such fractionation. A similar situation was reported by Lalu and Zannoni [12] for the enzyme system responsible for the oxidation of p-hydroxyphenyl pyruvate to homogentisate, in which case the component enzymes were readily separable in dog liver extracts but not in rat liver extracts. The dog liver sulfite oxidase was activated by added lipoate while the rat liver enzyme was not. Finally the rat liver enzyme showed no requirement for hypoxanthine in the methylene blue reduction assay while the dog liver enzyme exhibited an obligatory requirement for hypoxanthine under the same conditions [2]. Investigation of the mechanism of the enzymatic oxidation of sulfite in these tissues is being continued.

Summary.

An enzyme capable of catalyzing the oxidation of sulfite has been found in rat liver extracts. It has been purified fifty fold and found to catalyze the reduction of oxygen or such dyes as methylene blue and 2,6 dichlorobenzenone indophenol by sulfite. Aerobic oxidation of sulfite by the enzyme appears to be accompanied by the production of hydrogen peroxide. Hypoxanthine accelerates the aerobic oxidation of sulfite by this enzyme. The enzyme is compared to the previously described dog liver sulfite oxidase.

Résumé.

Un enzyme capable de catalyser l'oxydation du sulfite a été trouvé dans des extraits de foie de rat. Il a été purifié cinquante fois, et il catalyse la réduction de l'oxygène ou de colorants tels que le bleu de méthylène et le dichloro-2,6 benzophénone-indophénol par le sulfite. L'oxydation aérobique du sulfite par l'enzyme semble s'accompagner de formation d'eau

oxygénée. L'hypoxanthine accélère l'oxydation aérobique du sulfite par cet enzyme. L'enzyme est comparé à la sulfite-oxydase du foie de chien décrite précédemment.

ZUSAMMENFASSUNG.

Es wird ein Enzym beschrieben, das von uns im Rattenleberextrakt gefunden wurde und das die Fähigkeit besitzt, Sulfit zu oxydieren. Es wurde fünfzigfach gereinigt ; wir beobachteten, dass es die Reduktion von Sauerstoff und Farbstoffen wie Methylenblau und 2,6-Dichlorobenzonindophenol durch Sulfit katalysiert. Aerobe Oxydation von Sulfit durch das Enzym scheint von einer Produktion von Wasser stoffperoxyd begleitet zu sein. Hypoxanthin beschleunigt die aerobe Oxydation von Sulfit durch dieses Enzym. Das Enzym wird mit der kürzlich beschriebenen Hundeleber-Sulfitoxydase verglichen.

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QUANTITATIVE STUDIES ON URINARY THIOSULFATE EXCRETION BY HEALTHY HUMAN SUBJECTS*

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(Received for publication, December 28, 1951)

Since Schmiedeberg (1) first reported the finding of thiosulfate in cat and dog urine, its presence in normal human urine has been the subject of considerable controversy (2, 3). Many of the earlier reports (4, 5) leave the impression that its occurrence was irregular or that it was present in amounts below the sensitivity of the methods in use at that time. Moreover, it has been known since 1861 that thiosulfate was partly oxidized to sulfate when fed to normal men and that the degree of oxidation varied inversely with the amount administered (6). This was confirmed by Lasch (5) and Nyiri (7), but Fromageot recently reported that, in a limited number of subjects, small amounts of thiosulfate are normally present in human urine (8).

All of the quantitative procedures that have been suggested for thiosulfate determination involve complex reactions carried out directly in the urine (3, 9–11). None of these workers succeeded in separating thiosulfate from the other reducing substances present. In 1932, Spacu and Spacu (12) demonstrated that triethylenediamine nickel(II) was a specific precipitant for thiosulfate, and Chamot and Brickenkamp (13) used it in their analytical scheme for sulfur anions.

This reagent was adapted to the quantitative determination of thiosulfate in rabbit urine (14) and applied by Vassel, Partridge, and Crossley to dog urine (15). Preliminary reports on the application of this procedure to the quantitative determination of thiosulfate in human urine (16) and the results in a limited number of subjects (17) have been made. This report is an extension of this series in normal, healthy adults in order to provide a better background for the evaluation of the metabolic origin of thiosulfate.

Methods

The 24 hour specimens were preserved with chloroform and after collection were refrigerated until the analyses were completed. No attempt

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was made to record or control the dietary intake of these subjects, but, in order to evaluate the variations in their diet and metabolism, determinations were made of total urinary nitrogen by the Koch-McMeekin micro-Kjeldahl procedure (18) and of total urinary sulfur by adaptation of Denis and Reed's method (19) to the photoelectric colorimeter.

Thiosulfate Determination

Reagents—

Triethylenediamine nickel(II) nitrate. The crystalline compound was prepared according to the method of Werner (20) by the addition of ethylenediamine (Eastman Kodak 95 to 100 per cent) to nickel nitrate hexahydrate (Baker's c.p. grade).[1] The complex salt was recrystallized four times from aqueous solutions, air-dried, pulverized, and stored in a desiccator over solid sodium hydroxide.

Wash solution. A 1:1 mixture of absolute ethanol and water[2] containing 50 mg. per ml. of triethylenediamine nickel nitrate was prepared and kept refrigerated.

Dilute ammonium hydroxide. 5 ml. of concentrated ammonium hydroxide (c.p.) were diluted to 100 ml. with water.

Approximately 9 N sulfuric acid. A 1:3 dilution of concentrated sulfuric acid (c.p.) is used.

Dilute sulfuric acid. 6.13 gm. of sulfuric acid (c.p.) per liter.

Potassium iodide solution. 5 gm. of potassium iodide (Baker's c.p.) diluted to 100 ml. with water and prepared fresh whenever required.

Stock potassium iodate solution. 0.446 gm. of potassium iodate (Baker's c.p.; oven-dried and stored in a desiccator) is dissolved in water and made to 100 ml. in a volumetric flask.

Dilute potassium iodate (working standard). Stock potassium iodate is diluted 10-fold with water.

Standard iodine solution. 5 ml. of the dilute potassium iodate are added directly to 10 ml. of 5 per cent potassium iodide and 1.0 ml. of dilute sulfuric acid, the flask is swirled, and the volume made up to 1 liter. At this concentration, complete iodine liberation is not attained if the iodate is added after partial dilution. This solution deteriorates slowly when stored in a dark bottle, but its optical density is routinely measured each time before use and the new factor determined from the curve for concentration against optical density prepared from the same batch of stock potassium iodate or by serial dilution with blank solution.

Blank solution. This is a reagent blank containing the same concentrations of potassium iodide and sulfuric acid as the standard iodine solution

[1] Freshly distilled ethylenediamine is not required, as the principal impurity appears to be cobalt from the nickel nitrate.

[2] Wherever water is indicated, fresh, double distilled water was used.

ietary intake of these subjects, but, in their diet and metabolism, determina- rogen by the Koch-McMeekin micro- urinary sulfur by adaptation of Denis oelectric colorimeter.

Determination

rate. The crystalline compound was Werner (20) by the addition of ethyl- 100 per cent) to nickel nitrate hexahy- complex salt was recrystallized four ied, pulverized, and stored in a desic-

absolute ethanol and water[2] contain- mine nickel nitrate was prepared and

ml. of concentrated ammonium hy- ml. with water.

A 1:3 dilution of concentrated sul-

sulfuric acid (c.p.) per liter.

. of potassium iodide (Baker's c.p.) prepared fresh whenever required.

0.446 gm. of potassium iodate (Baker's ccator) is dissolved in water and made

3 standard). Stock potassium iodate

the dilute potassium iodate are added ssium iodide and 1.0 ml. of dilute sul- the volume made up to 1 liter. At liberation is not attained if the iodate his solution deteriorates slowly when al density is routinely measured each determined from the curve for concen- pared from the same batch of stock with blank solution.

blank containing the same concentra- ic acid as the standard iodine solution

not required, as the principal impurity rate.

double distilled water was used.

but with the iodate omitted. This solution is stable for months if kept in a stoppered container in a dark cabinet when not in use and not exposed to sunlight.

Double strength blank solution. 20 ml. of 5 per cent potassium iodide and 2 ml. of dilute sulfuric acid are diluted to 1 liter. This must be prepared fresh when needed.

Absolute ethanol. United States Industrial Alcohol (U. S. P.) as purchased in 1 gallon cans or bottles. Aldehyde-free ethanol prepared according to the method of Palmer (21) may be used.

Chloroform, c.p.

Indicator papers. pH papers covering the range from 1.0 to 3.0 and 5.0 to 8.0 are used.

Procedure

Refrigerated specimens are allowed to come to room temperature and, when sufficient sample is available, a portion is removed for adjustment of pH with ammonium hydroxide to 7.0 to 7.5 by use of indicator papers. Alkaline urines are used without further treatment.

5 ml. aliquots are pipetted into conical Pyrex centrifuge tubes (pH can be adjusted here if insufficient material is available for other analyses). 5 ml. of absolute ethanol are added, the solutions are thoroughly mixed, and the tubes are stoppered and refrigerated between 5–10° for a minimum of 1 hour. After centrifugation at 2500 to 3000 r.p.m. (5.5 inch radius) for 5 minutes, the supernatant solutions are decanted into other conical centrifuge tubes correspondingly marked and 100 mg. portions of powdered triethylenediamine nickel(II) nitrate are added to each tube. Complete solution and mixing are effected with glass rods kept separate for subsequent use in washing and titration. A mauve-colored precipitate usually begins to form upon addition of the precipitating reagent to the urine-alcohol mixture. Complete precipitation is obtained by placing the corked centrifuge tubes in the refrigerator overnight.

After refrigeration the tubes are centrifuged at high speed for 5 minutes, or until the precipitate is firmly packed, and the supernatant fluid is decanted and discarded.[3] The tubes are inverted over a piece of absorbent paper and allowed to drain for 10 minutes. The precipitates are each now washed once with 1 ml. of cold wash solution, with stirring rods to effect thorough washing, and the tubes refrigerated for 0.5 hour before

[3] Where low concentrations of sulfate and thiosulfate are encountered, more reproducible results are obtained by rubbing the crystals off the wall of the tube with a rubber policeman before centrifuging and pouring the supernatant and wash solution through a sintered glass funnel which is subsequently washed with the solution used to dissolve the precipitate in the tubes. The reason for this precaution is the tendency of the small crystals to float or remain suspended.

recentrifugation. The wash solution is discarded and the tubes inverted to drain free of excess wash solution.

The complex salt is dissolved in a small measured volume of blank[4] solution which can be varied in accordance with the amount of thiosulfate expected or found on a trial run, and a drop or two of 9 N sulfuric acid[5] is added from a small diameter dropper to a pH of 1.5 to 2.0 (with indicator paper). The mauve and blue colors of the different complexes are dissipated and the pale green color of the nickelous ion appears. The amount of acid required will depend upon the amount of precipitate, but seldom is it more than several small drops (0.04 to 0.06 ml.).

Estimation of the thiosulfate content of the dissolved precipitate is carried out immediately by the procedure of Sendroy and Alving (22), except that we have used the Beckman model DU spectrophotometer with 1 cm. silica cuvettes at 355 mμ. The steps usually followed are as follows: 5 ml. of standard iodine are diluted with 1 ml. of blank and the optical density is measured as R_s.[6] Next, two 1 ml. aliquots of the dissolved precipitate are pipetted into separate tubes. To one are added 5 ml. of blank solution, the contents are mixed, and the optical density noted; this is R_b.[7] To the remaining tube are added 5 ml. of standard, and after mixing, its optical density, R_r, is immediately read.

[4] The precipitate may be dissolved in either blank solution or water. If the latter is used, a volume of double blank equal to that of the water must be added. This is necessary to maintain the same iodide concentration as is present in the standard iodine solution; otherwise the "effective" color varies, as shown by Sendroy and Alving (22). We have found greater solution stability at lower iodide concentrations than they recommend, and since we did not require the color augmentation they obtained, we sacrificed increased optical density for solution stability.

[5] Due to coprecipitation of triethylenediamine nickel(II) sulfate by ethanol, the recovery of thiosulfate is improved but the bulk of the precipitate is larger. Additional acid is therefore required to dissolve it and to obtain the pH necessary for stoichiometric reaction of iodine and thiosulfate at the concentrations used. When phosphoric acid was used as recommended in the original procedure (22), the excess acid required caused nickel phosphate precipitation; hence sulfuric acid was substituted throughout the procedure.

[6] Serious errors may be introduced in measurements of the optical density of iodine solutions in the ultraviolet region unless readings are made almost instantaneously. This is true both for the standard iodine solution and the residual iodine unchanged by thiosulfate. Decreases in optical density at 355 mμ average about 2 per cent after 20 seconds and 6 per cent after 80 seconds in the light path. Such slight errors can be an appreciable proportion of the final result which is obtained by difference between the reading of the standard and the unknown.

[7] Optical density readings made on acidified solutions of the precipitate disclosed a significant absorption of light at the wave-length used due either to thiosulfate itself or to substances adsorbed on the complex. Therefore all values reported in this paper have been corrected by our R_b reading and no attempt has been made to determine the cause.

is discarded and the tubes inverted

a small measured volume of blank[4]
dance with the amount of thiosulfate
l a drop or two of 9 N sulfuric acid[5]
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Calculations are the same as for any colorimetric determination with suitable corrections for the volume aliquots used. A concentration-optical density curve was run for each batch of standard iodine prepared and found to be identical within the limits of error of measurement.

Testing Method—Since triethylenediamine nickel(II) thiosulfate has a solubility of 2.5 mg. per cent of thiosulfate sulfur in ethanol-water (1:1) at 20° (23), it was necessary to show that thiosulfate is completely precipitated under the conditions used in the determination; *i.e.*, at lower temperature, in the presence of excess precipitating agent and with the coprecipitation of triethylenediamine nickel(II) sulfate.

The data shown in Table I are typical of the results obtained from four

TABLE I

Recovery of Thiosulfate from Aqueous Solutions Containing Sulfate

Standard solutions of sodium thiosulfate containing 22.5 mg. per cent of sulfate sulfur to insure complete thiosulfate precipitation are used. The theoretical concentration of thiosulfate sulfur present was determined by direct iodometric titration for comparison with the amount found by the method described herein. Control titrations with the sulfate solutions alone gave no iodine-titratable material.

Thiosulfate sulfur, mg. per cent		Per cent recovery
Present	Found	
0.44	0.40	91
0.22	0.20	90
0.10	0.11	110

series of recovery experiments carried out in duplicate at eleven different concentrations over a range from 1.0 to 0.05 mg. per cent of thiosulfate sulfur. The average of all the recoveries was 95 per cent and is indicative of complete precipitation from water solutions in the presence of sulfate. Similar experiments, without added sulfate, indicate complete recovery only at concentrations of 0.5 mg. per cent of thiosulfate sulfur or more. This was to be expected from the tendency of the complex thiosulfate salt to become supersaturated (23); this appears to be prevented by the simultaneous precipitation of the complex sulfate. Coprecipitation of triethylenediamine nickel(II) sulfate is therefore essential to make the procedure, as described, quantitative down to concentrations of 0.1 to 0.05 mg. per cent of thiosulfate sulfur. The exact amount of sulfate necessary for this purpose has not been determined, though it has been established that any concentration from 5 to 50 mg. per cent of sulfate sulfur suffices for complete precipitation of thiosulfate and, since the concentrations found in

normal human urines are within this range, the problem was not pursued further.

The limiting factor in the procedure as described is the colorimetric iodine determination which can be extended by increasing the chromogenic equivalence of the iodine, as described by Sendroy and Alving (22), or by increasing the volume of urine from which the thiosulfate is precipitated.

The completeness of recovery of thiosulfate from human urine was proved by the addition of radioactive thiosulfate as the triethylenediamine nickel salt to urine and a determination of the radioactive thiosulfate made on an

TABLE II

Recovery of Urinary Thiosulfate Measured with Added Radioactive Thiosulfate

0.2 ml. of triethylenediamine nickel(II) thiosulfate, labeled with S^{35} and containing 4.56×10^{-1} mg. per ml., was added to a 20 ml. aliquot of each specimen after adjustment of the pH. 50 μl. of this material gave 860 c.p.m. after background correction. Each recovery shown is an average of triplicate determinations on 5 ml. aliquots of the specimens carried through the usual procedure. Self-absorption corrections were made by comparison counts on similar aliquots of the dissolved precipitate from another portion of each specimen to which an equivalent amount of radioactive material was added after the triethylenediamine nickel(II) thiosulfate was dissolved. All the counts were made with a thin, mica, end window Geiger-Müller tube, on the same shelf, spread over the same area, and dried.

Specimen No.	Observed	Corrected	Per cent recovery
	c.p.m.	*c.p.m.*	
5	647	825	96
6	563	809	94
7	517	890	104
8	517	745	93
9	527	825	97

aliquot of the precipitate. Table II shows that precipitation of thiosulfate is quantitative under the conditions of our procedure.

Additional tests of the specificity of the procedure were attempted by precipitation of the triethylenediamine nickel(II) sulfate complex from water solutions containing thirty different compounds (commercially available) of biological interest or containing chemical groups that might react similarly. Thiosulfate was not added in these tests so that the values found by iodimetric titrations would represent apparent thiosulfate or adsorbed iodone-titratable material. Both singly and in groups the following compounds gave negative results in concentrations shown in parentheses (as mg. per cent) after each substance: acetoacetic acid (40), DL-alanine (15), alloxan (20), arginine hydrochloride (10), ascorbic acid (40), L-asparagine (12), creatinine (40), cysteine hydrochloride (20), L-cystine (16), dithiodigly-

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Added Radioactive Thiosulfate

sulfate, labeled with S^{35} and con-
ml. aliquot of each specimen after
800 c.p.m. after background cor-
triplicate determinations on 5 ml.
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orrected	Per cent recovery
c.p.m.	
825	96
809	94
890	104
745	93
825	97

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col (saturated solution), fumaric acid (20), glutathione (10), glycine (10), hippuric acid (25), L-histidine hydrochloride (10), DL-homocystine (12), indole (10), D-lysine hydrochloride (10), methyldisulfide (saturated solution), methylmercaptan (saturated solution), niacin (38), sodium β-glycero-

TABLE III

24 Hour Urinary Thiosulfate, Total Sulfur, and Nitrogen Excretion by Healthy Adults

No. of samples	Sulfur				Total nitrogen	
	S_2O_3		Total			
	Average	Range	Average	Range	Average	Range
	mg.	mg.	mg.	mg.	gm.	gm.
8	11.5	14.9–9.6	1085	1200–1000	14.35	17.2–10.8
8	9.8	16.6–6.2	937	987– 900	13.3	16.7– 5.7
11	10.3	14.8–5.1	858	898– 805	13.5	16.0– 9.6
9	10.2	13.3–5.7	727	760– 700	12.3	14.6– 9.3
12	7.8	12.3–4.7	641	680– 601	10.9	13.6– 9.2
8	6.5	10.8–2.7	582	599– 560	10.2	14.8– 6.7
7	5.6	7.1–3.2	446	490– 404	9.6	12.0– 7.7
7	5.3	9.0–2.1	302	393– 210	8.8	12.9– 6.4

The correlation of thiosulfate sulfur with total nitrogen and total sulfur, assuming multiple linear regression, was evaluated by the method of analysis of variance (24) and the F value obtained was highly significant.

Analysis of Variance

Source	D.F.	Sum of squares	Mean square
Regression, $R^2\Sigma z^2$	2	3894.86	1947.43
Error of estimate, $(1 - R^2)\Sigma z^2$	67	2024.38	30.21
	69	5919.24	

$F = 1947.43/30.21 = 64.46$; $P < 0.01$.

phosphate (34), sodium oxalate (20), sodium thiocyanate (300), taurine (25), thiamine hydrochloride (25), L-tryptophan (12), L-tyrosine (10), urea (120), and uric acid (33).

Results

Table III shows the average values for 70 samples from twenty-eight subjects grouped in descending order of total sulfur excreted. Above a total sulfur excretion of 700 mg. a significantly higher excretion of thio-

sulfate is usually observed. Below this value, with four exceptions out of thirty-four samples, all the results lie well below the means in the first four groups.

TABLE IV

Typical Effect of Varied Protein Intake on Thiosulfate Excretion

24 hour urine collections were made before the start of the diet period and 3 days after returning to the usual diet. Diet period collections were started simultaneously with increased or decreased protein intake.

Subject	Diet	Sulfur		Total nitrogen
		S_2O_3	Total	
		mg.	*mg.*	*gm.*
K. D.	Normal	5.2	625	13.6
	Decrease	2.7	560	11.3
	"	2.1	270	8.0
	"	2.9	580	7.3
J. H.	Normal	5.7	720	13.1
	"	7.4	987	11.6
	Increase	9.6	1010	13.6
	"	12.3	1200	14.1
	"	14.8	898	16.0
	Normal	8.0	588	12.2

TABLE V

Comparison of Thiosulfate Concentration in Preserved and in Untreated Aliquots of Same Specimen

Freshly excreted, random urine specimens from six individuals were pooled and divided into two portions. Chloroform was added to one, which was immediately refrigerated. The other stood at room temperature, which varied from 25–35° during the period of observation.

Time	Thiosulfate sulfur, mg. per cent	
	Refrigerated	Room temperature
days		
0	0.328	0.325
1	0.325	0.308
2		0.291
4	0.319	0.262
5		0.251
6	0.328	0.236
21	0.275	

Seven of these subjects were then asked to vary their protein intake. Table IV shows typical results obtained in two instances during 3 day periods of decreasing and increasing protein intake respectively. Again

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..d collections were started simultane-
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Sulfur		Total nitrogen
	Total	
	mg.	*gm.*
	625	13.6
	560	11.3
	270	8.0
	580	7.3
	720	13.1
	987	11.6
	1010	13.6
	1200	14.1
	898	16.0
	588	12.2

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..rom six individuals were pooled and
..dded to one, which was immediately
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..sulfate sulfur, mg. per cent	
	Room temperature
	0.325
	0.308
	0.291
	0.262
	0.251
	0.236

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it is noted that the thiosulfate falls with lowered urinary sulfur and nitrogen and *vice versa*. The typical lag in nitrogen excretion is noted. A sample on the 4th day following return to their customary diet clearly indicates the dependence of the thiosulfate excretion on the dietary protein.

Typical results on the effect of chloroform and refrigeration for preservation of the specimens are shown in Table V. Additional observations have indicated that the preserved specimens retain their original thiosulfate concentration for 7 to 10 days. Thereafter the loss is slow and variable with some specimens showing one-fourth of their original content as long as 31 weeks later. Moreover, pooled, fresh specimens with chloroform added immediately show no difference in 24 to 48 hours from refrigerated portions. This parallels the conditions used herein. The decreased content of unpreserved specimens with time and the lack of any increase in 24 hours are interpreted as an indication that the thiosulfate found is not the result of bacterial action in urine after excretion.

DISCUSSION

These data show that thiosulfate is uniformly present in the urine of healthy adults but that there is a wide range of variability in the amount excreted. These variations generally correlate with the protein metabolism as reflected in the urinary excretion of nitrogen and sulfur. However, no evidence has been obtained to explain why some individuals show lower or higher thiosulfate values while excreting comparable levels of total sulfur. This may be due to metabolic variations, varied activity of the intestinal flora, or varied intake of non-thiosulfate-forming sulfur compounds. Preliminary studies[8] on increased thiosulfate excretion following cystine and methionine ingestion support the thesis that the source of the urinary thiosulfate is the sulfur amino acid content of the dietary protein.

Comparison of the range of values found in this series with those in the literature shows all the previous results to be in the lower range of our data. Three possibilities suggest themselves as explanations: (1) lower protein sulfur intake; (2) bacterial oxidation in the samples during or after collection; (3) differences in methods. Since no information is given on the dietary protein or urinary nitrogen and sulfur by the earlier workers, nor on the preservation of the urine, it is not possible to reconcile these differences at this time.

While the number of female subjects is small compared to the males, no consistent differences have been observed that cannot be explained by the urinary total sulfur and nitrogen variations. Likewise in the age range (20 to 43 years) of the subjects, no correlation with age was observed.

[8] Gast, J. H., and Arai, K., unpublished observations.

SUMMARY

Quantitative data have been presented which demonstrate the consistent presence of 2 to 17 mg. of thiosulfate sulfur in 24 hour urine specimens of healthy young adults. Concomitant studies on urinary total nitrogen and sulfur indicate that the urinary thiosulfate sulfur probably has its origin in the sulfur of the dietary protein and varies with the latter. A method for the determination of thiosulfate in urine has been described.

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M. Gaté. — Avec mon assistant M. Cuilleret, nous avons expérimenté le cyanure de mercure dans le traitement de 20 cas environ d'orchites blennorragiques. Nous avons également observé parfois la sédation très rapide de la douleur et l'évolution accélérée vers la guérison. A côté de ces cas, il en est d'autres qui sont insensibles à cette thérapeutique, ou même qui paraissent aggravés par elle. J'ai donc la même opinion que M. Pétouraud : efficacité intéressante, mais inconstante.

M. Lebeuf. — Dans les orchi-épididymites gonococciques j'ai fait autrefois du lait, de l'autohémothérapie avec de bons résultats. J'ai même obtenu le même effet sédatif avec des injections d'eau distillée.

M. Gaté. — Le scepticisme de notre collègue, M. Lebeuf, parfaitement défendable dans les orchi-épididymites gonococciques n'est plus de mise dans le traitement des arthrites blennorragiques et il semble que sur ce terrain M. Pétouraud a eu des succès. Personnellement nous n'avons pas essayé le cyanure dans le rhumatisme blennorragique.

M. Saby. — Dans 50 o/o des cas, d'après mon expérience personnelle, on obtient dans les orchi-épididymites blennorragiques, avec le cyanure de mercure, la sédation des douleurs dans l'heure qui suit l'injection et la résorption de l'inflammation en deux ou trois jours.

M. Carle. — J'ai fait autefois comme l'a conseillé M. Lévy-Bing des arsénobenzènes. Actuellement je préfère les vaccins. Il semble, [illegible]es orchi-épididymites gonococciques sont à l'heure [illegible] sévères. Il faut aussi proscrire les suspensoirs mal [illegible]mpriment au lieu de soutenir.

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Thiosulfate

Un cas d'œdème important des membres inférieurs au cours d'un traitement par l'hyposulfite de soude,

Par MM. Gaté et A. Fribdel.

Nous vous présentons un cas d'œdème au cours d'un traitement par l'hyposulfite de soude pour eczéma. M. Nicolas et ses collaborateurs ont rapporté précédemment plusieurs cas semblables. Par ailleurs, M. Nicolas et l'un de nous (Dr Gaté) ont rapporté une observation où un œdème des membres inférieurs et une scapulalgie, voire même une véritable arthrite de l'épaule, avaient été déclenchés par l'hyposulfite de soude et avaient alterné ultérieurement avec les poussées

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d'eczéma. Dans ce cas il semblait bien qu'on pouvait parler de métastase ou si l'on veut de phénomènes de résorption protéinique.

L'observation que nous rapportons est différente de cette dernière et peut se rapprocher de celles rapportées par M. Nicolas et ses élèves.

Il s'agit d'un malade de 45 ans présentant de l'eczéma aigu surtout marqué au visage et aux membres, mais ayant tendance à se généraliser. On entreprend un traitement par l'hyposulfite de soude. On lui fait six injections (1 gr. 50, 3 gr., puis 4 gr. 50 par injection). A ce moment le sujet nous signale un œdème des membres inférieurs, remontant au-dessus du genou, œdème dur, blanc lisse, donnant cependant le godet.

Il accuse également une augmentation de poids de six kilos, son eczéma n'est pas amélioré.

On arrête le traitement, d'autant plus que les urines présentent un disque d'albumine qui, au premier abord, a paru très important. Une analyse ultérieure a montré que la quantité d'albumine, renfermée dans les urines, était de 0 gr. 08 par litre, donc assez restreinte.

Il s'est agi, en somme, d'un œdème au cours d'un traitement par l'hyposulfite de soude, sans aucun rapport avec l'évolution de la dermatose. Nous nous sommes permis de rapporter cette observation, pour la rapprocher des cas déjà signalés à cette Société.

Ostio-folliculites avec eczéma séborrhéique secondaire du cuir chevelu et parakératose pityriasiforme consécutive des téguments à la suite de l'épilation radiothérapique pour teigne microsporique chez six enfants. Actinobiotropisme ?

Par MM. J. Gaté, P. Guilleret et A. Feindel.

Il est assez fréquent de voir après épilation radiothérapique, apparaître sur le cuir chevelu des *ostio-folliculites* de courte durée, qui cèdent rapidement à l'application de pommade soufrée. Par contre, il est plus rare d'observer les faits que nous allons rapporter concernant six enfants (parmi 11 petits malades que nous avions fait épiler aux rayons X pour une teigne microsporique apparue dans une colonie de vacances).

Au cours de la troisième semaine qui suivit l'irradiation, alors que l'épilation commençait à peine, nous avons vu se constituer chez six de nos jeunes malades, des ostio-folliculites banales du cuir chevelu, phénomène fréquemment observé après un tel traitement. Contrairement à ce qui a lieu d'habitude (alors que les cinq autres se comportaient normalement), ces six enfants firent dans un second stade un

eczéma séborrhéique typique du
se développer progressivement d'
les une parakératose pityriasiform
lisa à tout le corps, s'accompagna
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Dans les pustulettes des ostio-
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phylocoques, avec lesquels nous
de ces petits malades ont été traité
avoir donné de résultats remarqu
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Si le rôle du terrain ne peut êtr
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résumer toute la pathogénie de ces
être agi dans ces cas d'un véritable a
rement dermo-épidermite microbie
vant la terminologie actuelle.

LAVAL. — IMPRIMERI

Bull. Soc. Franc. Dermat. Syph. 41, April 1934

A CASE OF SEVERE EDEMA OF THE LOWER EXTREMITIES OCCURRING DURING SODIUM THIOSULFATE TREATMENT

by Messrs. Gaté and A. Freidel

We should like to present to you a case of edema occurring during sodium thiosulfate therapy for eczema. M. Nicolas et al. have previously reported several similar cases. In addition, M. Nicolas and one of us (Dr. Gaté) reported on one case in which edema of the lower extremities and scapulalgia, even true arthritis of the shoulder, had been induced by sodium thiosulfate and had later alternated with episodes of eczema. In this case it appears that one should speak of metastasis or, if desired, of protein absorption phenomena.

It involves a 45-year-old patient with acute eczema especially pronounced on the face and extremities, but which has a tendency to generalize. Sodium thiosulfate therapy was administered. Six injections were given (1.50 g, 3 g, then 4.50 g per injection). At this time the subject pointed out edema of the lower extremities which rose above the knee, a hard, white, smooth edema which formed a depression.

He also showed a 6-kg increase in weight, and his eczema had not improved.

Therapy was discontinued, particularly since the urine showed a disk of albumin which first appeared to be large. A later analysis showed that the amount of albumin found in the urine was about 0.08 g/liter, or very small.

In summing up, this was edema which appeared during sodium thiosulfate therapy and which was not related to the evolution of the dermatosis. We have reported this case to compare it with cases already cited by this Society.

Klin. Wochensch. 27: 457-459 (1949).

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DER HISTAMINABBAU IM MENSCHLICHEN BLUT IM RAHMEN DER VEGETATIVEN REGULATION*.

Von

thiosulfate

Wilhelm Jacob und Günther Stüttgen.

Aus dem Pathologischen Institut (Direktor: Prof. Dr. P. Huebschmann) und der Hautklinik (Direktor: Prof. Dr. H. Th. Schreus) der Medizinischen Akademie Düsseldorf.

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☐ NOT REQUIRED ____
☒ OTHER no response

Das biologisch bedeutsame Histamin kann dadurch in seiner Wirkung geschwächt werden, daß seine Receptoren blockiert werden. Daneben besteht aber das Prinzip einer Inaktivierung des Histamins infolge seines oxydativen Abbaues durch Diaminooxydase = Histaminase (Zeller, Best).

Dem Wechsel der Intensität dieses Histaminabbaues soll im folgenden, soweit er im menschlichen Blut nachzuweisen ist, an Hand ausgewählter Versuche mit ihren jeweils besonderen Bedingungen nachgegangen werden.

Werle und Koch wiesen bei der Lungentuberkulose eine Verminderung der Histaminase im Blut nach und vermuten eine Abhängigkeit der Histaminase von der jeweiligen Immunitätslage. Werle und Effkemann benutzten ihre Beobachtung des erhöhten Histaminasespiegels im menschlichen Blut während der Gravidität zu einer biologischen Schwangerschaftsreaktion, die in ihrer relativ schnellen Auswertung eine sichere Diagnose gestattet. Albus stellte bei Allergikern einen verminderten Histaminasespiegel im Blut fest. Uns interessiert die Frage, inwieweit die Fermentaktivität durch Einwirkungen wie Pyriferinjektion, Elektroschock und Ultraviolettbestrahlung verändert werden kann, die als zentrale und periphere Reize im Rahmen der Umstimmung (Hoff) ihre Wirkung zeigen.

Methodik.

Wir hielten uns in der Methodik der Histaminasebestimmung an die von Werle angegebene Versuchsanordnung: 3 cm³ defibrinierten Venenblutes werden mit 3 γ Histamin einer Lösung 1 : 100 000 versetzt und mit einer Tyrodelösung auf 4 cm³ aufgefüllt. Die mit dieser Lösung gefüllten Glasröge werden an einem Krebsarm befestigt und unter konstantem O_2-Strom 90 Min. in einem Wasserbade von 37° C geschüttelt. Nach dieser Zeit werden die Glasröge auf Eis gestellt, und somit wird der fermentative Prozeß gehemmt. Die Auswertung des Blutes geschieht am atropinisierten Meerschweinchendünndarm. Die Fermentaktivität ist der im Blut noch nachweisbaren Histaminmenge umgekehrt proportional. Die durch das gleiche Blut ohne Histaminzusatz hervorgerufene Darmkontraktion muß bei der Auswertung berücksichtigt werden.

Schwankungsbreite der Histaminasewerte: Wir unterscheiden einerseits die individuelle Schwankungsbreite und andererseits die Fehlerbreite der Versuchsmethodik. Zur Feststellung der individuellen Schwankungsbreite untersuchten wir über 24 Stunden zweistündlich entnommenes Blut von Patienten, die keinerlei Behandlung erfuhren. Wir erhielten dabei eine Aktivitätsänderung der Histaminase von nicht über ± 5%. Die Fehlerbreite der Versuchsmethodik wurde durch Doppelbestimmungen eingeengt, wie auch die Methodik als solche in der gleichzeitigen Bearbeitung aller Ansätze der einzelnen Versuchsreihen keine wesentliche Fehlerbreite zuläßt. Die Genauigkeit unserer Methodik wurde in Schwangerschaftsuntersuchungen bestätigt. Uns erschien eine Patientenzahl von jeweils 10 Fällen für eine Versuchsreihe ausreichend, da die Ergebnisse eindeutig weit außerhalb der individuellen Schwankungen und der methodischen Fehlerbreite liegen und durch mehrfache Kontrollen bestätigt werden konnten.

* Herrn Prof. Dr. Paul Huebschmann zu seinem 70. Geburtstag gewidmet.

1. *Die Histaminbeeinflussung durch Pyriferinjektionen.*

Bei Patienten, die aus therapeutischen Gründen Pyrifer erhielten, wurde vor und mehrmals nach der Pyriferinjektion Venenblut entnommen und die Histaminaseaktivität bestimmt. Die günstige Beeinflussung allergischer Krankheiten durch Pyrifer machte eine Aktivitätsänderung der Histaminase als Grundlage des therapeutischen Effektes wahrscheinlich, zumal Intensitätsschwankungen der Cholinesterase während der Pyriferwirkung bereits bewiesen sind (Stüttgen). Aus der weiter unten gezeigten Tabelle 1 ist ersichtlich, in welchem Maße sich die Histaminaseaktivität während der künstlichen Fieberreaktion verändert. Es zeigt sich eine individuelle Reaktionsverschiedenheit der Histaminase auf Pyrifer, die nicht unbedingt an den jeweils wechselnden Verlauf der Temperaturkurve gebunden ist. Der Charakter der Aktivitätsänderung des Fermentes ist biphasisch. Will man diese Schwankung in Beziehung zu einem etwaigen therapeutischen Erfolg bringen, so

Tabelle 1. *Die Aktivitätsänderungen der Histaminase während der Pyrifereinwirkung in absoluten Zahlen dargestellt. Die absoluten Zahlen entsprechen den abgebauten Mengen Histamin.* Das Fiebermaximum ist mit einem * bezeichnet.

	Vor	2	4	6	Maximale Aktivitätsänderung %
	Stunden nach Injektion				
1. Ekzem . .	50	150	130*	100	etwa +200
2. Ekzem . .	100	100	120	120*	+ 20
3. Tabes . . .	80	130	120*	120	+ 40
4. Ekzem . .	50	50	60	50*	+ 20
5. Lues . . .	150	170	200*	220	+ 45
6. Gonorrhoe .	130	150	170*	150	+ 30
7. Lues . . .	110	105	160*	150	+ 45
8. Ekzem . .	70	90	110*	110	+ 55
9. Ekzem . .	100	90	120*	160	+ 55
10. Ekzem . .	90	90	90	90*	—

möchten wir darauf hinweisen, daß ein Histaminaseendwert erst nach einem „Auspendeln" dieser Schwankungen erreicht ist. Wir haben des öfteren in den letzten Bestimmungen im Verlauf über 24 Stunden einen gleichbleibenden Histaminasewert gefunden, der deutlich unter dem Wert vor der Pyriferinjektion lag.

2. *Die Histaminasebeeinflussung durch Elektroschock*[1].

Die physiologischen Änderungen nach einer elektrischen Hirnreizung sind vielgestaltig, zeigen aber doch insgesamt eine Steuerung aller vegetativen Komponenten (Hoff). Da die Histaminase in diesen Komplex gehört, bestimmten wir vor und nach dem Elektroschock an 10 Patienten die Histaminasewerte des Blutes. Wir sehen auch beim Elektroschock zumeist eine Fermentschwankung. Während aber bei der Pyriferreaktion eine Aktivierung der Histaminase die Regel ist, beobachten wir beim Elektroschock eine Aktivitätsminderung. Die Intensitätsänderung der Histaminase bewegt sich während den

[1] Den Herren Medizinalräten Hölzer und Seidel haben wir für die freundliche Überlassung von Patientengut zu danken.

Tabelle 2. *Die Aktivitätsänderung der Histaminase nach Elektroschock in absoluten Zahlen dargestellt. Die absoluten Zahlen entsprechen den abgebauten Mengen Histamin.*

	Vor	1	2	4	Maximale Aktivitätsänderung %
	Stunden nach Schock				
1.	100	80	80	100	— 20
2.	90	90	80	80	— 10
3.	110	110	120	120	+ 9
4.	70	60	50	70	— 28
5.	100	90	90	70	— 30
6.	120	120	110	120	— 8
7.	115	115	100	110	— 15
8.	100	100	90	100	— 10
9.	90	75	60	80	— 33
10.	120	120	80	100	— 30

von uns gewählten Untersuchungszeiten schwerfälliger und geringgradiger als beim Pyrifer. Aus der isolierten Betrachtung der Histaminase dürfen wir auf eine Verschiedenheit der beiden zentralen Reizformen schließen (Tabelle 2).

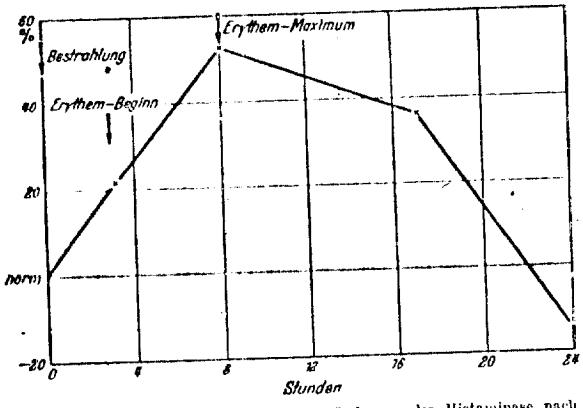

Abb. 1. Kurve 1 zeigt die Aktivitätsänderung der Histaminase nach Ganzbestrahlung mit Ultraviolettlicht des Falles 4 (Pat. Sl. z. B. Go.).

Die Ausgangswerte beider Tabellen müssen „schwanken", denn es handelt sich nicht nur um Normalwerte sondern um die Ausgangswerte bei verschiedenen Erkrankungen und Individuen. Es ist bekannt, daß die „Normalwerte" individuell schwanken und ihre Abweichung bei verschiedenen Krankheitszuständen wechselnd ist. Entscheidend ist hier, daß die Werte im Verhältnis zu den Ausgangswerten jeweils gleichsinnige Veränderungen aufzeigen, die außerhalb der Schwankungs- und Fehlerbreite liegen.

3. *Die Histaminasebeeinflussung durch Bestrahlung mit Ultraviolettlicht.*

Bei der Beobachtung der Cholinesteraseaktivierung durch Ultraviolettbestrahlung wurde von Stüttgen eine gleichzeitige Histaminaseaktivierung angenommen, da hierdurch möglicherweise das Ausbleiben einer Hemmung der Cholinesterase durch das sicherlich entstehende Histamin erklärbar ist.

Wir bestrahlten 10 Patienten in Form einer Ganzbestrahlung, und zwar 10 Min. ventral und 10 Min. dorsal. Die Bestimmung der Histaminaswerte erstreckte sich über die Maxima der Erythemreaktionen hinaus.

Insgesamt beobachteten wir bei den Patienten eine deutliche Histaminaseschwankung in gleichbleibender Abhängigkeit vom Erythembeginn. Die Histaminaseaktivität steigt im Beginn der Erythemreaktion und auch schon vor einer sichtbaren Erythembildung. Nach 24 Stunden hingegen war die Histaminaseaktivität gegenüber den Ausgangswerten vermindert. Wir erkennen also auch hier einen biphasischen Ablauf einer vegetativen Funktion, der an anderen Daten von Schneider und Dietzel gezeigt wurde (Abb. 1).

Unseren Untersuchungen an Patienten ging eine Bestrahlungsserie von 20 Meerschweinchen voraus. Diese zeigten gegenüber Kontrolltieren schon nach ungefähr 5minütigem Einfluß des Ultraviolettlichtes einen so deutlich verstärkten Histaminabbau, wie er sonst bei Meerschweinchen kaum erzielbar ist[1].

Auf die sich aus dieser Feststellung, daß eine tiefgehende Wirkung von einem Reiz auf die Peripherie aus möglich ist, ergebende Frage, welcher Art die die Vermittlung dieser Wirkung ist, kann hier nicht eingegangen werden. Es ist möglich, daß celluläre Stoffwechselprodukte auf dem Blutwege einen zentralen Reiz ausüben, Reizwirkungen zentripetal über das Nervensystem geleitet werden, oder aber, daß Stoffwechselveränderungen der Peripherie eben dortselbst Fermentaktivierungen verursachen. Es darf angenommen werden, daß dieser Reiz eine Steuerung zentralnervöser Art auslöst.

Durch eine einfache, wahrscheinlich nur auf Elektronenverschiebung bestehende Milieuänderung ist es möglich, eine Aktivitätsbeeinflussung der Histaminase und Cholinesterase zu erzielen. Dies zeigen unsere Versuche mit Natriumthiosulfat.

4. *Die Beeinflussung der Histaminase- und Cholinesteraseaktivität durch Natriumthiosulfat.*

Wir beobachteten nach ausgiebigen Natriumthiosulfatgaben bei allergischen Erkrankungen (Ekzeme) eine Aktivierung der Histaminase im Verlauf der Ekzembesserung. Da diese Histaminaseaktivierung allein durch den Ablauf des Ekzems bedingt sein konnte, Natriumthiosulfat dann also nicht in direktem Zusammenhang mit der Histaminaseaktivität stehen würde, führten wir folgende Versuche aus.

Es wurden 2,0 g Natriumthiosulfat intravenös Patienten injiziert. Die Werte der Histaminase und hier auch der Cholinesterase wurden vor der Injektion und 15 Min. nach der Injektion bestimmt. Die Histaminaseaktivität war bei den von uns untersuchten Patienten im Mittelwert um 40%, die der Cholinesterase um 30% erhöht.

Eine Fermentaktivierung durch Redoxpotentialveränderung, etwa durch SH.-Glutation ist bekannt (Nachmannsohn und Lederer, Keeser). Diese Ergebnisse führen die therapeutische Wirksamkeit des Natriumthiosulfats auch außerhalb des Gebietes seiner Schwermetall-Allergie einer Erklärung näher.

5. *Cocarboxylase in Beziehung zu Histamin-Histaminase.*

Im Verlaufe unserer Studien über den fermentativen Abbau des Histamins entstand die Notwendigkeit, Verbindungen von bekannter histaminase-

[1] Inauguraldissertation von Ernst Knopp, Pathologisches Institut der Medizinischen Akademie Düsseldorf 1948.

hemmender Eigenschaft in ihrer Bedeutung für den Komplex Histamin-Histaminase näher nachzugehen.

Zeller, Schär und Staehlin schrieben dem Aneurin eine beträchtliche histaminasehemmende Wirkung zu. Wir stellten bei Einwirkung von Aneurinphosphorsäure, also von Cocarboxylase in einer Konzentration von 20,0 γ je Kubikzentimeter Blut eine 35%ige *Hemmung* der Histaminase fest. Intravenöse Injektionen von 100 mg Benerva zeigten 15 Min. nach der Injektion allerdings keinen obligaten Hemmungseffekt. Bei Zusätzen von 10 γ Cocarboxylase zu einer intracutan injizierten Histaminlösung 1:10 000 (Histamin sowie Cocarboxylase wurden in 0,9% NaCl-Lösung gelöst, Injektionsmenge 0,1 cm³) war hingegen eine Einschränkung der urticariellen Histaminreaktion im Vergleich zur Reaktion auf Histaminlösung gleicher Konzentration ohne Zusatz feststellbar. Auch die Resorptionsdauer der Histaminquaddel war nicht etwa verlängert. Besonders eindrucksvoll war im Verlauf der Reihenversuche (Stüttgen) die Hemmung der Histaminreaktion auf Beflavin (Hoffmann-La Roche).

Wir ziehen den Schluß, daß bei dem Zusatz von Cocarboxylase zu dem fermentativen Ablauf der Histamininaktivierung bei momentaner Histaminfreisetzung die Eigenschaften der Cocarboxylase, die eine Einschränkung der Reaktion auf Histamin im Gewebe bewirken, größer sind, als die den Histaminabbau hemmenden Eigenschaften. Es kommt also auf die Summierung aller physiologischen Eigenschaften bei der Betrachtung eines Wirkstoffes an[1].

[1] Wir sind den Herren Prof. Dr. Derra, Direktor der Chirurgischen Klinik, und Herrn Obermedizinalrat Dr. Schubert, Direktor der Psychiatrischen Klinik der Medizinischen Akademie Düsseldorf für ihre bereitwillige Hilfe bei der Durchführung dieser Arbeit dankbar. Wir danken besonders Herrn Prof. Dr. Koch, Oberarzt des Pathologischen Instituts der Medizinischen Akademie Düsseldorf, da ohne seine weitgehende Unterstützung der experimentelle Teil dieser Arbeit nicht durchführbar gewesen wäre.

Zusammenfassung. **Wir konnten in vorliegender Arbeit Histaminaseaktivitätsänderungen durch tiefgehende Reize wie Pyriferfieber, Elektroschock und Ultraviolettbestrahlung nachweisen, wie auch durch intravenöse Zufuhr von Natriumthiosulfat. Die individuelle Schwankungsbreite über einen Zeitraum von 24 Stunden wurde bei Versuchspersonen ohne bestimmte Beeinflussung im Mittel von ± 5% gefunden; die durch die oben genannten Eingriffe festgestellten Schwankungen liegen meistens bei Werten um 30%.**

1. Pyriferfieber und Ultraviolettbestrahlung zeigen eine gleichsinnige biphasische Aktivitätsschwankung in den von uns untersuchten Zeiträumen, wobei besonders die *Aktivierung* der Histaminase deutlich ist.

2. Elektroschock bewirkt ebenfalls eine Histaminaseaktivitätsänderung, die in ihrer Intensität schwächer als bei Pyriferfieber und Ultraviolettbestrahlung ist. Darüber hinaus steht beim Elektroschock die *hemmende* Wirkung auf die Histaminase im Vordergrund.

3. Durch intravenöse Natriumthiosulfatgaben ist eine Aktivierung der Histaminase wie auch der Cholinesterase feststellbar.

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ÜBER DIE REFLEKTORISCHE BEEINFLUSSUNG DES HERZENS IN IHRER ABHÄNGIGKEIT VON DER REAKTIONSLAGE DES VEGETATIVEN NERVENSYSTEMS* **.

Von
Otto Bayer.

Die Herztätigkeit wird reflektorisch weitgehend von anderen Organsystemen beeinflußt. Als reflexogene Zonen kommen neben der Lunge, bzw. Pleura, vor allem der Magen-Darmkanal in Betracht. Es ist das Verdienst Roemhelds auf die engen Zusammenhänge zwischen Verdauungstrakt und Herz aufmerksam gemacht und die dadurch bedingten Korrelationsstörungen unter dem Begriff „gastrokardialer Symptomenkomplex" zusammengefaßt zu haben. Durch die Bergmannsche Schule, sowie von Hochrein und Mitarbeitern wurde die reflektorische Beeinflussung der Herztätigkeit bei Oberbauchaffektionen, so vor allem bei der Hiatushernie, beim Geschwürsleiden und Gallenwegsaffektionen enger umrissen. Für diese Fragestellung besonders aufschlußreich waren die experimentellen Untersuchungen von Dietrich und Schwiegk, die beim Hunde durch Dehnung des unteren Ösophagus eine meßbare Verkleinerung der Coronardurchblutung feststellen konnten, was sie als Folge einer über einen Vagusreiz zustande gekommenen Coronarverengerung deuteten.

Die Tatsache, daß nur ein kleiner Teil der Kranken mit abdominellen Störungen Erscheinungen von seiten des Herzens aufweist, ließ vermuten, daß für ihr Zustandekommen eine zusätzliche vegetativ-nervös bedingte Bereitschaft, wohl in Richtung der Vagotonie, wie Hochrein u. a. annehmen, vorliegen müßte.

Von diesem Gedanken ausgehend, überprüfte ich mit meinem Mitarbeiter Seynold systematisch die reflektorische Beeinflußbarkeit der Herztätigkeit und der Blutdruckregulation bei Menschen verschiedener vegetativer Struktur durch Setzen eines vegetativen Reizes im Magen. Wir gingen dabei so vor, daß wir eine mit einem Condom versehene Magensonde einführten, an deren oberen Ende ein Gebläse und im Nebenschluß ein Quecksilbermanometer angeschlossen war, wodurch es möglich war, den Magen aufzublähen und den dabei ausgeübten Druck abzulesen. Es wurde vor und nach Aufblähung des Magens auf einen Druck von 20 mm Hg das Ekg und der Blutdruck überprüft. Die Beurteilung der vegetativen Reaktionslage geschah nach klinischen Gesichtspunkten, wobei uns Anamnese und klinischer Befund wesentliche Hinweise gaben. Wir teilten danach unsere 40 Versuchspersonen, die im Alter zwischen 20

* Herrn Prof. Dr. E. Hoden zum 65. Geburtstage gewidmet.
** Nach einem Vortrag, gehalten auf der Tagung der Rheinisch-Westfälischen Gesellschaft für innere Medizin am 30. 6. 48.

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thiosulfate

DECOMPOSITION OF HISTAMINE IN HUMAN BLOOD IN THE FRAME OF AUTONOMIC REGULATION

Wilhelm Jacob and Guenther Stuettgen

The biologically significant histamine can be attenuated in its effect by blocking its receptors. Other than this, there is the principle of inactivation of histamine as a consequence of its oxidative decomposition by diaminooxidase = histaminase (Zeller, Best).

The variation in the degree of histamine breakdown shall be demonstrated as for as it is demonstrable in human blood by means of the methods selected, with their respective particular restrictions.

Werle and Koch point out a reduction in blood histaminase with tuberculosis and assume the state of immunity at the time to be dependent on histaminase. Werle and Effkemann utilized their observation of an elevated histaminase level in human blood during gravidity in a biological pregnancy reaction, which allowed a reliable diagnosis with a relatively rapid interpretation. Albus established a diminished histaminase level in the blood during allergy. The question interests us, in which way the enzymatic activity can be altered by the effects of pyrogenic injection, electric shock and ultraviolet radiation, which exhibit their effect as central and peripheral stimulants in the framework of altering the activity (Hoff).

METHOD

We kept to the method of histaminase determination as stated in the procedure by Werle: 3 cc of defibrinated venous blood is mixed with 3 gamma of a 1:100,000 histamine solution and brought to 4 cc with Tyrode solution. A glass tank, filled with this solution is fastened to a clamp and shaken in a water bath at 37°C for 90 minutes under constant stream of oxygen. After this, the tank is put on ice to inhibit the enzymatic process. The blood is evaluated on guinea pig small intestine, treated with atropine. The enzymatic activity in the blood is inversely proportional to the detectable amount of histamine. Intestinal contractions brought on by blood without added histamine must be considered in the evaluation.

Variable range of histaminase values: We differentiated on the one hand, the individual fluctuation range and on the other hand, the margin of error of the experimental methods. To establish the individual fluctuation ranges, every two hours we took blood from patients who were experiencing no form of treatment. We got a change in histaminase activity of not over 1.5%. The margin of error in the experimental method was narrowed by duplicate determinations, as the method allows no substantial margin of error as such in the simultaneous processing of all items in the series of experiments. The precision of our method was confirmed in pregnancy tests. 10 patients seemed sufficient to us for a series of experiments, since the results can clearly by broadly controlled outside of individual fluctuations and of methodical margin of error, by multiple controls.

1. THE INFLUENCE OF PYROGENIC INJECTIONS ON HISTAMINE

From patients who received pyrogens for therapeutic purposes, venous blood was drawn before and repeatedly after the injection of pyrogen, and the histaminase activity was determined. The favorable influence of pyrogens on allergic conditions provavly has as its foundation of therapeutic effect changes in the activity of histaminase, especially since the fluctuation of cholinesterase under the influence of pyrogen has already been demonstrated (Stuettgem). Further down, on Table 1, it is obvious to what degree the histaminase activity changes during the artificial fever reaction. An individual difference reaction appears, which is not unconditionally tied to the changing course of the temperature curve at the time. The character of the modification in the activity of the enzyme is biphasic. If these fluctuations are to brought into relation to a possible therapeutic consequence, then we would like to indicate that a final histaminase value is

is attained just after as "outward swing" in these fluctuations. We have found more frequently in the past determinations, a histaminase value remaining constant over 24 hours, which is clearly due to the pyrogen injection.

2. THE INFLUENCE OF ELECTRIC SHOCK ON HISTAMINE .

The physiological changes after an electrical stimulation of the brain are manifold, but show collectively, an actuation of all autonomic components (Hoff). Since histaminase belongs in this complex, we determined the histaminase values in the blood of 10 patients before and after electric shock. We also saw, for the most part, an enzymatic fluctuation from electric shock. While activation of histaminase by pyrogenic reaction is the rule, we observed a reduction in activity with electric shock. The change in the intensity of the histaminase induced during the experimental period of time selected by us, were slacker and to a lesser degree than with pyrogen. From the isolated analysis of histamine, we might conclude a difference in both central forms of stimulation (Table 2). The initial values of both tables must 'vary', for it is a matter not only of normal values but of the values in different illnesses and individuals. It is known, that the "normal values" vary individually and their deviation is altered by various unhealthy states. It is decisive here that the values from to time show changes in proportion to the initial values which lie outside of the fluctuation range and margin of error.

3. THE INFLUENCE ON HISTAMINASE BY IRRADIATION WITH ULTRAVIOLET LIGHT

By observing the cholinesterase activation from ultraviolet radiation, a simultaneous histaminase activation was assumed by Stuettgen, since, this way, perhaps the absence of an inhibition of cholinesterase is explicable through the histamine undoubtedly produced.

We irradiated 10 patients totally, 10 minutes ventrally and 10 minutes dorsally. The determination of the histaminase values extended beyond the maxima of an erythematous reaction.

We observed in the patients collectively a distinct fluctuation in constant dependence on the beginning of an erythematous reaction, and also before a perceptible erythema. On the contrary, after 24 hours the histaminase activity diminished, opposed to the initial values. We alse recognized here a biphasic decline of an autonomous function, which was shown in other data from Schneider and Dietzel (Fig 1).

Our investigations on patients preceded a series of irradiations on 20 guinea pigs. These showed, contrary to the control animals, a distinct increase in histamine breakdown after almost 5 minutes under the influence of ultraviolet light, as is barely producible in guinea pigs (1). From establishing that a profound effect on the periphery is possible from a stimulation, the question raised as to the manner in which this result is supplied can not be gone into here. It is possible that cellular metabolic products exert a central irritation on the blood stream. The effects of the irritation are conducted centripetally over the nervous system, or metabolic products in the periphery themselves cause the enzymatic activation. It may be assumed that this stimulation releases a central nervous system control.

By a simple change in enviornment, probably existing in an electron displacement, it is possible to attain an effect on the activity of histaminase and cholinesterase. This points to our experiment with sodium thiosulfate.

(1) Inaugural dissertation of Ernst Knigge, Pathological Institute of the Medical Academy of Duesseldorf, 1948

4. THE EFFECT OF SODIUM THIOSULFATE ON HISTAMINASE AND CHOLINESTERASE ACTIVITY

After a strong administration of sodium thiosulfate in allergic conditions (eczema), observed an activation of histaminase in the course of improvement of the eczema. se this histaminase activation can be conditional to the termination of the exzema lone, sodium thiosulfate then would not stand in direct connection with the histaminase ctivation, we performed this experiment:

2.0 g of sodium thiosulfate was intravenously injected into patients. The histaminase alues and also the cholinesterase were determined before the injection and 15 minutes fter the injection. In the patients examined by us the average value of histaminase as 40% and that of the cholinesterase was elevated to 30%.

An enzymatic activation from changes in redox potential is known, perhaps through H-glutathion (Nachmannsohn and Lederer, Keeser). These experiments lead to an explanation n greater detail of the therapeutic efficacy of sodium thiosulfate outside the realm f heavy metal allergy.

5. COCARBOXYLASE IN RELATION TO HISTAMINE-HISTAMINASE

In the course of our studies on the enzymatic decomposition of histamine, the necessity arose to investigate compounds known to have histamine inhibiting properties in their importance to the histamine-histaminase complex.

Zeller, Schaer and Staehlin ascribe to aneurine a considerable histaminase retarding effect. We established a 35% reduction of histaminase from the effect of aneurinephosphoric acid and also of cocarboxylase in concentrations of 20.0 gamma each per cc of blood. Intravenous injections of 100 mg of Benerva showed no obligate retardation effect 15 minutes after the injection. On addition of 10 gamma of cocarboxlyase to an intracutaneously injected 1:10,000 histamine solution (histamine as well as cocarboxylase was solved in 0.9 NaCl solution; amount of injection was 1 cc), on the contrary, there was a reduction of the urticaral histamine reactine in comparison to the reaction established with histamine of the same concentration but without any supplement. Also the resorption time of the histamine skin eruption was not prolonged. The inhibition of the histamine reaction in the course of the series of experiments (Stuettgen) by Beflavin (Hoffmann-La Roche) was most impressive.

We draw the conclusion that with an addition of cocarboxylase to the enzymatic activation of histamine, with momentary histamine liberation, the properties of cocarboxylase which effect a reduction of the reaction on histamine in the tissues are greater than the inhibiting properties to the histamine breakdown. A consideration of the active principle also depends on an accumulation of all physiological properties.

SUMMARY

In the present work we could demonstrate changes in histaminase reactivity through extensive stimulation as pyrogen fever, electroshock and ultraviolet radiation as also by the intravenous administration of sodium thiosulfate. The individual fluctuation ranges over a 24 hour period were found to be with experimental subjects without definite influence ± 5%; in those who underwent the above named treatments had fluctations of 30%.

1. Pyrogenic fever and ultraviolet radiation exhibit a similar biphasic fluctuation in activity during the time of our experiments, with which the activation of histaminase is especially distinct.

2. Electroshock also effects a change in histaminase activity which is weaker in its intensity than that of pyrogenic fever and ultraviolet radiation. Moreover, an inhibitory effect on histaminase by electroshock comes to the fore.

3. An activation of histaminase through intravenously administered sodium thiosulfate is demonstrable, as it is with cholinesterase.

Table 1. The changes in activity of histaminase under the influence of pyrogen presented in absolute figures. The absolute figures correspond to the amount of histamine decomposed. The fever maximum is indicated by *.

	Before	2	4	6	Maximal activity change
	Hrs. post injec.				
1. Eczema	1.. 60	130	130*	100	circa +100
2. Eczema	2.. 100	100	120	150*	+20
3. Tabes	3.. 80	130	120*	120	+40
4. Eczema	4.. 60	50	60	80*	+20
5. Lues	5.. 150	170	200*	220	+45
6. Eczema	6.. 130	150	170*	150	+30
7. Lues	7.. 110	105	160*	150	+45
8. Eczema	8.. 70	90	110*	110	+55
9. Eczema	9.. 100	90	120*	160	+55
10. Eczema	10.. 90	60	60	80*	—

Table 2. The changes in activity of histaminase following electroshock presented in absolute figures. The absolute figures correspond to the amount of histamine decomposed.

	Before	1	2	4	Maximal activity change
	Hrs. post injec.				%
1.	100	80	80	160	— 20
2.	90	90	80	80	— 10
3.	110	110	120	120	+ 9
4.	70	60	60	70	— 28
5.	100	90	90	70	— 30
6.	120	120	110	120	— 8
7.	115	115	100	110	— 15
8.	100	100	90	100	— 10
9.	90	75	60	80	— 33
10.	120	120	80	100	— 30

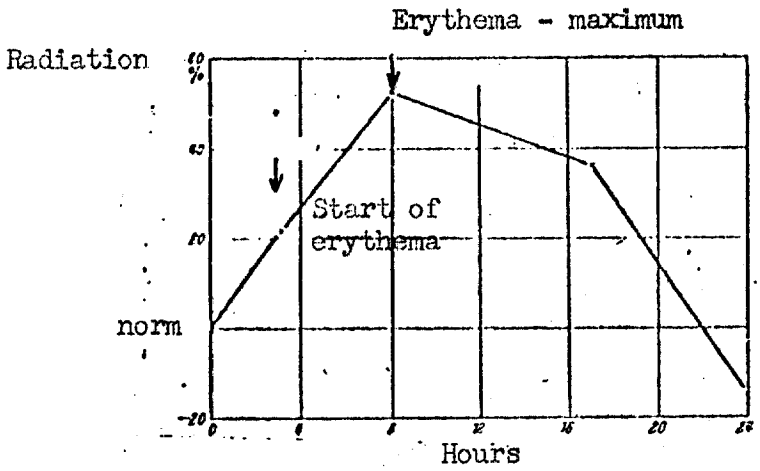

Fig. 1. Curve 1 shows the activity changes of histamine following the total irradiation of 4 cases with ultraviolet light

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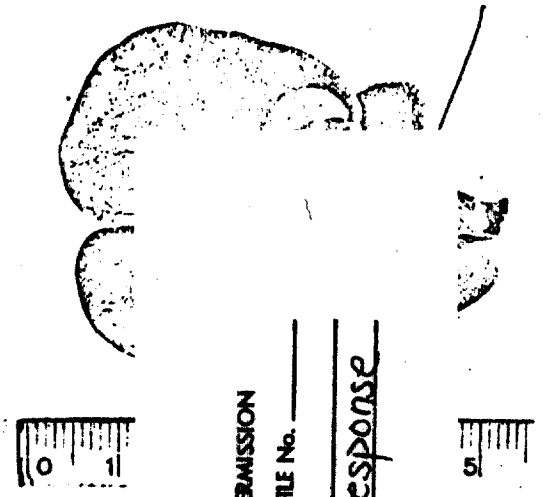

Abb. 1. Zahlrei... der Leber
na...

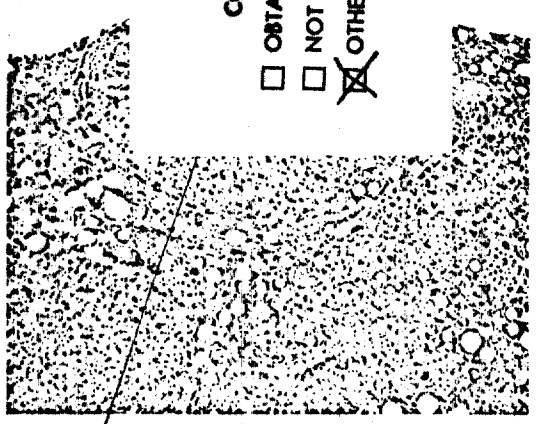

Abb. 2. Adenomartige Struktur eines Knötchens an der Leberoberfläche nach dreimonatiger cholinarmer Diät. In der Umgebung grobtropfige Verfettung und Riesenleberzellen.

chenförmige adenomatöse Bildungen auf, welche den Charakter von Regeneraten haben. Aber auch das übrige Lebergewebe zeigt Zeichen von gesteigertem Umbau. Neben starker fein- und grobtropfiger Verfettung weisen die Leberzellen auffallende Größenunterschiede auf. Man kann von eigentlichen Riesenleberzellen sprechen, deren Kerne ebenfalls teilweise sehr groß, gelegentlich pyknotisch sind. Das interstitielle Gewebe ist diffus leicht vermehrt und zellreich. Es finden sich Lymphozyten, Plasmazellen sowie auch Vermehrung von Histiozyten. Manchmal sieht man, in stark dystrophischem Lebergewebe eingelagert, ganze Herde, die aus kleineren und großen adenomartigen Bezirken aufgebaut sind. Im umliegenden Gewebe liegen wabige, wie Schaumzellen aussehende Gebilde, in denen noch kleine Kerne zu erkennen sind. Im Fettrotpräparat ergeben diese Schaumzellen intensive feinkörnige Verfettung.

Bis jetzt wurden 106 Ratten beiderlei Geschlechts in den Versuch eingesetzt. Davon gingen 20 an den Folgen des akuten Cholinmangels (subkapsuläre Nierenblutungen, Nierenrindennekrose, Leberverfettung, intraokulare Blutungen[1] usw.) ein. Es handelt sich also um Tiere, die trotz der geringen Cholinzufuhr den Zustand der »hämorrhagischen Degeneration« nicht überstehen konnten. Von den übrigen 86 Ratten gingen 17 z.T. an Infektion der oberen Luftwege, z.T. an den Folgen des chronischen Cholinmangels ein. 10 von diesen Ratten wiesen Adenome der Leber auf. Wir halten es nicht für ausgeschlossen, daß sich diese Leberadenome nach einer genügend langen Cholinmangelperiode zu Adenokarzinomen weiterentwickeln.

Die Versuche wurden aus Mitteln der Emil-Barell-Stiftung zur Förderung der medizinisch-wissenschaftlichen Forschung durchgeführt.

H. STAUB, G. VIOLLIER und A. WERTHEMANN

Eiweißlaboratorium der medizinischen Klinik und Pathologische Anstalt der Universität Basel, den 9. März 1948.

Summary

Hepatoma-like neoplasmas were found in the liver of rats that had been on a choline-deficient diet for 3 to 4 m... the work of COPELAND and SALMON is c... ...r experiments, however, the lesions were o...

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...und G. VIOLLIER, Helv. physiol. acta 6, C 3 (1948).

The Influence of Sodium Thiosulfate on Experimental Tumor Induction

In a recent paper we described the influence of sodium thiosulfate on the proteolytic action of papain and related plant enzymes[1]. While partly purified and inactivated enzyme preparations were reactivated by low concentrations of this salt, an inhibitory action was observed in the presence of thiosulfate when the activity of proteolytically active plant saps was tested. It was suggested that the thiosulfate ion is able to replace the active part of the ferment which contains an SH-group.

Considerable comparative work has been done on the catheptic activity of tissue and serum of normal and tumor-bearing animals and humans[2]. In general, an increased catheptic activity of the serum of tumor bearing animals and tumor tissue has been found. As some cathepsins resemble the papain-like plant enzymes in their behavior toward activators and inhibitors, it was thought to be of interest to study the action of sodium thiosulfate on experimental carcinogenesis.

Two months old albino mice of a strain showing 0·5% spontaneous sarcomas at the age of 1 year and 15% spontaneous pulmonary adenomas at the age of 6 months were used in the following experiments. In the treated groups, finely ground crystalline sodium thiosulfate was added to the stock diet in the amount of 0·4%, and this supplemented diet was fed 1 week before the carcinogenic treatment and through the whole experimental period. The results presented in table I and II show that the number of tumors produced by methyl-cholanthrene

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Table I

Comparison of the percentages of mice developing local tumors within 4 months after a single subcutaneous injection of methylcholanthrene and fed a diet with or without added sodium thiosulfate.

Dose of methylcholanthrene	Diet	Number of animals	% of animals with local tumors
0·9 mg	stock	119	63
0·9 mg	stock + 0·4% thiosulfate	44	34
0·45 mg	stock	86	64
0·45 mg	stock + 0·4% thiosulfate	49	51

Table III

Comparison of the number of rats with liver tumors after 15 months on a diet containing 0·015% of p-dimethylaminoazobenzene with or without added sodium thiosulfate

Diet	Effective total	Number of rats with liver tumors	Number of rats with other tumors
stock + 0·015% p-dimethylaminoazobenzene	38	10	6
stock + 0·015% p-dimethylaminoazobenzene + 0·4% thiosulfate . .	40	1	2

or urethane was smaller when thiosulfate was fed with the diet. In an experiment with a transplantable mouse sarcoma there was no difference in takes in 72 mice on the experimental diet and 60 controls.

In another experiment 2 months old albino rats were fed the stock diet with 0·015% of p-dimethylaminoazobenzene. Moreover, one group received 0·4% of sodium thiosulfate with the diet. The result is presented in table 3 and shows an impressive reduction of the number of liver tumors in the treated group after 15 months on the diet. Histological changes in the livers other than tumors were also reduced in the animals receiving thiosulfate.

No detrimental effect of the thiosulfate diet could be detected in the treated groups and in 2 control series receiving the same diet without carcinogenic treatment. Individual food intake was not recorded but gross determinations did not reveal any difference in food consumption or between final weights of the treated and control animals. The fact that the number of takes of tumor implants in mice was not reduced by the thiosulfate diet may indicate that its action is perhaps exercized during the stage of malignant transformation, although the experiments presented do not allow to decide whether the treatment only delays tumor development or whether it definitely inhibits it. The influence on tumor growth was not studied. It seems especially noteworthy that the effect of the thiosulfate treatment was observable with 3 completely different and differently acting carcinogens, because most of the known anticarcinogenic agents are rather specific in their effect, probably because they act on the metabolic destruction or excretion of the carcinogenic agent. The fact that thiosulfate is active with the different carcinogens used may be an indication that its action is more closely related to the process of carcinogenesis. Any attempt to explain its action would yet be purely speculative.

It may be mentioned that thiosulfate has been used in clinical cases of cancer and that encouraging results have been reported[1]. The present experiments can not be taken as evidence for a therapeutical value of this compound as its effect on established tumors has not been studied. Its counteraction of the tendency of urethane to produce pulmonary tumors in mice may be of practical interest, as this compound, although definitely carcinogenic[2], has some use in human therapy and has been applied lately in clinical cases of leukemia[3] and prostatic tumors[4].

The experiments were made in the Instituto Quimico-Biológico, Caracas. We are indepted to Dr. Rudolf Jaffé for the histological study of the tumor slices.

W. G. Jaffé

Departamento de Química, Ministerio de Agricultura y Cría, Caracas, Venezuela, April 4, 1948.

[1] K. Stern and R. Willheim, The Biochemistry of Malignant Tumors (Reference Press, Brooklyn, N.Y., 1943), pp. 70 and 290.
[2] W. G. Jaffé, Cancer Res. 7, 107 (1947).
[3] E. Paterson, A. Haddow, A. Thomas, and J. M. Watkinson, Lancet I, 677 (1946).
[4] C. Huggins, S. T. Ye, and R. Jones, Science 106, 147 (1947).

Table II

Comparison of the percentages of mice developing ethylurethane-induced pulmonary adenomas when fed a diet with or without added sodium thiosulfate

Route of application and dose of urethane	Diet	75 days after beginning of treatment		Mean number of tumor nodules	110 days after beginning of treatment		Mean number of tumor nodules
		Effective total	% of tumor bearing mice		Effective total	% of tumor bearing mice	
0·15 mg injected	stock	87	68	2·7	22	92	5·4
0·15 mg injected	stock + 0·4% thiosulfate	76	59	1·8	25	58	2·8
0·15 % in the diet	stock + urethane	85	51	3·9	50	98	12
0·15 % in the diet	stock + urethane + 0·4% thiosulfate	16	47	1·3	22	92	11
0·075% in the diet	stock + urethane	10	80	1·5	14	100	11
0·075% in the diet	stock + urethane + 0·4% thiosulfate	10	60	1·5	34	68	3·2

The mean number of pulmonary adenoma nodules was calculated for the tumor bearing animals only.

Zusammenfassung

Mäuse wurden mit einer Diät ernährt, die 0,4 % Natriumthiosulfat enthielt. Nach Injektion von Methylcholanthren entwickelten sich dann weniger lokale Tumoren als bei den Kontrolltieren. Ebenso gab es seltener Lungenadenome nach Verabreichung von Urethan. Dasselbe zeigte sich bei Ratten: durch Buttergelb wurden weniger Hepatome erzeugt, wenn gleichzeitig Natriumthiosulfat gegeben wurde.

Cathétérisme intracardiaque en clinique

Importance de la méthode

Le cathétérisme des cavités du cœur, déjà pratiqué depuis longtemps sur l'animal par les physiologistes, a été effectué chez l'homme pour la première fois en 1929. FORSSMANN[1] réalisa sur lui-même cette expérience, introduisant une sonde jusque dans son oreillette droite, par une veine du bras mise à nu.

Depuis lors, de nombreux chercheurs[2] ont utilisé cette méthode d'investigation; ils ont pu se convaincre de son innocuité. Les travaux les plus importants sur ce sujet ont été publiés par MONIZ[3] (Lisbonne), COURNAND[4] (New-York), MCMICHAEL[5] (Londres), LENÈGRE[6] (Paris), CHAVEZ (Mexico) et leurs élèves.

Alors que la plupart des opérateurs dénudent la veine jugulaire droite et utilisent des cathéters semi-rigides, d'autres, dont LENÈGRE et MAURICE[6], introduisent une sonde souple par un trocart placé dans une veine du pli du coude, la basilique de préférence. Il s'agit donc d'une ponction veineuse transcutanée, ce qui constitue en fait un procédé relativement simple. Venant des grandes veines, la sonde gagne l'oreillette droite, le ventricule droit et dans les cas favorables remonte jusque dans l'artère pulmonaire. Il arrive aussi qu'elle pénètre dans l'oreillette gauche par le trou de Botal et atteigne ainsi l'orifice d'une veine pulmonaire dans laquelle elle s'engage (fig. 1).

Jusqu'ici nous avons eu la possibilité d'explorer 18 fois l'oreillette droite, 13 fois le ventricule droit, 2 fois l'oreillette gauche, 1 fois une veine pulmonaire, 2 fois l'artère pulmonaire.

Le sondage permet de multiples investigations:

1° Mesure de la pression dans les cavités cardiaques et les vaisseaux pulmonaires et caves.

2° Enregistrement d'électrocardiogrammes intracardiaques à tous les points du parcours de la sonde.

3° Prélèvement d'échantillons de sang dans les cavités explorées, en vue du dosage de l'O_2 et du CO_2 (calcul exact du débit cardiaque; diagnostic de communications anormales).

4° Prélèvement d'échantillons de sang dans la veine cave inférieure, dans les veines sus-hépatiques ou rénales (étude chimique des fonctions hépatiques et rénales).

5° Radiographie des cavités cardiaques et des gros vaisseaux par l'injection d'un liquide radio-opaque,

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[5] J. MCMICHAEL et E. P. SHARPEY-SCHAFER, Brit. Heart J. *6*, 33 (1944).

[6] J. LENÈGRE et P. MAURICE, Paris médical, *129*, 21, 23 (1945). Acta cardiolog. *2*, 1 (1947).

Fig. 1. La sonde introduite par la veine basilique droite a gagné l'oreillette droite, puis a pénétré dans l'oreillette gauche en franchissant le trou de Botal. Elle s'est engagée dans une veine pulmonaire gauche. Femme 75 ans. Infarctus pariétal, R. M.

méthode connue sous le nom d'angiocardiographie (moyen de diagnostic indispensable chez les congénitaux destinés à la correction chirurgicale).

A côté de l'intérêt physiologique évident de ces applications du cathétérisme, c'est avant tout la portée clinique de la méthode qui a retenu notre attention. L'un de nous a introduit récemment à la Clinique médicale le procédé spécial de sondage tel qu'il est pratiqué par LENÈGRE[1]. Cet auteur a déjà démontré que l'élévation de la *pression ventriculaire droite* précède le plus souvent tous les signes classiques de la décompensation. On conçoit l'intérêt lié à la connaissance de cette nouvelle mesure manométrique. Citons par exemple sa valeur chez des malades appelés à subir une intervention chirurgicale ou chez des femmes enceintes atteintes de cardiopathie. La mesure des pressions endocavitaires fournit aussi un appoint utile à la reconnaissance des malformations congénitales (COURNAND[2]). Elle facilite le diagnostic différentiel des pneumopathies et des cardiopathies chroniques.

Nous extrayons de nos premiers examens deux exemples:

1° Ba. Paul, 54 ans, voyageur, fréquentes bronchites, bronchopneumonie en 1946. Cyanose. Hb. 104%, Gl. r. 5218000. Dyspnée d'effort accusée. Capacité vitale 900 cm³. R. X.: augmentation globale du cœur. Tension artérielle: 140/90. ECG: déviation axiale droite. B.W. négatif. Le cathétérisme donne les chiffres de pression suivants: Or. dr. 5 cm; ventr. dr. 49 cm (chiffre normal 15 cm d'eau); Or. g. (traversée du trou de Botal) 9 cm.

L'élévation de la pression intraventriculaire droite indique nettement une surcharge imposée à cette moitié du cœur et l'influence néfaste de la pneumopathie chronique.

2° Weg. James, 24 ans, polisseur, pneumonie en 1943, bronchite en 1947 et dyspnée progressive. Maigreur et mauvais état général.

[1] J. LENÈGRE et P. MAURICE, Paris médical *129*, 21, 23 (1945).

[2] A. COURNAND, H. D. LAUSON, R. A. BLOOMFIELD, E. S. BREED et F. E. DE BALDWIN, Proc. Soc. Exp. Biol. a. Med. *53*, 34 (1944).

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nous sommes pas servis chez la Souris, mais seulement chez [illegible]mme), agit sur différents organites de la cellule tumorale, [illegible] sur un autre mécanisme défensif et il sera utile de les com[illegible]er pour obtenir le maximum d'action thérapeutique. Il est [illegible]able qu'on ne pourra obtenir la guérison complète que par [illegible] méthode, avec l'aide de l'organothérapie [spécialement or[illegible] réticulo-endothéliaux (réticulin), rate, thymus, foie et cor[illegible] surrénal]. Ces associations, l'ordre des injections et éventuel[illegible]ent le problème du régime doivent intervenir dans les expé[illegible]nces qui restent à faire.

Nos recherches montrent qu'il est possible d'agir sur la décom[illegible]sition de la tumeur ou sur le ralentissement de sa croissance [illegible]r les substances ci-dessus mentionnées ; les résultats sont d'au[illegible]nt meilleurs que le traitement est commencé plus tôt. Il en est [illegible] chimioth[illegible]rapie que pour les interventions chi[illegible] l'actinothérapie. L'utilisation en temps voulu [illegible]loréthyle amène la guérison complète.

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Thiosulfate

L'ACTION ANTIANAPHYLACTIQUE DU SÉRUM AU THIOSULFATE,

par J. KABELIK.

Comme sérum au thiosulfate j'ai utilisé le sérum de jeunes Chevaux qui reçoivent, par voie intraveineuse, 4 heures avant la saignée, une forte dose de thiosulfate de soude à 10 p. 100 (1*).

Mes expériences ont été faites sur le Cobaye. Les animaux étaient sensibilisés par injection intrapéritonéale de sérum humain ou bovin (dose sensibilisatrice 0,1 c.c.). Après 14 jours, le choc était toujours provoqué avec une dose de 1 c.c. (dose déchaînante) en injection intrapéritonéale. L'animal a toujours succombé en 10 minutes. $S_2O_3Na_2$ est capable d'inhiber le choc (lumière). S_2O_3Mg est près de 2 fois 1/2 plus actif, mais aussi 5 fois plus toxique, ce qui est sans importance, par suite de la faible toxicité du thiosulfate (la dose toxique de $S_2O_3Na_2$ pour un Mammifère est d'environ 3-4 gr. par kgr.). Dans nos expériences il était possible, avec une régularité mathématique, d'inhiber le choc par l'injection intrapéritonéale de 0.5 c.c. de $S_2O_3Na_2$ à 10 p. 100, si ce produit était administré 20 minutes au moins avant l'injection du sérum. En effet il suffit déjà, d'après nos expériences, d'ajouter 2 p. 100 de glucose et 2 p. 100 de thiosulfate au sérum pour empêcher le choc mortel, mais il vaut mieux

(1) Ce sérum a été préparé à l'Institut d'hygiène de l'Etat, à Prague, par [illegible] du Dr Feierabend.

cependant injecter le thiosulfate auparavant. Si on injecte le sérum et que les premiers symptômes du choc apparaissent, aucune injection de thiosulfate n'est plus capable de sauver l'animal. L'antianaphylaxie persiste pendant quelques jours, et 4 jours après l'injection de thiosulfate, nous ne pouvons déchaîner le choc. Après 24 heures l'immunisation semble être la plus haute. Nous n'avons pas eu l'occasion de constater si elle dure plus de 4 jours. Nous n'avons pas fait non plus d'épreuves quantitatives, de sorte que nous ne pouvons dire combien il faut injecter de $S_2O_3Na_2$ pour qu'il se développe une antianaphylaxie envers une certaine dose déchaînante variable.

Le sérum au thiosulfate possède les mêmes propriétés ; 2 c.c. développent la même antianaphylaxie, plus parfaite même, sans le moindre symptôme clinique. Les conditions de temps sont les mêmes qu'après l'administration de thiosulfate pur. Le sérum normal de Cheval est sans effet anaphylactique. L'antianaphylaxie par le sérum au thiosulfate ne peut pas être causée par la présence mécanique du thiosulfate, car on ne peut déceler chimiquement de traces de cette substance. Après 4 heures, plus de la moitié en est éliminée de l'organisme du Cheval, l'autre partie est chimiquement modifiée et même si toute la dose de $S_2O_3Na_2$ injecté restait dans le sang, elle serait diluée au-dessous de la dose active.

Il serait nécessaire à l'avenir d'opérer aussi avec le sérum du même Cheval avant et après l'injection de thiosulfate, pour préciser le moment où il sera le plus actif, et de faire aussi des épreuves avec d'autres animaux, pour voir s'ils ne conviennent pas mieux que le Cheval.

Nous pouvons aussi montrer qu'une petite quantité de $S_2O_3Na_2$ ou de sérum au thiosulfate ajoutée au sérum normal sensibilisant humain ou bovin, ne peut pas empêcher la sensibilisation. Il faut aussi établir si une forte dose n'en est pas capable, et rechercher si, chez le Cobaye sensibilisé par le sérum de Cheval, le sérum de Cheval au thiosulfate peut provoquer le choc.

Le facteur antianaphylactique peut être extrait du sérum par l'alcool. On ajoute à 100 c.c. de sérum au thiosulfate 50 c.c. d'alcool concentré, on laisse 14 jours ; on évapore ensuite les 10 c.c. de liquide pur surnageant le précipité de protéines : on dissout le résidu dans de la solution physiologique et on l'injecte au Cobaye. Il a une action antianaphylactique, très faible 2 minutes après l'injection intrapéritonéale, déjà marquée après 20 minutes, très forte après 22 heures. Nous n'avons pas fait d'expériences plus prolongées ; par analogie avec les expériences mentionnées, nous pouvons nous attendre à une action, même après plusieurs jours. L'extrait du sérum normal de Cheval, préparé de la même manière, était sans effet. Il en était de même de

l'extrait alcoolique de rate normale de Cheval, malgré ses propriétés antianaphylactiques qu'a établies Birke (2*) et bien qu'il s'agisse d'un organe riche en système réticulo-endothélial. Mais il est possible qu'un extrait splénique à l'eau, à l'éther ou à l'éther de pétrole aurait une action antianaphylactique. Il est aussi probable qu'on pourrait retrouver l'activité antianaphylactique dans un extrait dont la fraction lipoïdique gênante, par exemple, serait éliminée par l'acétone.

Une méthode convenable d'extraction nous permettrait peut-être de concentrer ou d'isoler directement la substance antianaphylactique du sérum au thiosulfate ou d'un autre organe.

Nos expériences sont donc en rapport étroit avec les travaux de Moldovan (3*) qui, en excitant le système réticulo-endothélial par des injections intraveineuses d'encre de Chine, cherche à accumuler l'hormone hypothétique réticulo-endothéliale, qu'il appelle réticuline M, dans le sang et à l'en extraire par l'alcool. Je considère que les injections de thiosulfate conviennent mieux à cet effet; la solution hypertonique (20 p. 100 de ce sel) peut provoquer à elle seule une forte sécrétion du système réticulo-endothélial; de plus l'action eucolloïdale du thiosulfate élève fortement l'eucolloïdalité du plasma (voir les travaux de mon institut) et, par cela, sont préparées les conditions les plus favorables au fonctionnement du système réticulo-endothélial. Il est aussi très probable que la réticuline est très proche des substances comme l'insuline, les hormones de la circulation sanguine, qui abaissent la pression sanguine, ainsi que de l'hormone du cortex surrénal. Ces substances sont en lien étroit avec le métabolisme du soufre et on sait que le soufre bivalent est directement la partie principale de leurs molécules. Le thiosulfate pourrait alors constituer un excellent matériel d'édification.

Il serait très intéressant d'étudier le rapport des propriétés antianaphylactiques avec la phagocytose, qui peut être un rapport de causalité réciproque. Lebduska (4*) a montré que le thiosulfate élève la phagocytose de près de 100 p. 100 et Schliephake (5*) a aussi trouvé que l'extrait (sans protéine ni lipoïde) de la rate, le « Prosplen », peut activer très fortement la phagocytose.

Le meilleur fonctionnement du système réticulo-endothélial active non seulement la capture des Bactéries du sang, mais aussi la phagocytose des leucocytes neutrophiles. Parallèlement à la régulation de l'eucolloïdalité du plasma sanguin évoluent la régulation de la sédimentation des érythrocytes, l'amélioration du fonc-

(2*) Birke. *Münch. med. Wochenschr.*, 1928, 2099.
(3*) Moldovan. *C. R. de la Soc. de biol.*, 1928, t. *98*, p. 1617, etc.
(4*) Lebduska et Cirvinka. *C. R. de la Soc. de biol.*, 1930, t. *103*, p. 366.
(5*) Schliephake. *Deutsch. Arch. f. klin. Med.*, 1931, t. *171*, fasc. 2.

tionnement du système réticulo-endothélial, la phagocytose et la moindre sensibilité anaphylactique.

Tout cela nous montre l'importance pratique de ces expériences. Ainsi, par exemple, on peut très sensiblement améliorer les sérums thérapeutiques, si l'on injecte au Cheval immunisé avant le prélèvement du sang, une forte dose de thiosulfate, ce qui diminue le danger des accidents d'anaphylaxie et rehausse leur action antibactérienne. Les sérums préparés par cette méthode ou d'autres substances obtenues par extraction pourraient renforcer l'action des médicaments chimiothérapiques (comme le fait le thiosulfate pour le salvarsan) et trouver probablement leur utilisation dans la thérapeutique biologique des tumeurs malignes.

Nombre de chromosomes et cellules sexuelles polyvalentes chez l'Homme,

par K. Precechtel.

Le nombre diploïde de chromosomes, dans les cellules sexuelles de l'Homme, n'est pas encore établi avec précision. Plusieurs auteurs (Duesberg, Branca, Gutherz, Guyer, Montgomery, Jordan, Niemann) ont trouvé 12 chromosomes dans le groupe haploïde dans les cellules sexuelles mâles ; Moore et Arnold ont trouvé 16, Vilcox 18 chromosomes. Pour le groupe diploïde H. v. Winiwarter et Oguma ont trouvé 47. Paintner, Ewans et Swezy 48, Stieve 40-55 chromosomes. L'évaluation du nombre de chromosomes dans les cellules humaines somatiques n'a donné aussi aucun résultat positif ; Flemming et Schachov en ont compté 24 pour le groupe diploïde de cellules somatiques ; O. Grosser une fois 45-54, une autre fois seulement 30-36 ; Rappeport indique 32-53 chromosomes.

Si nous comparons tous les résultats des numérations de chromosomes dans les cellules sexuelles mâles, nous obtenons une série de nombres (24, 32, 36, 47 ou 48), qui indiquent un nombre diploïde de chromosomes dont la majeure partie est multiple du nombre 12. Les différents résultats chez les auteurs mentionnés s'expliquent facilement en admettant que les uns ont compté les chromosomes dans des cellules diploïdes, les autres dans des cellules triploïdes ou tétraploïdes.

Nous sommes arrivés à cette conclusion en calculant les volumes d'œufs humains (follicules de Graaf mûrs) de différentes grandeurs et de leurs noyaux dont les diamètres sont connus

Comptes Rendus Soc. Biol. 110: 397-400 (1932).

THE ANTIANAPHYLACTIC ACTION OF SERUM TO THIOSULFATE

by J. Kabelik

As the thiosulfate containing serum we used serum obtained from young horses who had received a strong dose of sodium thiosulfate (10%) administered intravenously four hours before the animals were bled. (1*)

My experiments were carried out on guinea pigs. The animals were sensitized by an intraperitoneal injection of human or bovine serum (the sensitizing dose was 0.1 cc). After 14 days, shock was always produced by a dose of 1 cc (discharge dose) injected intraperitoneally. The animal always died within 10 minutes. $S_2O_3Na_2$ is capable of inhibiting the shock (light). $S_2O_3Mg_2$ is nearly two and a half times more active; however, it is also five times more toxic but this is of no importance since thiosulfate has very low toxicity (the toxic dose of $S_2O_3Na_2$ for a mammal is about 3-4 grams per kilogram). In our experiments it was possible, with mathematical regularity, to inhibit the shock by an intraperitoneal injection of 0.5 cc of $S_2O_3Na_2$ in a concentration of 10 per cent, provided that this compound was administered at least twenty minutes before the injection of serum. Thus, according to our experiments, it was sufficient to add 2 per cent glucose and 2 per cent thiosulfate to the serum in order to prevent fatal shock; however, it was better to inject the thiosulfate beforehand. When the serum was injected and the first symptoms of the shock appeared, no injection of thiosulfate was capable of saving the animal from

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1*) This serum was prepared by the State Institute of Hygiene, at Prague by Dr. Feieranbend.

fatal shock. Nevertheless, the anti-anaphylaxis did continue for several days and four days after the injection of thiosulfate we were not able to discharge the shock. After 24 hours, the immunization appeared to be highest. We did not have the opportunity of testing if the immunity lasted longer than four days. Furthermore, we did not carry out any quantitative tests of the sort that we could state how much of $S_2O_3Na_2$ to inject in order to see if anti-anaphylaxis would develop around a certain variable releasing or discharging dose.

The thiosulfate serum possessed the same properties; 2 cc produced the same anti-anaphylaxis, even without the least clinical symptom. The conditions of time were the same as after the administration of pure thiosulfate. Normal horse serum had no anaphylactic effect. The anti-anaphylaxis produced by the thiosulfate containing serum could not be due to the mechanical presence of thiosulfate because we could not chemically detect traces of this substance. After four hours more than one-half of the substance was excreted from the organism (horse); the remaining portion was chemically altered, and even if the entire amount of injected $S_2O_3Na_2$ remained in the blood, it was diluted beyond the active dose.

In the future, it would be advisable to use the serum of the same horse before and after the injection of thiosulfate, in order to determine the time when the serum is most active; tests using other animals should also be made, in order to see if they are more suitable for these experiments than the horse.

We should be able to show that a small quantity of $S_2O_3Na_2$ or of thiosulfate containing serum added to normal sensitized

human or bovine serum did not prevent the sensitization. It should also be established if a high dose was not capable of preventing the sensitization; we should also attempt to see if in guinea pigs sensitized by horse serum, horse serum containing thiosulfate may produce shock.

The anti-anaphylaxis factor may be extracted from the serum by alcohol. Fifty cc of concentrated alcohol are added to 100 cc of thiosulfate containing serum and the mixture is left to stand for 14 days. Then 10 cc of the pure liquid containing the supernatant of precipitated proteins are evaporated; the residue is dissolved in physiological saline and injected into guinea pigs. The solution has an anti-anaphylaxis action, which is very weak two minutes after the intraperitoneal injection, but is quite marked after 20 minutes and very strong after 22 hours. We did not carry out longer experiments. However, by analogy to the experiments mentioned, we believe that we would observe an effect even after several days. The extract of normal horse serum, prepared in the same manner, has no effect. The same is true for an alcohol extract of horse spleen, despite the anti-anaphylactic properties established by Birke (2*), and despite the fact that the spleen is a rich organ of the reticuloendothelial system. However, it is possible that an extract of spleen prepared from water, ether, or with petroleum ether might have an anti-anaphylactic action. It is also probable that an extract with its interfering lipid fraction removed by acetone, could also possess anti-anaphylactic activity.

(2*) Birke, Munch. Med. Wochensehr., 1928, 2000.

An expedient method of extraction would be to concentrate or separate directly the anti-anaphylactic substance of serum containing thiosulfate or serum obtained from another organ.

Our experiments, are therefore in agreement with the studies conducted by Moldovan (3*); by exciting the reticulo-endothelial system with repeated intravenous injections of china ink, this investigator was able to study the accumulated hypothetical reticuloendothelial hormone which he named reticulin M; this substance was present in the blood and could be extraced with alcohol. I believe that injections of thio-sulfate are more suitable for this purpose; a hypertonic solution (20 per cent of this salt) may be used for inducing marked secretion of the reticuloendothelial system. It is also very probably that reticulin very closely resembles a substance such as insulin, and the hormones present in the circulating blood, which lower blood pressure as well as the hormones secreted from the adrenal cortex. These substances are quite closely associated with the metabolism of sulfur and it is a known fact that bivalent sulfur is the direct principle portion of their molecules. Thus, thiosulfate would be an excellent substance to study.

It would be very interesting to study the relationship between the anti-anaphylactic properties and the phenomenon of phagocytosis; the relationship may be a causal, reciprocal one. Lebduska (4*) showed that thiosulfate increases phagocytosis by

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(3*) Moldovan, C.R., de al Soc. de Biol., 1928, t. 98, p. 1617, etc.

(4*) Lebduska and Cirvinka, C.R., de la Soc. de biol., 1930, t. 103, p. 366.

nearly one hundred per cent; Schliephake (5*) also observed that an extract from the spleen (without any protein or lipid) known as "Prosplen" could markedly increase phagocytosis.

The best function of the active reticuloendothelial system is not only the capture or engulfment of bacteria in the blood, but also phagocytosis of neutrophilic leukocytes. Parallel to the regulation of the eucolloid of the blood plasma, there results regulation of erythrocyte sedimentation, improvement of the reticuloendothelial functions, phagocytosis and decreased sensitivity to anaphylaxis.

All these facts simply indicate the practical importance of these experiments. Thus, for example, it is possible to improve appreciably the therapeutic sera, by injecting a high dose of thiosulfate into the horse before the blood sample is collected; this shall decrease the danger of anaphylaxis and increase the antibacterial action. Sera prepared by this method or other substances obtained by this method may be used to strengthen the action of chemotherapeutic agents (for example, thiosulfate may strengthen salvarsan) and will probably be found very useful in the therapeutic biology of malignant tumors.

(5*) Schleiphake, Deutsch. Arch. f. klin. med., 1931, t. 174, fasc. 3.

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ORIGINAL COMMUNICATIONS

SODIUM THIOSULPHATE IN THE TREATMENT OF ALLERGY*

REPORT OF SIX CASES

By JACOB E. KLEIN, M.D.
Normal, Ill.

The relation of alterations in the colloid state of the tissues to allergic manifestations is worthy of further study, since this may aid in understanding and treating allergic reactions. In a previous report[1] on allergic epilepsy I briefly mentioned Lumiere's experimental studies on alterations of the colloid state in anaphylaxis. On the basis of these studies I used sodium thiosulphate in the treatment of a patient with allergic epilepsy. The convulsive seizures disappeared as did the asthma. The patient, who had previously been much undernourished, gained markedly in weight. This was a clinically successful application of Lumiere's theories on anaphylaxis.

*From Soldiers' and Sailors' Children's School, Normal, Ill.

In 1921 Lumiere[2] published his brilliant essay on the rôle of colloids in biology. As stated in my first report, and reiterated now, this work deserves to receive serious consideration, since it is based on experimental evidence and seems of great practical significance. Lumiere maintains that anaphylactic phenomena are due to an alteration in the colloids of the tissue fluids and blood plasma wherein the usually finely dispersed colloid particles are agglutinated and form precipitates in the perivascular lymphatics and tissue spaces. A flocculation of these altered colloid particles in the lungs causes asthmatic symptoms; in the brain, it results in epilepsy; in the skin, it induces urticaria, etc. Lumiere suggested that treatment should aim at dissolving or breaking up this abnormal state of the colloids. On the basis of experimental studies he suggested that sodium thiosulphate might be effective for this purpose.

In colloid phenomena surface tension and electric charge are important factors. As Bayliss[3] has stated, colloidal particles possess electric charges and are prevented from aggregating and precipitating by the mutual repulsion of these charges. In the precipitation of colloids bivalent ions are more effective than univalent, and trivalent ions more than bivalent. This definitely demonstrates the importance of electric charge in precipitation phenomena. For our present purpose it is sufficient to point out that certain physical phenomena, such as surface tension and particle charge of colloids, are significant physical changes which may alter colloid states and reactions. When flocculation or precipitation occurs in the body the normal colloidal state is disrupted and there results local or even general alterations in the tissues which may vary in severity from a temporary local anaphylactic reaction to a general shock terminating in death.

Allergy then may be considered one of the modes of response of the tissues to a foreign substance. This is not a fixed mode of reactions as Rössle[4] has justly indicated. He designates these altered tissue states as pathergies, among which he recognizes allergic, anergic and parallergic types of reaction. Let us rather stress the importance of understanding the tissue changes in allergy and logically control them through basic knowledge of these phenomena. Let us not be confused by the infinite number of allergens.

The following brief clinical reports are offered as examples which in practice have demonstrated the efficiency of the sodium thiosulphate treatment of various allergic manifestations, including migraine, urticaria, Arthus's phenomenon after repeated serum injections, and serum anaphylaxis.

CASE REPORTS

Case 1: C. S., female, age 10 years, observed at the Pediatric Clinic, Northwestern University (No. 63486) was admitted October 5, 1933 with complaints of headache and pain in the abdomen. The headache was hemicranial in type and most severe in the right frontal region. Vomiting occurred when the headaches were severe. The headaches had been occurring daily and had become so severe as to keep the child from school the week before coming to the clinic. Abdominal pains of a colicky nature had occurred intermittently for the past two years, approximately every 10 days to two weeks, and accompanied the attacks of headache. The bowel movements were normal.

Family History. The father died of asthma. Also a grandfather and a grandmother had died of asthma. The entire family is hypersensitive to tomatoes. One brother of the patient has epilepsy.

Birth History. The patient was born at home in normal labor after two and one-half hours. The birthweight was seven pounds. No history of asphyxia or convulsions.

Feeding History. Breast fed up to five weeks. Then refused the breast and was placed on artificial feeding formula.

Developmental History. Sat up at 7 months; talked at 11 months; walked at 14 months; had first tooth at 9 months.

Previous Illnesses. Mumps at 2 years; measles at 5 years; chickenpox at 8 months. Smallpox vaccination at 6 years. Received toxin-antitoxin for diphtheria.

Physical Examination. The general condition was fair, although the child was somewhat malnourished. Head: No noteworthy defects were observed in the examination of the eyes, ears, nose and throat. Neck: The middle lobe of the thyroid was slightly enlarged. Heart and lungs were normal. Abdomen presented normal findings. There was no localized tenderness or rigidity; nor was there any evidence of distention or unusual

peristalsis. Extremities: There were no defects. The reflexes were normal.

Diagnosis. Migraine with abdominal symptoms on an allergic constitutional basis.

Treatment: Sodium thiosulphate was given by mouth as per the following prescription:

℞ Sodium thiosulphate 14.0
Aqua destillata 120.0
Sig. One teaspoonful every 4 hours.

Progress Notes. October 19, 1933—the abdominal pains had ceased. The headaches now occurred 2 to 3 times weekly, instead of daily. The general condition had improved markedly. The patient was advised to continue the oral sodium thiosulphate treatment. No further notes are available on the end results as the patient was not seen at the clinic any more.

Discussion. This was a classical example of severe migraine with concomitant abdominal symptoms in a child with an allergic family background and an allergic constitution. Sodium thiosulphate by mouth induced definite clinical improvement and amelioration.

Case 2. H. R., male, age 40 years.

Complaint. Severe migraine seizures which cause the patient to take to bed for a day or two and interfere with his working efficiency. This has been occurring at irregular intervals since adolescence. The attacks vary in frequency but occur at least once a week. The patient has resorted to aspirin, bromoseltzer, etc. for symptomatic relief. In this instance there was no history of food allergy, nor evidence of other types of allergy.

On January 5, 1937 the patient was observed when suffering from a particularly excruciating attack of migraine. He walked into the office with pale, drawn features and clenched teeth, and seemed very much in agony. He was immediately given an intravenous injection of seven and one-half grains sodium thiosulphate. Within 15 minutes he was completely relieved of his headache and had regained his normal color and appearance. The treatment acted like a specific in relieving the attack.

Discussion. An adult with excruciating attack of migraine received rapid relief from intravenous administration of sodium thiosulphate. In my opinion migraine is similar to epilepsy, except

that the one induces sensory reactions and the other causes motor phenomena. Applying Lumiere's colloid theories on allergy, one might conceive of an allergic flocculation or precipitation of the tissue colloids in the sensory regions of the cerebral cortex with an associated vasomotor disturbance and edema.

Objective findings and descriptions of cerebral changes in migraine are rare. For this reason Goltman's[5] description of a craniocerebral exploration during a typical attack of migraine is of great interest. The patient had been under observation since a cranial operation performed in 1927. The migraine attacks were accompanied by generalized vasomotor spasm. The dura was tense and did not pulsate. The brain was edematous, and the spinal fluid was under increased pressure. In the brain there was secondary vascular dilatation with resulting edema. Also hypersecretion of cerebrospinal fluid occurred, followed by hyperabsorption.

It seems that in persons suffering from migraine the sensory region of the brain has been biologically altered so that it responds in an allergic manner to the particular allergen which provokes the reaction. I suspect that the cerebral changes in migraine are analagous to the tissue alterations observed in urticaria. When we recall that nerve tissues are ectodermal in origin, this view does not seem entirely unreasonable. Therefore, the sodium thiosulphate treatment should be as effective in the one as in the other condition, as the following case report would seem to indicate.

Case 3. M. G., male, age 13 years, weight 105 pounds, height 61.8 inches. Admitted to the hospital November 15, 1936.

Present Complaint. Severe generalized urticaria (giant hives). General health excellent. No history of previous allergic manifestations.

Previous Illness. Upper respiratory infections—influenza. Received toxin-antitoxin, August 29, 1926; scarlet fever immunization, March 1, 1927.

Physical Examination. General health good. Head: Tonsils removed. Teeth—normal. Heart—functional systolic blow. Lungs—normal. Abdomen—no localized tenderness, rigidity or distention. Skin—large itching hives over the face and entire body.

Diagnosis. Generalized urticaria—immediate cause undetermined.

Treatment. Oral administration of calcium and hypodermic administration of adrenalin—five minims, gave no relief in six hours. Then 7½ grains of sodium thiosulphate was given intravenously. Within 20 minutes all of the hives, erythema and itching had disappeared. This was practically a specific therapeutic reaction. The boy was discharged from the hospital the next day, (November 16, 1936) and has suffered no recurrence since then.

Discussion. In this instance there was a rapid therapeutic result without any knowledge of the causative allergen. Such a treatment should prove particularly useful in those unfortunate persons who are hypersensitive to a multiplicity of allergens. This treatment apparently alters the tissue reaction to the offending allergen and restores a state of normality for an unknown period of time.

Case 4. A. L., male, age 16 years. Admitted to the hospital October 26, 1936 with severe swelling of the left arm, from the shoulder to the elbow. The week before he had suffered a dirty laceration of the right index finger which had necessitated giving 1,500 units of tetanus antitoxin. He now presented himself with a severe local reaction at the site of the antitetanus injection.

Previous Illness. 1928—frequent upper respiratory infections; 1929—pneumonia. September 3, 1936—serum reaction after tetanus antitoxin. Toxin-antitoxin was given in 1925 for diphtheria prevention.

Physical Examination. Revealed a well developed muscular boy, who was very careless about his personal hygiene. General health was excellent. Throat—normal; heart and lungs—normal. There was a healing wound on the right index finger. A marked brawny swelling of the left arm was noted from the shoulder to the elbow with local redness and heat, but no fluctuation.

Progress Notes. October 26, 1936—on admission the marked local induration was treated with cold magnesium sulphate compresses. Adrenalin, 7 minims, was given subcutaneously; also calcium lactate (gr. 10) every 4 hours; ultraviolet treatments over the swollen area for 5 minutes. October 27, 1936—the condition was unimproved. The indurated swelling had extended so that now the right arm was swollen from the shoulder down to

the back of the hand. There was considerable redness and local heat.

Diagnosis. Arthus's phenomenon at the site of injection of tetanus antitoxin.

The arm was most alarmingly swollen now (about twice the normal size), and there was a brawny edema. The condition was distinctly alarming and there was a possibility of impending gangrene due to interference with the circulation. October 27, 1936, 8:30 P.M.—7½ grains sodium thiosulphate given intravenously. October 28, 1936, 8:00 A.M.—the swelling was definitely reduced and the edema was less brawny. The patient felt much better. 11:00 A.M.—sodium thiosulphate (7½ grains) given intravenously. October 29, 1936—the swelling and redness had rapidly subsided and the arm was swollen only slightly. The bony landmarks about the elbow joint could be readily distinguished now. October 30, 1936—the arm was now normal in appearance, and function was normal. The patient was discharged as well.

Comment. A severe Arthus's phenomenon in the left arm, as an allergic reaction to tetanus antitoxin, threatened the circulation of the entire arm. The effect of sodium thiosulphate intravenously was brilliant and specific. I feel that it saved the boy's arm. The other and customary treatments, calcium, adrenalin, cold magnesium sulphate packs and ultraviolet radiation, had induced no noteworthy improvement.

Case 5. J. E., male, age 15 years, was admitted to the hospital with a crushing laceration of the right middle and ring fingers. A prophylactic dose (1,500 units) of tetanus antitoxin was immediately given. Twelve hours after this injection the patient began to complain of intense headache, substernal pressure, malaise and a severe generalized urticaria associated with severe pruritus. Temperature 103°, pulse 104, respiration 22. The patient was in considerable distress and most uncomfortable.

Sodium thiosulphate (15 grains) was given intravenously. Within 10 minutes the patient was markedly relieved. Within a half hour the intense headache had disappeared, the substernal pressure sensation had vanished, and the urticaria had subsided. The general condition of the patient was restored to normal.

Case 6. J. G., female, age 16 years. Admitted to hospital

April 16, 1937 with a deep laceration over the internal aspect of the right thigh immediately above the knee joint. This was sutured. Tetanus antitoxin (1,500 units) was given intramuscularly. A week later the patient developed an intense headache, intense pains about the right shoulder joint, and generalized urticaria with severe pruritus. Calcium, calomine lotion, etc. were of no help. Sodium thiosulphate (15 grains) was given intravenously. Within 30 to 45 minutes all of the allergic symptoms had disappeared. There had been a swelling and marked redness at the site of the tetanus antitoxin injection in the right upper arm. Within 30 minutes after the injection of sodium thiosulphate, this red, swollen area became blanched and the skin resumed its normal appearance, as did all the other areas of urticaria. This was a truly objective demonstration of the effect of sodium thiosulphate on the abnormal tissue reaction (edema and redness).

SUMMARY

This brief series of clinical studies indicates definitely that sodium thiosulphate is a most valuable drug in the treatment of allergic reactions. It may be given intravenously and orally for allergic epilepsy, migraine, urticaria, severe serum reactions and in the treatment of the Arthus's phenomenon. I suggest that it may be given orally and hypodermically for hay fever and for gastro-intestinal food allergy; also in allergic reactions seen during and after scarlatinal immunization with scarlatinal toxin. I can not too emphatically indicate the brilliancy of the therapeutic results, especially as concerns the almost immediate relief of the allergic manifestations.

The mode of action of sodium thiosulphate in allergy has not yet been clarified. Lumiere's studies indicate that it reduces the size of the flocculates and precipitates; it helps restore the normal colloid state. How this is achieved is not explained. It is highly probable that this is accomplished through changing the electric charge on the colloid particles by liberation of the H-ion, or through the acid sulphate radicle liberated on decomposition of the sodium thiosulphate.

It is desirable to emphasize the importance of colloid state and colloid particles in health and disease. Perhaps some of the reactions observed after intravenous medication (salvarsan, etc.)

have been due to neglecting the determination of the size of the colloid particles and the degree of their dispersion. In the future, better methods and precautions will no doubt be developed to insure normal colloid reactions in therapy of disease and in maintenance of health.

CONCLUSIONS

1. An attempt is made to explain allergic reactions on the basis of alterations in colloid state.
2. Lumiere's theories and experiments on colloids are re-emphasized.
3. The beneficial therapeutic action of sodium thiosulphate is demonstrated in six different clinical types of allergy.

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THYROID GRAFT IN INFANTILE MYXEDEMA. (Presse Médicale, Paris, Dec. 11, 1937). Le Fort reports the history of a child who, in 1925 at the age of 2½ years, showed the symptoms of myxedematous idiocy. There was severe deficiency, perhaps complete absence, of thyroid secretion. Palpation of the neck gave no indication of the presence of a thyroid. It was decided to try transplantation of human thyroid tissue. About one-half of the right lobe of the thyroid was obtained from an executed criminal and about two thirds of this tissue was immediately transplanted into the abdominal rectus muscle of the child. The author describes the post-operative course and the changes that took place in the child in the years following. Four days after the operation the child had lost much weight and its facial expression was already greatly changed. Dentition, which had been greatly retarded before the intervention, was accelerated. The modification of the intelligence was surprising. From the point of view of the physical and mental development, the efficacy of the thyroid graft was gradually lessened as the years advanced. Examination in November 1936 revealed that the child, now 14, showed the development of a child of nine. However, the development, although slow, had not ceased. —*Journal A. M. A.*

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Aus dem Tuberkulose-Forschungsinstitut Borstel
(Direktor: Prof. Dr. Dr. E. FREERKSEN).

Über die reversible Inaktivierung des thyreotropen Hormons.

Von
JOACHIM KRACHT.

174
Thiosulfate

Mit 1 Textabbildung.

(Eingegangen am 30. November 1951.)

Die physiologische Bedeutung des thyreotropen Hormons (TSH) beruht auf der Aktivierung der Schilddrüse in Form einer proteolytischen Hydrolyse des gespeicherten Thyreoglobulins und vermehrten Thyroxinausschüttung. Damit verbunden ist eine konsekutiv gesteigerte Jodidaufnahmefähigkeit und erhöhte Thyroxinsynthese. Die entsprechenden histologischen Kriterien bestehen in der Verflüssigung und Rückresorption des Kolloids mit nachfolgender Hypertrophie und Hyperplasie der Acinusepithelien.

Die TSH-Sekretion des Hypophysenvorderlappens wird normalerweise durch den Blut-Thyroxinspiegel in Form eines relaisartigen homeostatischen Regulationsmechanismus gesteuert. Unübersichtlicher liegen die Verhältnisse bei der Thyreotoxikose im Hinblick auf die Frage ihrer ausschließlich hypophysär-zentralen Genese. Es entsprechen nämlich nicht nur die cytologischen Befunde an Hypophysen menschlicher Thyreotoxikosen dem Normalbild (WEGELIN), sondern der TSH-Nachweis im Serum und Harn bei echten Thyreotoxikosen ist auch negativ (RAWSON, PURVES und GRIESBACH). Man muß daraus folgern, daß die pathogenetische Bedeutung einer Hypersekretion von TSH entweder überschätzt wurde oder noch andere Mechanismen (wie z. B. die Sensibilisierung der Schilddrüse auf normale Thyreotropinimpulse oder chemische Reaktionen zwischen TSH und Schilddrüse) hinzukommen.

Erste Hinweise dafür, daß chemische Beziehungen zwischen Jod und TSH bestehen, gab neben den therapeutischen Erfahrungen der Jod-Jodkalibehandlung nach PLUMMER der invitro-Befund einer direkten thyreotropininaktivierenden Wirkung von Schilddrüsengewebe (RAWSON u. Mitarb.). Auch Thymus und Lymphknoten haben diese Fähigkeit, wenn auch in geringerem Maße, allen übrigen Organen fehlt sie vollständig. Als Folge einer TSH-Inaktivierung durch Jod sind auch unsere Ergebnisse zur Therapie der Schreckthyreotoxikose beim Wildkaninchen zu deuten, denn bei kombinierter Methylthiouracil- und Jodbehandlung zeigte sich eine deutliche, von der Dosis elementaren Jods abhängige histologische Ruhigstellung der Schilddrüse trotz gleichzeitig vermehrter Thyreotropinsekretion. ALBERT u. Mitarb. haben außerdem auf eine in vitro-Inaktivierung von TSH durch Jod und auf die Reversibilität des Prozesses durch Reduktionsmittel (besonders antithyreoidale Substanzen) hingewiesen. Diese Autoren stellten fest, daß

die Jodierung von TSH zu einem braunen Präcipitat führt, welches seine Aktivität in Abhängigkeit von der zugeführten Joddosis weitgehend oder total eingebüßt hat. Es enthält insgesamt 40 γ J pro Einheit TSH, von denen 24 γ locker gebunden sein und sich durch Aceton auswaschen lassen sollen. Diese nur noch schwach braun-gelb gefärbte Fraktion hatte die Ausgangsaktivität pro Einheit TSH wiedererlangt. Man folgerte daraus, daß Inaktivierung nur durch Jodüberschuß zu erzielen sei und die restlichen 16 γ fester gebundenes Jod keine inaktivierende Potenz besitzen. Die TSH-Inaktivierung durch Jod wurde dabei als Oxydationsvorgang aufgefaßt und die Reversibilität durch antithyreoidale Substanzen auf deren reduzierende Eigenschaften zurückgeführt. Fundierte Aussagen über den chemischen Vorgang selbst waren den Autoren in Anbetracht des noch unbekannten chemischen Aufbaus des thyreotropen Hormons nicht möglich.

Mit den vorliegenden Untersuchungen wird versucht, zur weiteren Aufklärung der reversiblen Inaktivierung von TSH unter in vitro- und in vivo-Bedingungen beizutragen.

Jeweils 1 Amp. Pretiron-Schering* (28,6 mg Protein = 500 Junkmann-Schoeller- oder Meerschweincheneinheiten = MsE) in 1 ml H_2O gelöst, wurde mit 20 ml Lugolscher Lösung (1 g J und 2 g KJ ad 100 ml H_2O) versetzt und das Gemisch auf 28 ml mit H_2O aufgefüllt. Es entstand sofort eine braungefärbte Fällung, die nach 2 Std durch Schütteln gleichmäßig verteilt, in 5 gleichen Teilen abzentrifugiert wurde und für alle folgenden Versuche als Ausgangsmaterial diente. Das braune Präcipitat wurde für sich untersucht, aber auch jeweils zu gleichen Teilen von 100 MsE mit verschiedenen Substanzen versetzt. Der Zusatz von 2,5 ml n/100 $Na_2S_2O_3$ ließ die braune Farbe des Jodaddukts verschwinden und eine weiß-trübe Flüssigkeit entstehen. 0,03 ml einer 10%igen Ascorbinsäure entfärbte das braune Präcipitat spontan zu einer klaren, farblosen Flüssigkeit, die sich nicht vom gelösten Original-TSH unterschied. Zu der gleichen Aufhellung führte der Zusatz von 3 ml einer 1%igen NH_3-Lösung. In Aceton löste sich das braune Präcipitat ohne sinnfällige Farbänderung; auch durch Verdampfen des Lösungsmittels im Vakuumexsiccator, erneutes Aufnehmen des Rückstandes mit Aceton und dessen nochmalige Entfernung gelang es nicht, eine im Aussehen wesentlich schwächer gefärbte Substanz zu erhalten. Der Jodnachweis mit Stärke fiel in den Fraktionen mit $Na_2S_2O_3$, Ascorbinsäure und NH_3 negativ, in den aceton-behandelten Proben bzw. nach zehnmaliger H_2O-Auswaschung noch eindeutig positiv aus.

In einer weiteren Versuchsreihe wurden 500 MsE Pretiron in 8 ml H_2O gelöst und gleiche Anteile von 100 MsE sowohl in reiner Form angewandt als auch nach Hinzufügen von 0,03 ml 10%iger Ascorbinsäure und in einer weiteren Fraktion von 2 ml Bromwasser; dabei kam es im Gegensatz zum Jodversuch zu keiner Fällung. Die TSH-Bromwasser-Fraktion wurde außerdem nach Durchleiten von H_2 während einer Dauer von 10 min untersucht. Ein weiteres Material ergab sich durch die Beobachtung, daß sich der jodierte TSH-Komplex nach 5 Tagen zu einer weiß-trüben homogenen Verbindung entfärbte, die mit Stärke keine Jodreaktion mehr ergab.

Für den Tierversuch benutzten wir etwa 250 g schwere Meerschweinchen, deren Schilddrüsen während einer Dauer von 14 Tagen vor Versuchsbeginn durch eine täglich oral pipettierte Lugolsche Lösung (1 ml mit 250 γ freiem Jod) ruhiggestellt wurden. Die mittlere Zellhöhe (mZ.) des gleichmäßig flachkubischen

* Der Schering-A.-G., Berlin, danken wir für Versuchsmengen von Pretiron, der Fa. C. F. Boehringer Söhne, Mannheim, für die Überlassung von Methylthiouracilampullen.

Follikelepithels betrug bei diesen in der Folge unbehandelten Kontrolltieren ohne signifikante Schwankungen 5,9 μ, so daß ein einheitliches Epithelniveau als Ausgangsmaterial vorausgesetzt werden konnte. Von jeder Fraktion erhielten jeweils 3 Tiere an 3 aufeinanderfolgenden Tagen je 5 MsE täglich s.c. verabfolgt. Die Tötung erfolgte am 4. Tag. Die Schilddrüsen wurden gewogen, nach BOUIN fixiert, in Paraffin eingebettet und nach GOLDNER gefärbt. Zur Bestimmung der mittleren Zellhöhe wurden mit einem Schraubenokularmikrometer je 200 Epithelien pro Tier, und zwar an 50 Follikeln bei 3, 6, 9 und 12 des Uhrzeigersystems, ausgemessen und aus den Werten von 3 Tieren der Mittelwert bestimmt.

Die histometrischen Ergebnisse sind in Tab. 1 und 2 zusammengefaßt. Auf eine Angabe des Schilddrüsengewichts wurde verzichtet, da die Meerschweinchenschilddrüse großen physiologischen Größenschwankungen unterworfen ist. Tab. 1 zeigt als Gegenpol zur geplummerten Ruheschilddrüse (mZ. 5,9 μ) unter TSH ein hyperaktives Bild mit hochzylindrisch-papillärem Epithel, Kolloidverflüssigung und -rückresorption, intracellulären Kolloidtröpfchen und erhöhtem Mitoseindex bei einer mZ. von 15,2 μ. Das jodierte TSH (braunes Präcipitat) hat indessen seine thyreotrope Aktivität weitgehend verloren; das kubische, durchweg regelmäßig vacuolisierte Epithel (mZ. 7,2 μ) gehört gerade noch in das Bild der Ruheschilddrüse. Gleichartig verhält sich das mit Bromwasser versetzte TSH (mZ. 6,8 μ), während Ascorbinsäure die thyreotrope Aktivität nur geringfügig negativ beeinflußt. Es zeigt sich also eine eindeutige Inaktivierung von TSH durch Jod und Brom, die prozentual, verglichen mit der Ausgangslage, 86 bzw. 89% beträgt (Abb. 1).

Tabelle 1. *In vitro-Inaktivierung von TSH.*

Material	mZ. in μ
Kontrolle	5,9
3 mal 5 MsE TSH	15,2
3 „ 5 „ TSH + Ascorbinsäure	13,5
3 „ 5 „ jodiertes TSH	7,2
3 „ 5 „ bromiertes TSH	6,8

Tabelle 2. *In vitro-Reaktivierung von inaktiviertem TSH.*

Material	mZ. in μ
3 mal 5 MsE jodiertes TSH + $Na_2S_2O_3$	12,5
3 „ 5 „ „ TSH + Ascorbinsäure	10,4
3 „ 5 „ entfärbtes jodiertes TSH	10,45
3 „ 5 „ jodiertes TSH, ausgewaschen in H_2O	8,9
3 „ 5 „ „ TSH, behandelt mit Aceton	8,7
3 „ 5 „ „ TSH + NH_3	6,0
3 „ 5 „ bromiertes TSH, nach Durchleiten von H_2	10,45

Die grundsätzliche Möglichkeit einer Reversibilität dieses Aktivitätsverlustes zeigt Tab. 2, wonach der Zusatz von $Na_2S_2O_3$ zur jodierten Verbindung eine Reaktivierung um 55% zur Folge hat. Diese Fraktion

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führt mit einer mZ. von 12,5 μ bei zylindrisch-vacuolisiertem Epithel erhöhter Mitosezahl und entsprechenden Kolloidbefunden zum Bilde der aktivierten Schilddrüse. Einen gleichartigen, wenn auch schwächeren Effekt bewirkt Zusatz von Ascorbinsäure (mZ. 10,4 μ) zur jodierten Verbindung, während deren Behandlung mit Aceton (mZ. 8,7 μ) und H_2O (mZ. 8,9 μ) wesentlich weniger eindrucksvolle Ergebnisse zeitigt.

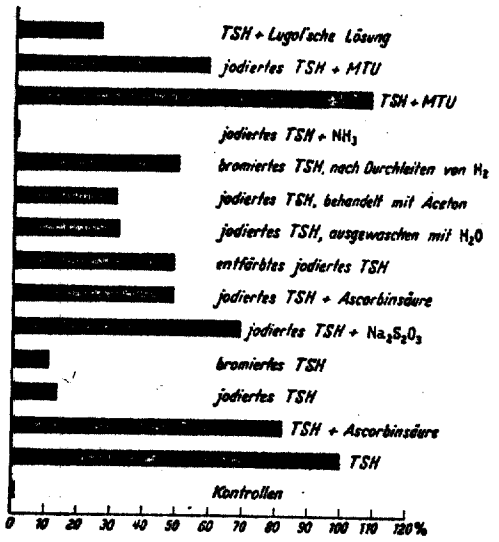

Abb. 1. Thyreotrope Aktivität aller Versuchsgruppen in prozentualer Darstellung.

NH_3 ist bezüglich einer Reaktivierung des jodierten TSH vollkommen unwirksam. Das Epithel- (mZ. 6,0 μ) und Kolloidbild entspricht dem der geplummerten Kontrollen und läßt selbst örtliche geringfügige Aktivitätszeichen wie beim brauen Präcipitat vermissen. Dagegen hat das Durchleiten von Wasserstoff durch die bromierte Verbindung eine Reaktivierung um fast 40% zur Folge. Überraschend erscheint die wesentlich stärkere Aktivität des nach einigen Tagen jodfreien entfärbten brauen Präcipitats mit einer mZ. von 10,45 μ gegenüber 7,2 μ des frisch bereiteten Gemisches. Eine Übersicht der unterschiedlichen morphokinetischen Effekte der verschiedenen Versuchsgruppen unter- und zueinander gibt nach prozentualer Auswertung der histometrischen Ergebnisse Abb. 1.

Zur Beurteilung, inwieweit diese Resultate auch in vivo Gültigkeit besitzen, wurden die entgegengesetzten Prinzipien unabhängig voneinander den Tieren verabfolgt, und zwar jeweils 3mal 5 MsE reinen oder

jodierten Thyreotropins und 1 ml einer 5%igen Methylthiouracillösung (MTU-BOEHRINGER) s.c. bzw. 1 ml einer LUGOLschen Lösung mit 250 γ freien Jods oral (Tab. 3). MTU verstärkt, wenn auch wegen der schwachen Dosis nur geringfügig, die Wirkung des TSH (mZ. 16,06 μ), obwohl eine

Tabelle 3. *In vivo-Reaktionen von aktivem und inaktiviertem TSH mit Methylthiouracil bzw. Jod.*

Material	mZ. in μ
3mal 5 MsE TSH + MTU	16,05
3 „ 5 „ jodiertes TSH + MTU	11,4
3 „ 5 „ TSH + LUGOLsche Lösung (250 γ J)	8,32

hypophysär kompensatorische Thyreotropinsekretion wie nach längerer Applikation antithyreoidaler Substanzen entfällt. Jodiertes TSH wird durch MTU deutlich reaktiviert (mZ. 11,4 μ), und LUGOLsche Lösung inaktiviert das Original-TSH (mZ. 8,32 μ) ähnlich wie unter invitro-Bedingungen. Als Parallele hierzu können die Epithelwerte der behandelten Wildkaninchenschilddrüsen angeführt werden, deren mZ. unter MTU 11,67 μ, nach MTU + Lugol (100 γ Jod) 10,38 μ, nach MTU + Lugol (500 γ J) 6,98 μ und nach alleiniger Behandlung mit LUGOLscher Lösung (500 γ J) 6,18 μ betrug.

Den in vitro- und in vivo-Verhältnissen gemeinsam ist die dosisabhängige TSH-inaktivierende Potenz des freien Jods, wogegen Jodide die thyreotrope Aktivität weder entscheidend hemmen noch in vitro zu einem schwerlöslichen Proteinkomplex mit TSH — ähnlich dem braunen Präcipitat — führen. Es darf als gesichert gelten, daß lediglich elementares Jod für die Aktivitätsminderung von Bedeutung ist. Sie tritt nur bei Jodüberschuß ein und ist stets dann am deutlichsten, wenn kleine Quanten TSH mit großen Jodmengen in Reaktion gebracht werden. Dieses Moment ließe daran denken, daß Jod per se, d. h. allein durch seine Anwesenheit und im Überschuß, wirkt, zumal nach ALBERT und Mitarbeitern Parallelen zwischen Inaktivierung und Jodabsorption nicht gegeben sind. Außerdem konnten diese Autoren weder eine theoretisch mögliche Thyroxinentstehung im jodierten Protein noch einen Aktivitätsverlust des TSH durch simultan zugesetztes Thyroxin nachweisen. Auch eine Beeinträchtigung der Reaktionsfähigkeit der Schilddrüse durch den Jodüberschuß bzw. gestörte Absorptionsverhältnisse sind in Anbetracht der Versuchsdauer und der gleichzeitig unbeeinflußten gonadotropen Aktivität (Hodengewicht) jodierter Hypophysenextrakte unwahrscheinlich. Die Bedeutung des Jodüberschusses für die Inaktivierung geht aus dem Vergleich der epitheliotropen Effekte des braunen Präcipitats mit 86% gegenüber 51% Aktivitätsverlust der entfärbten jodfreien Verbindung hervor. Hieraus ist abzuleiten, daß eine Jodadditionswirkung zwar wesentlich ist, aber nicht ausschließlich vorliegen

kann, da die volle thyreotrope Aktivität durch Entfernung von Jod nicht wieder hergestellt wird, obwohl eine Denaturierung in Anbetracht der Reversibilität des Prozesses auszuschließen ist. Die entfärbte Verbindung nimmt aktivitätsmäßig vielmehr eine Mittelstellung zwischen jodiertem und Original-TSH ein (Abb. 1). In gleicher Weise führt $Na_2S_2O_3$ trotz Reaktion mit dem additiv gebundenen Jod lediglich zu einer partiellen Reaktivierung. Es ist deshalb ein zusätzlicher Mechanismus anzunehmen, der für den Hauptanteil der Reaktivierung verantwortlich ist und sowohl in einer Jodsubstitution (vgl. oben) als auch in einer chemischen Umsetzung des TSH selbst liegen könnte. Der Inaktivierungsvorgang wäre demnach in 3 Gruppen zu unterteilen, nämlich:

1. Jodsubstitution, 2. Jodaddition, 3. Oxydativer Vorgang.

Jodaddition und oxydativer Prozeß sind dabei dominierend, während der Jodsubstitution nach den erwähnten Untersuchungen von Albert keine Bedeutung zukommt. Die additive Jodwirkung könnte — vergleichbar mit der Jodfällungsreaktion der Alkaloide — auf einer lockeren Anlagerung von Jod an basische Stickstoffgruppen beruhen, womit auch die Schwerlöslichkeit des Präcipitats erklärbar wäre.

Für die Annahme einer oxydationsbedingten Inaktivierung spricht, daß sowohl elementares Jod als auch Brom starke Oxydationsmittel sind und der Inaktivierungsvorgang durch Reduktionsmittel reversibel ist.

Eine weitere Stütze für diese Annahme liegt in der Wirkung des Zusatzes von Bromwasser und der Aktivitätsregeneration nach Durchleiten von H_2. Nach Albert wirken auch andere Reduktionsmittel (KSCN, Ascorbinsäure) und besonders antithyreoidale Substanzen reaktivierend, aber nicht durch ihre Anwesenheit, weil ihre Entfernung nach erfolgter Reaktion durch Dialyse oder Fällung mit Ammonsulfat keine Verminderung des wiedergewonnenen Aktivitätsanteils nach sich zieht. $Na_2S_2O_3$ wirkt durch seine Reaktion mit Jod ($J_2 + 2\,Na_2S_2O_3 \rightarrow 2\,NaJ + Na_2S_4O_6$) und hatte in unseren Untersuchungen die stärkste reaktivierende Potenz. Die Wirkung der Ascorbinsäure erscheint in dreifacher Hinsicht bedeutungsvoll, als einmal eine direkte Reaktion mit Jod ($C_6H_8O_6 + J_2 \rightarrow C_6H_6O_6 + 2\,HJ$) und eine Reduktion des oxydierten TSH möglich und auf der anderen Seite auch eine oxydative Wirkung gegeben ist, wie die Aktivitätsminderung des Original-TSH zeigt. Letztere könnte auf eine H_2O_2-Entstehung zurückzuführen und für die geringgradige Inaktivierung bei Zusatz zum Original-TSH verantwortlich sein. Oxydations- und Reduktionspotential wirken bei der Reaktivierung der jodierten Verbindung durch Ascorbinsäure einander entgegen, ohne daß es möglich wäre, für die einzelnen Vorgänge prozentuale Zahlen anzugeben. Die Epithelwerte nach mehrmaliger H_2O-Auswaschung oder Lösen des braunen Präcipitats in Aceton zeigen lediglich die generelle Richtung einer Reaktivierung an. Die Reaktivierung der jodierten Verbindung durch Aceton ist kleiner als nach den Ergebnissen von Albert, aber deutlich zu erkennen. Es ist anzunehmen, daß durch das mehrmalige Auswaschen mit H_2O bzw. durch das Abdampfen des Acetons der Anteil locker gebundenen Jods vermindert und diese Differenz im

Jodüberschuß für den beobachteten Reaktivierungsgrad verantwortlich ist. Das Ausbleiben einer Reaktivierung nach NH_3-Zusatz muß ähnlich dem Resultat von ALBERT mit NaOH auf eine Eiweißdenaturierung zurückgeführt werden. Das Ausmaß des beschriebenen Oxydationsmechanismus wird im wesentlichen vom Redoxpotential des zugefügten Substrats bestimmt. Die hiermit verbundenen chemischen Reaktionen sind weitgehend unbekannt. Gesichert ist nach den Untersuchungen von CIERESZKO, daß das gereinigte Protein 1,2% Schwefel enthält. Da die Inaktivierung von Proteinen und Fermenten vielfach auf einer Oxydation von SH-Gruppen zu den entsprechenden Disulfidverbindungen beruht, ist für die Inaktivierung des TSH, wie schon ALBERT vermutete, ein gleichartiger Effekt anzunehmen. Umgekehrt wäre die Reaktivierung als Reduktion des Disulfids zur SH-Gruppierung aufzufassen.

Ob auch unter physiologischen Bedingungen die TSH-inaktivierende Wirkung von Jod und Schilddrüsenexplantaten (RAWSON) von Bedeutung ist, bleibt offen, da die in vitro-Verhältnisse nicht mit dem komlizierteren Wirkungsmechanismus im lebenden Organismus identisch zu sein brauchen. Bekannt ist jedoch die involvierende Wirkung von elementarem Jod auf thiouracil- und TSH-behandelte Schilddrüsen, wogegen Jodide keine Wirkung zeigen. Der Schwerpunkt der antithyreoidalen Wirkung liegt entweder in der Fähigkeit, elementares Jod unmittelbar oder bei seiner Entstehung zu reduzieren bzw. ein hypothetisches jodid-oxydierendes Enzym zu inhibieren. Das Jod-TSH-Gleichgewicht wird dadurch zugunsten des TSH verschoben, wobei für die initiale Epithelaktivierung eine kompensatorisch vermehrte Ausschüttung aus dem Hypophysenvorderlappen nicht gefordert zu werden braucht. Durch die unterbundene Jodidoxydation und den offenbar ebenfalls reduktionsbedingten Verstärkungseffekt der antithyreoidalen Substanzen (augmenting effect nach RAWSON) wäre die Schilddrüse für einen physiologischen TSH-Spiegel sensibilisiert. Da die aktivitätssenkende Jodwirkung dosisabhängig ist, scheint ein Hinweis gegeben, daß das TSH-Jod-Gleichgewicht erst durch einen Jodüberschuß durchbrochen wird, der nun seinerseits das thyreotrope Hormon auf oxydativem Wege bremst.

Nach den Untersuchungen von RAWSON vermag die thyreotoxische Struma doppelt so viel TSH zu inaktivieren als eine euthyreotische Schilddrüse; darüber hinaus kann sie vermehrt Jodide aufnehmen. Ohne Berücksichtigung einer zusätzlichen Beteiligung der Hypophyse am Jod-TSH-Chemismus erscheint es wesentlich, daß TSH in Serum und Harn thyreotoxischer Patienten nicht nachgewiesen wurde (RAWSON, PURVES und GRIESBACH), obwohl angenommen werden darf, daß ihre Schilddrüsen unter einer gesteigerten TSH-Stimulierung stehen. Dieser Effekt ist nur durch eine erhöhte TSH-Inaktivierung durch hyperplastisches Schilddrüsengewebe möglich, wobei zusätzliche Jodgaben etwa nach Art der Plummerung zu einer weiteren Aktivitätssenkung und damit zur Kolloidstruma mit wieder regelmäßigem Follikelepithel und normalem Jodgehalt führen.

Eigene Untersuchungen über den diskutierten Verstärkungseffekt wurden in vivo mit TSH + MTU durchgeführt, mußten aber aus

Gründen der nur geringen zur Verfügung stehenden Pretironmenge beschränkt bleiben. Danach ist die histologische Aktivierung noch extremer als nach TSH allein, was allerdings in den Epithelwerten (mZ. 16,05 μ : 15,2 μ) weniger zum Ausdruck kommt, die noch in den Rahmen physiologischer Streuungen gehören. Die gleichzeitige Applikation von TSH und Lugolscher Lösung führt als in vivo-Parallele zu den in vitro-Versuchen mit jodiertem TSH zu einem erheblichen Aktivitätsverlust, der analog den Ergebnissen von antithyreoidaler und Jodtherapie am Wildkaninchen bei weiterer Steigerung der Joddosis eine noch stärkere Aktivitätssenkung erwarten läßt. Andererseits führt die kombinierte in vivo-Applikation von jodiertem inaktiven TSH und MTU zu einer Aktivitätsregeneration, für die eine kompensatorische zusätzliche TSH-Ausschüttung durch die Hypophyse unwahrscheinlich ist; vielmehr kann der Reaktivierungsgrad auf die reduktive Potenz des MTU zurückgeführt werden. Die beiden letzten Versuchsanordnungen dürfen als Parallele zu den in vitro-Versuchen gewertet werden und erlauben den Schluß, daß im lebenden Organismus vielleicht ähnliche Inaktivierungs-Reaktivierungsmechanismen vorliegen.

Zusammenfassung.

Thyreotropes Hormon wird in vitro durch elementares Jod oder Bromwasser weitgehend inaktiviert. Die partielle Reversibilität dieses Prozesses durch $Na_2S_2O_3$ und Reduktionsmittel, wie Wasserstoff und Ascorbinsäure, läßt darauf schließen, daß für die Inaktivierung neben einer Jodadditionswirkung vor allem ein oxydativer, für die Reaktivierung ein Reduktionsvorgang verantwortlich ist, der möglicherweise auf einer rückläufigen Überführung von SH-Gruppen zum Disulfid beruht. Einzelheiten hierzu bleiben in Anbetracht des unaufgeklärten chemischen Aufbaus des thyreotropen Hormons offen. Parallelunterschungen deuten darauf hin, daß ein ähnlicher Jod-TSH-Chemismus auch im lebenden Organismus vorhanden ist und in der Physiologie des Jodstoffwechsels berücksichtigt werden muß.

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schwellen (Oliver[2])
berger[3] eine Cleara
Inulin-Clearance 159
liegen die von Ferro
Thiosulfat bei Mens
schweres Nierengift,
in den Harn gelangt,
dieser mehr oder min
daß dem Clearance-V
nämlich daß er die
ein solches Quantum
hat schnell allgemei
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Gegen diese Annah
liegen die Verhältnisse n
fließt, desto größer wäre
nach der Kreatininmeth
Kochsalz zurückresorbie
man bei der kindlichen
Harnstoff, Phosphat, Na
Erwachsenen die Inulin
Dieser Befund ist also n
vereinbar. Ferner fand
scheidung von p-Amino

* Herrn Prof. Dr. R.

Arch. Exper. Path. u. Pharmakol. 214: 433-440 (1952).

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Thyrotoxicosis

THE REVERSIBLE INACTIVATION OF THE THYROTROPIC HORMONE

The physiologic significance of the thyrotropic hormone (TSH) lies in the activation of the thyroid gland by way of proteolytic hydrolysis of the stored thyroglobulin and increased thyroxine release. Coupled with this is a consecutively increased ability to take in iodide and increased thyroxine synthesis. The corresponding histologic criteria are liquefaction and reabsorption of colloid with subsequent hypertrophy and hyperplasia of the acinus epithelia.

The TSH secretion of the adenohypophysis is normally controlled by the blood-thyroxine level in form of a release-like homeostatic regulation mechanism. The conditions in thyrotoxicosis are less perspicuous insofar as the question of their genesis exclusively in the center of the hypophysis cerebri is concerned. Not only are the cytologic findings of hypophyses of human thyrotoxicoses normal (WEGELIN), but also is the TSH demonstration in serum andurine in true thyrotoxicoses negative (RAWSON, PURVES and GRIESBACH). One has to conclude from this that the pathogenetic significance of hypersecretion of TSH has either been overestimated, or that additional mechanisms are involved (as i.g. sensitization of the thyroid gland to normal thyrotropine impulses or chemical reactions between TSH and thyroid gland).

KRACHT, MD, JOACHIM
Institute for Tuberculosis Research, Borstel (Director: FREERKSEN MD, E.)
Arch. exper. Path. u. Pharmakol., vol. 214, 433-440 (1952)

First indications that chemical relationships between iodine and TSH exist, resulted, in addition to the therapeutic experiences with iodine-potassium iodide treatment after PLUMMER, from in vitro findings of a direct thyrotropine inactivating action of pituitary tissue (RAWSON and collegues). The thymus and lymph nodes have this ability as well, although to a lesser degree, but it is completely absent in all other organs. Our results with the therapy of stress thyrotoxicosis in rabbits also have to be interpreted as a consequence of TSH inactivation by iodine, for combined treatment with methyl-thiouracil and iodine resulted in obvious histologic inactivation of the thyroid gland, dependent on the dosage of the elementary iodine, in spite of simultaneously increased thyrotropine secretion. In addition, ALBERT and collegues have demonstrated an in vitro inactivation of TSH by iodine and the reversibility of this process by reduction agents (especially antithyroid substances). These authors determined that iodizing of TSH leads to a brown precipitate, the actvity of which is largely or totally independent of the added iodine dosage. It contains a total of 40 γ I per unit TSH, 24 γ of which should be loosely bonded and washable by acetone. This fraction of only faint brown-yellow color had regained the initial activity per unit TSH. One concluded from this that inactivation could only be attained by iodine excess and that the remaining 16 γ of more tightly bonded iodine has no inactivating potency. The TSH inactivation by iodine was considered a process of oxydation, and the reversibility by antithyroid substances was attributed to their reducing actions. The authors were unable to make definite statements about the chemical process itself because of the still unknown chemical composition of the thyrotropic hormone.

With the following investigations we shall try to contribute to the further clarification of reversible inactivation of TSH under in vitro

and in vivo conditions.

1 ampule each of Pretiron - Schering* (28.6 mg protein = 500 JUNKMANN-SCHOELLER or guinea pig units = MsE), dissolved in 1 ml H_2O, was shifted with 20 ml LUGOL's solution (1 g I and 2 g KI ad 100 ml H_2O), and H_2O was added to the mixture up to 28 ml. Immediately, a brown colored precipitation developed, which was distributed evenly by shaking after 2 hours, centrifuged in 5 equal portions , and then served as basic material for all subsequent experiments. The brown precipitate was examined by itself and also shifted, with equivalent portions of 100 MsE each, with various substances. The addition of 2.5 ml n/100 $Na_2S_2O_3$ caused the brown color of the iodine adduct to disappear, and a white-cloudy liquid developed. 0.03 ml of 10 % ascorbic acid spontaneously decolorized the brown precipitate to a clear colorless liquid, which did not differ from the dissolved original TSH. The addition of 3 ml of 1 % NH_3 solution resulted in the same clearing up. When the brown precipitate was dissolved in acetone, no visible change in color followed; also by vaporizing the solution in the vacuum exsiccator and again dissolving the residue in acetone and removing the latter again, it was not possible to obtain a substance with an appreciably weaker color. Iodine demonstration with starch in the fractions with $Na_2S_2O_3$, ascorbic acid and NH_3 was negative; in the samples treated with acetone or washed 10 times with H_2O respectively, it was still unequivocally positive.

In another series of experiments, 500 MsE Pretiron was dissolved in 8 ml H_2O, and equal portions of 100 MsE were used in pure form as well as after addition of 0.03 ml of 10 % ascorbic acid and in an additional

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* We thank Schering-A.-G., Berlin, for sample amounts of Pretiron and C. F. Boehringer Söhne, Mannheim, for the supply of methyl-thiouracil ampules.

fraction of 2 ml of bromine water; in contrast to the iodine experiment, no precipitation resulted. The TSH-bromine water fraction was further examined during a period of 10 minutes after passing H_2 through it. Additional material resulted from the observation that the iodized TSH complex discolored after 5 days to a cloudy white homogenous compound which did no longer show an iodine reaction with starch.

For the animal experiment we used guinea pigs weighing about 250 mg. Their thyroid glands had been inactivated during a period of 14 days before the beginning of the experiment by daily administration of orally pipetted LUGOL's solution (1 ml with 250 γ free iodine). The median cell height (mZ.) of the evenly flat-cubical follicle epithelium in these subsequently untreated control animals was 5.9 μ without significant fluctuations, so that a uniform epithelium level could be presupposed as initial material. Each of the 3 animals received 5 MsE a day s.c. of each fraction on 3 consecutive days. On the 4th day they were killed. The thyroid glands were weighed, fixed after BOUIN, imbedded in paraffin and dyed after GOLDNER. In order to determine the medium cell hight, 200 epithelia per animal were measured with a screw eyepiece micrometer, i.e. clockwise in 50 follicles at 3, 6, 9 and 12 o'clock. The medium value was determined from the values of the 3 animals.

The histometric results are compiled in tables 1 and 2. We did not indicate the weight of the thyroid gland since the guinea pig thyroid gland is subject to great fluctuations of physiologic size. Table 1 shows as antipole to the artificially inactivated thyroid gland (mZ. 5.9 μ) under TSH a hyperactive picture with high cylindric-papillary epithelium, colloid liquefaction and reabsorption, intracellular colloid drops and increased mitosis index with a mZ. of 15.2 μ. The iodized TSH (brown precipitate), however, has largely lost its thyrotropic activity;

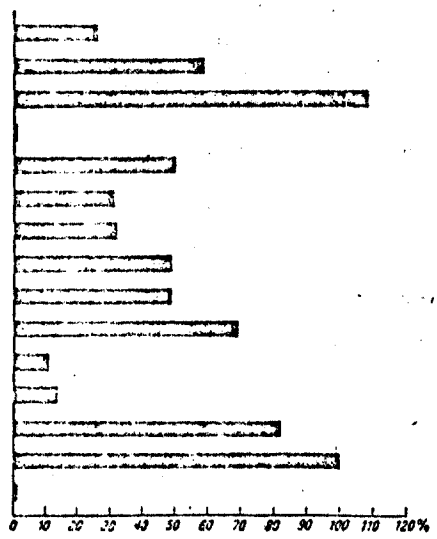
Table 1. In vitro inactivation of TSH

material	mZ. in μ
control	5.9
3 times 5 MsE of TSH	15.2
3 times 5 MsE of TSH + ascorbic acid	13.5
3 times 5 MsE of iodized TSH	7.2
3 times 5 MsE of brominized TSH	6.8

Table 2. In vitro reactivation of inactivated TSH

material	mZ. in μ
3 times 5 MsE of iodized TSH + $Na_2S_2O_3$	12.5
3 times 5 MsE of TSH + ascorbic acid	10.4
3 times 5 MsE of decolorized iodized TSH	10.45
3 times 5 MsE of iodized TSH, washed in H_2O	8.9
3 times 5 MsE of iodized TSH, treated with acetone	8.7
3 times 5 MsE of iodized TSH + NH_3	6.0
3 times 5 MsE of brominized TSH, after passing through of H_2	10.45

Chart 1. Thyrotropic activity of all test groups in percentile values



TSH + LUGOL's solution
iodized TSH + MTU
TSH + MTU
iodized TSH + NH_3
brominized TSH, after passing through of H_2
iodized TSH, treated with acetone
iodized TSH, washed with H_2O
decolorized iodized TSH
iodized TSH + ascorbic acid
iodized TSH + $Na_2S_2O_3$
brominized TSH
iodized TSH
TSH + ascorbic acid
TSH
controls

the cubical epithelium, vacuolated with consistent regularity (mZ. 7.2 μ), only just belonged into the picture of the inactive thyroid gland. The TSH shifted with bromine water (mZ. 6.8 μ) reacts in the same manner, while ascorbic acid only has a minor negative influence on the thyrotropic activity. An unequivocal inactivation of TSH by iodine and bromine, amounting to 86 and 89 % respectively compared to the original situation, is therefore demonstrated (chart 1).

Table 2 confirms that it is possible on principle to reverse this loss of activity, that the addition of $Na_2S_2O_3$ to the iodized compound results in a 55 % reactivation. This fraction with a mZ. of 12.5 μ with cylindrical vacuolated epithelium, increased mitosis and corresponding colloid findings reactivates the thyroid gland. An equal, although weaker effect results from the addition of ascorbic acid (mZ. 10.4 μ) to the iodized compound, while its treatment with acetone (mZ. 8.7 μ) and H_2O (mZ. 8.9 μ) shows considerably less impressive results. NH_3 is completely ineffective in reactivating the iodized TSH. The epithelium (mZ. 6.0 μ) and colloid picture concurs with the inactivated controls and even minimal local signs of activity, as in the brown precipitate, are absent. In contrast, passing hydrogen through the brominized compound results in reactivation of nearly 40 %. The considerably greater activity of the brown precipitate, free of iodine and decolorized a few days later, is surprising with its mZ. of 10.45 μ compared to the 7.2 μ of the freshly prepared mixture. Chart 1 shows a synopsis of the different morphokinetic effects of the varous test groupings in relationship to each other and the histometric results after percentile evaluation.

In order to assess to what extent these results are valid in vivo, the opposite principles were applied to the animals independent of each other, i.e. 3 times 5 MsE each of pure or iodized thyrotropine and 1 ml

of 5 % methyl-thiouracil solution (MTU - BOEHRINGER) s.c. and 1 ml of LUGOL's solution with 250 γ of free iodine orally (table 3) respectively.

Table 3. In vivo reaction of active and inactivated TSH with methylthiouracil and iodine respectively

material	mZ. in μ
3 times 5 MsE of TSH + MTU	16.05
3 times 5 MsE of iodized TSH + MTU	11.4
3 times 5 MsE of TSH + Lugol's solution (250 γ I)	8.32

MTU increases, although minimally because of the small dosage, the effectiveness of TSH (mZ. 16.06 μ), in spite of the fact that hypophyseally compensatory secretion of thyrotropine ceases as it does after prolonged application of antithyroid substances. Iodized TSH is obviously reactivated by MTU (mZ. 11.4 μ), and LUGOL's solution inactivates the original TSH (mZ. 8.32 μ), in a manner similar to in vitro conditions. Parallel to this are the epithelium values of the thyroid glands of the treated rabbits, with mZ. of 11.67 μ after MTU, 10.38 μ after MTU + LUGOL (100 γ iodine), 6.98 μ after MTU + LUGOL (500 γ I) and 6.18 μ after treatment with LUGOL's solution alone.

The fact that the TSH inactivating potency of the free iodine is dependent on the dosage is a condition common to both in vitro and in vivo situations. In contrast, iodides neither decisively inhibit the thyrotropic activity nor result in vitro in a protein complex with TSH which resists dissolution, similar to the brown precipitate. It is therefore certain that only elementary iodine is of importance in the reduction of

activity. It only results with excess iodine and is always most perspicuous when small quantities of TSH are made to react with large quantities of iodine. This fact might lead to the conclusion that iodine per se, i.e. solely by its presence and in excess, is effective, all the more since no parallels exist between inactivation and iodine absorption, according to ALBERT and collegues. In addition, these authors could not demonstrate that the development of thyroxine in iodized protein is theoretically possible or that the activity of TSH is reduced by simultaneous addition of thyroxine. It is also improbable, in view of the duration of the experiment and the simultaneously unaffected gonadotropic activity (testicular weight) of iodized hypophyseal extracts, that iodine excess influences the reaction capacity of the thyroid gland or that absorption is impaired. The importance of the iodine excess in reference to inactivation is illustrated by the comparison between the epitheliotropic effects of the brown precipitate of 86 % and the reduction of activity of the decolorized iodine-free compound of 51 %. One can conclude from this that the effect of iodine addition is significant but cannot be present exclusively, since complete thyrotropic activity cannot be restored by removal of iodine, although denaturation has to be excluded in view of the reversibility of the process. As far as activity is concerned, the decolorized compound assumes a central position between iodized and original TSH (chart 1). In the same manner, $Na_2S_2O_3$ only results in partial reactivation in spite of its reaction with the additively bonded iodine. It can therefore be assumed that an additional mechanism is present, which is responsible for the main portion of the reactivation and which might be contained in an iodine substitution (see above) as well as in a chemical transformation of TSH itself. The process of inactivation could accordingly be devided into 3 groups, i.e.:

1. iodine substitution, 2. iodine addition, 3. oxydation process.

Iodine addition and oxydation process are the dominating factors, while iodine substitution, according to the referred investigations by ALBERT, is of no importance. The effect of iodine addition could be due to a loose linkeage of iodine with groups of basic nitrogene - comparable to the iodine precipitation reaction of the alkaloids - , which would also explain the difficult solubility of the precipitate.

The assumption that inactivation is contingent on oxydation is supported by the fact that elementary iodine as well as bromine are potent oxydation agents and that the inactivation process can be reversed by reduction agents.

The assumption is further supported by the effect of the addition of bromine water and the regeneration of activity after passing through of H_2. According to ALBERT, other reduction agents (KSCN, ascorbic acid) and especially antithyroid substances also have a reactivating effect, but not by their presence, because their removal after reaction by dialysis or precipitation with ammonium sulfate does not result in reduction of the restored portion of activity. $Na_2S_2O_3$ is effective by its reaction with iodine ($I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$) and had the strongest reactivating potency in our experiments. The effect of ascorbic acid seems to be significant in a threefold sense, since on the one hand a direct reaction with iodine ($C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2HI$) and a reduction of the oxydized TSH is possible, and on the other hand oxydating effect exists, as demonstrated by activity reduction of the original TSH. The latter could be attributed to a development of H_2O_2 and could be responsible for the minimal inactivation when the original TSH is added. The potential for oxydation and reduction work against each other during reactivation of the iodized compound by ascorbic acid, and it is not

possible to quote the percentage ratio of the individual processes. Following several washings with H_2O or dissolution of the brown precipitate in acetone, the epithelium values only point towards the general direction of reactivation. The reactivation of iodized compounds by acetone is of a lesser degree than that in ALBERT's results, but clearly recognizable. We suppose that the portion of loosely bonded iodine is reduced by repeated washing with H_2O and by vaporizing the acetone respectively, and that this difference in iodine excess is responsible for the observed degree of reactivation. Failure of reactivation after addition of NH_3 must be attributed to a protein denaturation, similarly the result of ALBERT with NaOH. The extent of the described oxydation mechanism is primarily determined by the redox potential of the added substrate. The investigations of CIERESZKO confirmed that the purified protein contains 1.2 % sulfur. Since inactivation of proteins and ferments is frequently caused by oxydation of SH groups to the corresponding disulfide compounds, one can assume, as ALBERT did, that the inactivation of TSH is effected in the same manner. Reversely, the reactivation could be conceived as a reduction of disulfide to the SH grouping.

It has not been clarified whether the TSH inactivating effect of iodine and thyroid gland explants (RAWSON) is significant under physiologic conditions, since in vitro conditions are not necessarily identical to the complicated operation mechanisms in the living organism. However, it is known that elemetary iodine has an involutionary effect on thyriod glands treated with thiouracil and TSH, which cannot be counteracted by iodides. The focal point of the antithyroid action lies either in the ability to reduce elementary iodine immediately or during its development or to inhibit a hypothetic iodide oxydating enzyme respectively. The iodine-TSH equilibrium is thereby shifted in favor of TSH, which does not

necessarily require increased compensatory secretion from the antehypophysis for the initial activation of epithelium. By inhibited iodide oxydation and by the potentiating effect of the antithyroid substances ("augmenting effect" according to RAWSON) - evidently also contingent on reduction - , the thyroid gland would be sensitized for determination of the physiologic TSH level. Since the activity reducing effect of iodine depends on dosage, there seems to be an indication that the equilibrium of TSH and iodine is only disturbed by an iodine excess, which now in turn inhibits the thyrotropic hormone by way of oxydation.

According to the investigations of RAWSON, the thyrotoxic struma can inactivate the double amount of TSH than a euthyroid goiter; furthermore, it can take in increased amounts of iodides. Disregarding an additional participation of the thyroid gland in the iodine-TSH chemism, it seems important that TSH could not be demonstrated in the serum and urine of thyrotoxic patients (RAWSON, PURVES and GRIESBACH), although it can be assumed that their thyroid glands are under increased TSH stimulation. The only possible cause for this effect is increased TSH inactivation by hyperplastic thyroid parenchyma, in which additions of iodine lead to a further reduction of activity and with that to struma colloides with again regular follicle epithelium and normal iodine content.

We conducted our own in vivo investigations into the discussed augmenting effect with TSH + MTU; however, we were limited by the small amount of Pretiron available to us. According to these investigations, the histologic activation is more extreme than with TSH alone, although less obvious in the epithelium values (mZ. 16.05 μ : 15.2 μ), which are still within the normal physiologic range. As in vivo parallel to the in vitro experiments with iodized TSH, the simultaneous application of

TSH and LUGOL's solution results in a significant loss of activity, which can be expected to lead to further decrease of activity following additional increase of iodine dosage, analogous to the results of anti-thyroid and iodine therapy in rabbits. On the other hand, combined in vivo application of iodized inactive TSH and MTU results in regeneration of activity, for which compensatory additional TSH release by the thyriod gland is unlikely; the degree of reactivation can more probably be attributed to the reductive potency of MTU. The last two tests may be considered parallels to the in vitro experiments and therefore permit the conclusion that similar inactivation-reactivation mechanisms are probably present in the living organism.

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Aus dem Institut für Hygiene und Mikrobiologie der Medizinischen Akademie Düsseldorf (Direktor: Prof. Dr. W. Kikuth)

Über die Hemmung der Immunhämolyse durch Antirheumatika*)

Thiosulfate 184

Von A. Lange

Mit 3 Abbildungen und 3 Tabellen

(Eingegangen am 15. Oktober 1960)

Die meisten sogenannten Antirheumatika besitzen außer ihrer antiphlogistischen, antipyretischen und analgetischen auch eine im Tierversuch nachweisbare antiallergische Wirkung. Wenn ich die Corticosteroide einmal zu den Antirheumatika zählen darf, dann kann man sogar sagen, daß die Antirheumatika auch therapeutisch als wirksame Antiallergika eingesetzt werden können.

Die antiallergische Wirkung der Antirheumatika beruht nicht auf einer Antihistaminwirkung wie das bei den Antihistaminika der Fall ist. Manche allergische Reaktionen werden durch Antihistaminika nicht, wohl aber durch Antirheumatika beeinflußt. So kann die Entwicklung des Arthus-Phänomens beim Kaninchen und Meerschweinchen durch Antirheumatika verhindert werden, während Antihistaminika hier wirkungslos sind. Auch der inversanaphylaktische Schock des Meerschweinchens läßt sich, wie wir zeigen konnten, durch Antirheumatika, nicht aber durch Antihistaminika hemmen.

Aus diesen Befunden ist zu entnehmen, daß die antiallergische Wirkung der Antirheumatika mit Sicherheit auf einen anderen Mechanismus zurückzuführen ist als die der Antihistaminika. Wir untersuchten, ob die Antirheumatika bei der Antigen-Antikörper-Reaktion selbst oder bei einer der direkten Folgen der Antigen-Antikörper-Reaktion, nämlich bei der Bindung des Komplements zur Wirkung kommen.

Als Modell einer Antigen-Antikörper-Reaktion mit Komplementbindung und dadurch bedingter Zytolyse diente uns die durch den hämolytischen Ambozeptor und Komplement bewirkte Hämolyse von Schaferythrozyten. Nach dem in Tab. 1 dargestellten Schema untersuchten wir Antirheumatika, wie Natriumsalizylat, Phenylbutazon, Chloroquin, Prednisolon sowie verschiedene andere Substanzen auf ihre hämolysehemmende Wirkung, indem wir sie im Versuchsansatz 1 auf Komplement und Schaferythrozyten sowie im Versuchsansatz 3 auf Komplement und Ambozeptor einwirken ließen. Die Versuchsansätze 2 und 4 sind die jeweiligen Kochsalzkontrollen.

Nach einer Einwirkungszeit von 30 min bei 37° C wurden die zur Hämolyse notwendigen Faktoren, in Versuchsansatz 1 und 2 der Ambozeptor, in Versuchsansatz 3 und 4 die Schaferythrozyten, zugefügt. Lediglich im Versuchsansatz 3, in dem die Antirheumatika auf Komplement und Ambozeptor eingewirkt hatten, wurde eine vollständige Hemmung der Hämolyse beobachtet. Eine Wirkung auf die Erythrozyten war somit ausgeschlossen. Auch das freie Komplement konnte als Angriffspunkt nicht in Betracht kommen, da in beiden Versuchsansätzen Komplement vorhanden war und nur in einem die Hemmung der Hämolyse festgestellt werden konnte. Zu erklären war das nur damit, daß der Ambozeptor, also der Antikörper, durch die Ein-

*) Vorgetragen auf der Jahrestagung der Deutschen Gesellschaft für Rheumatologie vom 14. bis 15. Oktober 1960 in Bad Driburg.

Z. Rheumaforsch., 20: 203–208, 1961

Tab. 1. Wirkung von Natriumsalizylat (Sal) auf die spezifische Hammelblutkörperchen(HBK)-hämolyse

1. Wirkung von Sal auf Komplement (Ko) und HBK	2. Kochsalzkontrolle zu 1.	3. Wirkung von Sal auf Ko und Amboceptor (Amb)	4. Kochsalzkontrolle zu 3.
0,5 ml Ko 0,05 ml HBK 0,2 ml Sal	0,5 ml Ko 0,05 ml HBK 0,2 ml NaCl	0,5 ml Ko 0,05 ml Amb 0,2 ml Sal	0,5 ml Ko 0,05 ml Amb 0,2 ml NaCl
Die Mischungen werden für 30 min in ein H_2O-Bad von 37° C gestellt			
Es wurden dann zugefügt:			
0,05 ml Amb	0,05 ml Amb	0,05 ml HBK	0,05 ml HBK
Komplette Hämolyse oei 37° C nach Minuten			
25	10	nach 120 min keine Spur Hämolyse	15

wirkung der Antirheumatika inaktiviert worden war. Restitutionsversuche bestätigten diese Vermutung. Lediglich nachträglich zum System zugefügter Ambozeptor brachte die Hämolyse wieder in Gang, nicht aber nachträglich zugefügtes Komplement.

Weitere Versuche zeigten, daß zwischen Antikörper- und Antirheumatikakonzentration eine streng quantitative Beziehung besteht. Tab. 2 zeigt, daß steigende Antirheumatikumkonzentrationen entsprechend größere Ambozeptormengen inaktivieren.

Tab. 2. Wirkung verschiedener Natriumsalizylat-Konzentrationen auf Amboceptorverdünnungsreihen

Natriumsalizylat-konzentration %	Amboceptorverdünnung 1:									
	5000	7500	11 250	16 900	25 300	38 000	57 000		128 000	192 000
1,0	+	+	±	—	—	—	—	—	—	—
0,667	+	+	+	±	±	—	—	—	—	—
0,444	+	+	+	+	±	±	±	—	—	—
0,296	+	+	+	+	+	+	±	—	—	—
0,197	+	+	+	+	+	+	+	±	—	—
0,132	+	+	+	+	+	+	+	±	—	—
0,088	+	+	+	+	+	+	+	±	±	—
0,059	+	+	+	+	+	+	+	+	±	—
0,039	+	+	+	+	+	+	+	+	±	±
0,026	+	+	+	+	+	+	+	+	+	±
0,9 % NaCl-Lösung	+	+	+	+	+	+	+	+	+	±

+ vollständige, ± unvollständige, — keine Hämolyse.

In weiteren Versuchen wurde die Wirkung verschiedener Konzentrationen auf 1 hämolytische Ambozeptoreinheit (1 HE) untersucht. Als 1 HE Ambozeptor wurde die Ambozeptorkonzentration bezeichnet, die in Gegenwart von 1:10 verdünntem Komplement alle angebotenen Erythrozyten lysiert und damit eine 100%ige Hämolyse bewirkt. Die Ambozeptoraktivität beträgt dann 100 %. Es wurde untersucht, um welchen Prozentsatz die Ambozeptoraktivität durch verschiedene Konzentrationen der Antirheumatika herabgesetzt wird.

In Abb. 1 sind die Wirkungskurven von Prednisolon, Phenylbutazon, Chloroquin, Gentisinsäure, Salizylsäure und Kochsalz dargestellt. Während die vier ersten Pharmaka bereits bei molaren Endkonzentrationen in der Größenordnung von 10^{-3} wirksam sind, ist die Salizylsäure in Form von Natriumsalizylat erst bei Konzentrationen von 10^{-2} voll wirksam. Die volle Wirksamkeit von Kochsalz tritt erst bei einer molaren Endkonzentration von 10^{-1} ein. Auch Kochsalz hat eine inaktivierende Wirkung auf den Ambozeptor, die aber um 2 Zehnerpotenzen, genauer gesagt, etwa 300mal schwächer ist als die des Prednisolons, des in unseren Versuchen wirksamsten Pharmakons.

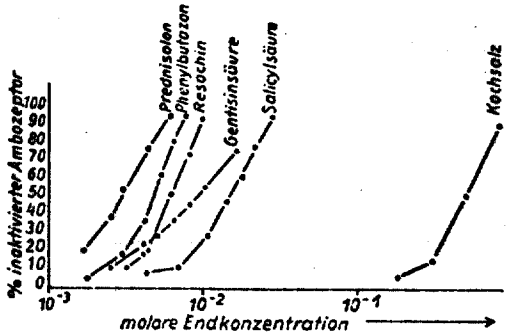

Abb. 1. Wirkung verschiedener Substanzen auf eine hämolytische Einheit Ambozeptor

Eine Reihe der verschiedensten Substanzen wurde auf ihre ambozeptorinaktivierende Wirkung untersucht. Das Ergebnis dieser Versuche zeigt Tab. 3. Vor allen hier aufgeführten Substanzen hatten Prednisolon, Phenylbutazon, Resochin, Solvoteben und Gentisinsäure die stärkste Wirkung. Es ist auffällig, daß gerade die Antirheumatika zu den wirksamsten ambozeptorinaktivierenden Pharmaka gehören. Lediglich das Amidopyrin bildet eine Ausnahme. Es ist nur sehr schwach wirksam.

Die für die Ambozeptorinaktivierung in vitro benutzten Konzentrationen der Antirheumatika können in vivo nicht erreicht werden. Es fragt sich deshalb, ob die in vivo erreichbaren Konzentrationen eine Antikörperinaktivierung in vivo bewirken können. Wir sensibilisierten 12 Kaninchen gegen Schaferythrozyten. Nach Abschluß der Sensibilisierungsperiode wurde der Ambozeptorgehalt in den Kaninchenseren durch Reihenverdünnung titriert. 6 Kaninchen wurden anschließend 100 mg/kg Butazolidin intravenös injiziert. Die anderen Tiere erhielten auf gleiche Weise das entsprechende Volumen physiologische Kochsalzlösung. Der Ambozeptortiter wurde in keiner Weise durch die Behandlung beeinflußt.

Tab. 3. Vergleich der amboceptorinaktivierenden Wirksamkeit verschiedener Substanzen

Gruppe I hauptsächlich wirksam bei molaren Endkonzentrationen von 10^{-3}	Gruppe II hauptsächlich wirksam bei molaren Endkonzentrationen von 10^{-2}	Gruppe III unwirksam oder wirksam bei molaren Endkonzentrationen von 10^{-1}
Prednisolon	Salizylsäure	Amidopyrin
Phenylbutazon	Adenosin	Antipyrin
Resochin	Natriumsulfat	Natriumchlorid
Solvoteben	Natriumthiosulfat	Natriumdihydrogenphosphat
Gentisinsäure	Evipan-Natrium	Natriumbikarbonat
		Kalziumchlorid
		Magnesiumchlorid
		Kaliumchlorid
		Ammoniumchlorid
		Kaliumtartrat
		Harnstoff
		Veronal-Natrium
		Coffein
		p-Aminobenzoesäure
		Elkosin
		Novocain
		Neoteben
		Kalziumglukonat
		Neo-Antergan
		Megaphen

Inzwischen hatten in-vitro-Versuche ergeben, daß sich der Antigen-Antikörper-Komplex trotz der Anwesenheit der Antirheumatika bildet. Somit konnte die Bindungsstelle des Ambozeptors für die Erythrozyten als Angriffspunkt der Antirheumatika ausgeschlossen werden. Ebenso verhielt es sich mit der Bindungsstelle des Erythrozyten-Ambozeptor-Komplexes für das Komplement. Nach Vorbehandlung des fertigen Antigen-Antikörper-Komplexes mit den Antirheumatika konnte sich trotzdem nachträglich angebotenes Komplement binden und so die Zellen lysieren.

Da das freie Komplement und die Erythrozyten von den verwendeten Antirheumatikakonzentrationen nicht angegriffen werden, blieb nur eine Möglichkeit für die Erklärung der ambozeptorinaktivierenden Wirkung. Sie mußte indirekt über die Inaktivierung bereits gebundener Komplementkomponenten erfolgen. Aus diesem Grunde wurde der Erythrozyten-Ambozeptor-Komplex gleichzeitig mit Butazolidin und Komplement behandelt. Bei geeigneten Komplement- und Butazolidinkonzentrationen blieb die Hämolyse aus. Nach Waschung des so behandelten Erythrozyten-Ambozeptor-Komplexes und nachträglicher Zugabe von Komplement war die Hämolyse gehemmt. Sie war um so stärker gehemmt, wie Abb. 2 zeigt, je größer die zur Vorbehandlung verwendeten Komplementkonzentrationen bei gleichbleibender Butazolidinkonzentration war. Wurde der Erythrozyten-Ambozeptor-Komplex gleichzeitig mit Butazolidin und inaktiviertem Komplement behandelt, so wurde die Hämolyse nicht gehemmt.

Die Untersuchungen hierüber sind noch nicht abgeschlossen. Die bisherigen Befunde können aber in der Weise gedeutet werden, daß Butazolidin nicht direkt den Ambozeptor angreift, sondern nur indirekt, nämlich über das an den Erythrozyten-Ambozeptor-Komplex gebundene Komplement. Das an den Komplex gebundene Komplement wird direkt inaktiviert und läßt eine weitere Komplementbindung nicht mehr zu.

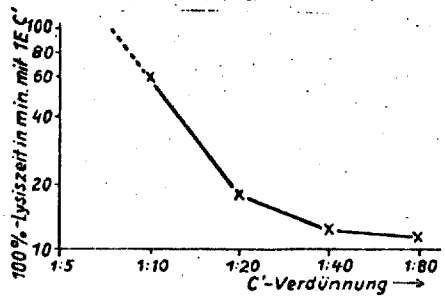

Abb. 2. EA vorbehandelt mit C'Verdünnung in Gegenwart von 0,3% Butazolidin

Auf Grund der Ergebnisse dieser in-vitro-Versuche nahmen wir zusammen mit Zaborski die Tierversuche wieder auf. Erneut wurden 12 Kaninchen gegen Hammelerythrozyten sensibilisiert. Nach Abschluß der Sensibilisierungsperiode wurde das nicht inaktivierte, also komplementhaltige Serum der Kaninchen auf seine hämolytische Aktivität gegenüber einer photometrisch standardisierten Suspension von Hammelerythrozyten geprüft und nicht, wie sonst üblich, der Titer des Ambozeptors im inaktivierten Serum bestimmt. Dabei wurde das Serum nur 1:2 mit der Erythrozytensuspension verdünnt. Nach vier Bestimmungen der hämolytischen Aktivität eines jeden Kaninchenserums zu verschiedenen Tageszeiten wurde am folgenden Tage 6 Kaninchen Butazolidin intravenös injiziert und den 6 anderen Kaninchen zur Kontrolle Kochsalz. Eine, vier, 8 und 24 Std. nachher wurde die hämolytische Aktivität der Seren photometrisch bestimmt.

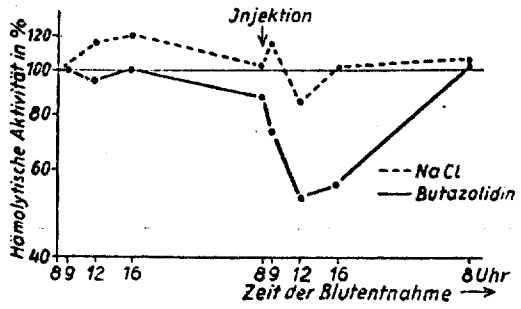

Abb. 3. Wirkung von Butazolidin (i. v. injiziert) auf die hämolytische Aktivität des Serums sensibilisierter Kaninchen

Abb. 3 zeigt das Ergebnis dieser Versuche. Die hämolytische Aktivität des Kaninchenserums wird durch Butazolidin eindeutig herabgesetzt, und zwar um etwa 46% im Vergleich zum Ausgangswert. Die Unterschiede zwischen den Mittelwerten des Vortages und denen des Behandlungstages sind mit einem $p < 0{,}01$ statistisch signifikant. Mit Kochsalz wurde ein solcher Effekt nicht erreicht. Mit einer geeigneten Methode läßt sich somit nachweisen, daß in vivo erreichbare Butazolidinkonzentrationen die durch Komplement und Ambozeptor bewirkte Hämolyse zu hemmen vermögen.

Damit wird es wahrscheinlich, daß die antiallergische Wirkung der Antirheumatika mindestens zum Teil durch eine Inaktivierung des an den Antigen-Antikörper-Komplex gebundenen Komplement zustandekommt, so daß von einer indirekten Inaktivierung des Antikörpers gesprochen werden kann.

Um nun den Zusammenhang zwischen der antiallergischen Wirkung der Antirheumatika einerseits und dem rheumatischen Geschehen andererseits aufzuzeigen, muß auf die Theorie KLINGES hingewiesen werden. Danach soll der rheumatische Gewebsschaden durch eine am Orte der Schädigung ablaufende Antigen-Antikörper-Reaktion zustande kommen.

Die Antirheumatika können das Fortschreiten des rheumatischen Gewebsschadens hemmen. Sofern die Theorie KLINGES zutrifft, kann man auch annehmen, daß diese Wirkung der Antirheumatika nicht nur auf ihre antiphlogistische, sondern auch auf ihre antiallergische Wirkung zurückzuführen ist.

Anschrift des Verfassers:
Dr. A. LANGE, Inst. f. Hygiene u. Mikrobiologie d. Med. Akademie, Düsseldorf, Witzelstraße 109

BUCHBESPRECHUNGEN

Les tractions vertébrales (Die Streckbehandlung der Wirbelsäule). Von J. LEVERNIEUX-Paris. Mit einem Vorwort von S. DE SÉZE-Paris. 124 Seiten mit 35 Abbildungen. (Paris 1960, Expansion Scientifique Française.) Preis: brosch. nffr. 18,–.

Durch bessere Kenntnisse der Anatomie, Physiologie und Pathologie der Wirbelsäule sind die seit dem Altertum geübten therapeutischen Versuche der Streckbehandlung von Wirbelsäulenschäden wieder zu Ehren gekommen. Die oft sehr eindrucksvollen Erfolge führten aber zu mißbräuchlicher Anwendung und brachten die Methode in Mißkredit. Dem vorliegenden Buch liegen die Erfahrungen der DE SÉZEschen Klinik in Paris seit 1946 zugrunde. Sie sind experimenteller und klinischer Natur. Die Versuche wurden am Präparat mit genauer Messung der Wirkung mechanischer Zugkräfte auf Zwischenwirbelscheiben durchgeführt und photographiert, Versuche in vivo wurden röntgenologisch kontrolliert. An etwa 12 000 Kranken wurden praktische Erfahrungen gesammelt und die Indikationen abgeleitet. Es handelte sich um verschieden lokalisierte Bandscheibenschäden und discoradiculäre Komplikationen. Es ließ sich noch nachweisen, daß durch die Traktionsbehandlung der Intervertrebralraum bis zu 1,5 mm vergrößert wird. Der Zug muß möglichst an beiden Enden im Bereich des kleinsten Vertebralsegments angreifen, kräftig genug sein, ohne zu verletzen. Er muß langsam zunehmend vergrößert und messend verfolgt werden. In dem Buch finden sich gute Abb. über die praktische Durchführung und die angewandte Apparatur. Es wird als unabdingbare Voraussetzung des Verfahrens eine vorherige detaillierte Diagnostik der Störung und ihrer Lokalisation mehrfach betont. Die Gefahren der Extensionsbehandlung sind bei Beachtung aller Spielregeln sehr gering. Von der Traktion ist die viel umstrittene Manipulation zu unterscheiden, welche ihre besonderen Indikationen hat und damit kombiniert werden kann. Die Ergebnisse der Traktionstherapie bezüglich der Schmerzbekämpfung sind gut, wenn sie in der vorsichtig meßbaren Weise durchgeführt werden.

Das Buch kann in seiner klaren und übersichtlichen Darstellung bestens empfohlen werden und eröffnet neue therapeutische Wege. R. SCHOEN (Göttingen)

Lehrbuch der speziellen pathologischen Anatomie. Von E. KAUFMANN †. 11.–12. Auflage von M. STAEMMLER-Aachen. Band III, Lieferung 7. Seite 854–1000 mit 76 Abbildungen. (Berlin 1960, Walter de Gruyter & Co.) Preis: brosch. DM 33,–.

Die 7. Lieferung des 3. Bandes der Neuauflage des berühmten Lehrbuches der speziellen pathologischen Anatomie von E. KAUFMANN umfaßt das Hör- und Gleichgewichtsorgan durch H. UFFENORDE-Göttingen. Die abgerundete Darstellung der Anatomie und Pathologie des Ohres wird vor allem neben dem Pathologen den HNO-Fachärzten willkommen sein. R. SCHOEN (Göttingen)

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Thiosulfate

THE INHIBITION OF IMMUNE HEMOLYSIS BY ANTIRHEUMATICS

Most of the so-called antirheumatics have, in addition to their antiphlogistic, antipyretic and analgesic effect, an antiallergic effect as well, which can be demonstrated by experiments with animals. If I may include the corticosteroids in the antirheumatics, it can even be said that antirheumatics may be employed as therapeutically effective antiallergics.

The antiallergic effect of antirheumatics is not due to an antihistaminic action as is the case with antihistamines. Some allergic reactions are not affected by antihistamines but are influenced by antirheumatics. Thus the development of Arthus's phenomenon in rabbits and guinea pigs can be prevented by antirheumatics but not by antihistamines. Inverse anaphylaxis of guinea pigs can also be inhibited by antirheumatics, but not by antihistamines, as we were able to demonstrate.

One can conclude from these findings that the antiallergic effect of antirheumatics evidently has to be attributed to a mechanism different from the one in antihistamines. We investigated whether the antirheumatics take effect during the antigen-antibody reaction itself or as one of the direct consequences of the antigen-antibody reaction, i.e. during binding with complement.

LANGE, MD, A.
Institute for Hygiene and Microbiology of the Medical Academy, Düsseldorf, Germany (Director: KIKUTH, MD, W.)
Z. Rheumaforsch., 20:203-208, 1961

As model for an antigen-antibody reaction with complement binding and consequential cytolysis we employed hemolysis of sheep erythrocytes produced by hemolytic amboceptor and complement. With the system depicted in table 1, we examined antirheumatics, such as sodium salicylate, phenylbutazone, chloroquine, prednisolone as well as various other substances for their hemolysis inhibiting effectiveness by allowing them to react on complement and sheep erythrocytes in test 1 and on complement and amboceptor in test 3. Tests 2 and 4 are the respective sodium chloride controls.

After a reaction time of 30 min. at 37° C, we added the factors required for hemolysis, in test 1 and 2 the amboceptor and in test 3 and 4 the sheep erythrocytes. Only in test 3, in which the antirheumatics had reacted on complement and amboceptor, we observed a complete inhibition of hemolysis. This excluded an affect on the erythrocytes, The free complement could not be considered as point of exertion either, since complement was present in both tests and inhibition of hemolysis could only be observed in one. This could only be explained by the fact that the amboceptor, i.e. the antibody, had been inactivated by the action of the antirheumatics. Restitution experiments confirmed this supposition. Hemolysis was reactivated only by supplementing the system with amboceptor, not with complement.

Additional tests showed that a strict interrelation exists between the concentration of antibodies and antirheumatics. Table 2 demonstrates that increasing concentrations of antirheumatics inactivate correspondingly greater amounts of amboceptor.

In further experiments, we examined the effect of various concentrations on 1 hemolytic amboceptor unit (1 HU). We designated as 1 HU amboceptor that concentration which, in the presence of complement diluted at

Table 1. Effect of sodium salicylate (Sal) on the specific sheep erythrocytes (SE) hemolysis

1	2	3	4
effect of Sal on complement (co) and SE	sodium chloride control to 1	effect of Sal on co and amboceptor (amb)	sodium chloride control to 3
0.5 ml co 0.05 ml SE 0.2 ml Sal	0.5 ml co 0.05 ml SE 0.2 ml NaCl	0.5 ml co 0.05 ml amb 0.2 ml Sal	0.5 ml co 0.05 ml amb 0.2 ml NaCl
The mixtures were placed for 30 min. into a H_2O bath of 37° C			
We then added:			
0.05 ml amb	0.05 ml amb	0.05 ml SE	0.05 ml SE
Complete hemolysis at 37° C in minutes			
25	10	after 120 min. no sign of hemolysis	15

Table 2. Effect of various sodium salicylate concentrations on the amboceptor dilution series

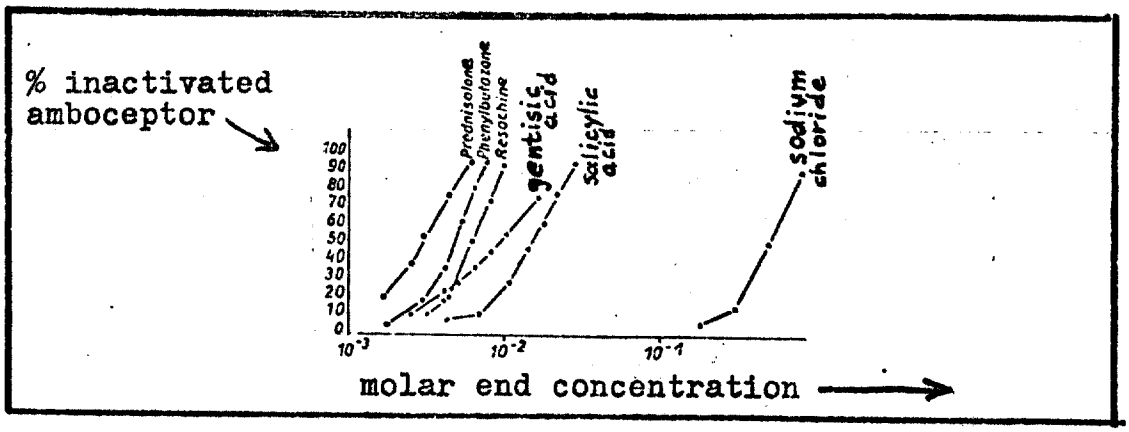
Sodium salicylate Konzentration %	amboceptor dilution 1:									
	5000	7500	11250	16900	25300	38000	57000		128000	192000
1,0	+	+	±	—	—	—	—	—	—	—
0,667	+	+	+	±	±	—	—	—	—	—
0,444	+	+	+	+	±	±	±	—	—	—
0,296	+	+	+	+	+	+	±	—	—	—
0,197	+	+	+	+	+	+	+	±	—	—
0,132	+	+	+	+	+	+	+	±	—	—
0,088	+	+	+	+	+	+	+	±	±	—
0,059	+	+	+	+	+	+	+	+	±	—
0,039	+	+	+	+	+	+	+	+	±	—
0,026	+	+	+	+	+	+	+	+	+	±
0,9% NaCl-solution	+	+	+	+	+	+	+	+	+	±

+ complete, ± incomplete, — no hemolysis

the ratio of 1:10, lyses all existing erythrocytes and therefore exerts a 100 % hemolysis. The amboceptor activity is then 100 %. We investigated how much the percentage ratio of the amboceptor activity is decreased by various concentrations of antirheumatics.

Chart 1 depicts the effectiveness curves of prednisolone, phenylbutazone, chloroquine, gentisic acid, salicylic acid and sodium chloride. While the first 4 agents are already effective in molar end concentrations in the scale of 10^{-3}, salicylic acid in the form of sodium salicylate is only fully effective at concentrations of 10^{-2}. The full effectiveness of sodium chloride is only reached at a molar end concentration of 10^{-1}. Sodium chloride also has an inactivating effect on the amboceptor, but it is 20 % or, more exactly, about 300 times weaker than that of prednisolone, the most effective agent in our experiments.

Chart 1. effect of various substances on 1 hemolytic unit amboceptor



We investigated amboceptor inactivating potency of a series of the most various substances. The results of these tests are compiled in table 3. Prednisolone, phenylbutazone, resochine, solvoteben and gentisic acid had the strongest effectiveness of all substances represented here.

Table 3. Comparison of the amboceptor inactivation potency of various substances

group I	group II	group III
mainly effective in molar end concentrations of 10^{-3}	mainly effective in molar end concentration of 10^{-2}	ineffective or effective in molar end concentration of 10^{-1}
prednisolone phenylbutazone resochine solvoteben gentisic acid	salicylic acid adenosin sodium sulfate sodium thiosulfate evipan-sodium	amidopyrine antipyrine sodium chloride sodium diphosphate soduim bicarbonate calcium chloride magnesium chloride potassium chloride ammonium chloride potassium tartrate urea veronal-sodium caffeine p-aminobenzoic acid elcosin novocaine neoteben sodium gluconate neo-antergan megaphen

It is conspicuous that just the antirheumatics belong to the agents with the highest amboceptor inactivation potency. Only amidopyrine is an exception. Its effectiveness is very low.

The concentration of antirheumatics used for amboceptor inactivation in vitro cannot be attained in vivo. The question therefore is, whether the concentration attainable in vivo can exert an antibody inactivation in vivo. We sensitized 12 rabbits against sheep erythrocytes. After conclusion of the sensitizing period, the amboceptor content in the serum of the rabbits was titrated by serial dilution. 6 rabbits were then given

100 mg/kg butazolidine by intravenous injection. The other animals received the corresponding amount of normal saline solution in the same manner. The amboceptor titer was in no way influenced by this treatment.

Meanwhile, in vitro experiments had demonstrated that the antigen-antibody complex forms in spite of the presence of antirheumatics. Therefore the point of connection of the amboceptor with the erythrocytes can be excluded as the point of exertion of the antirheumatics. The same applied to the point of connection of the erythrocyte-amboceptor complex with the complement. After pretreatment of the finished antigen-antibody complex with antirheumatics, susequently introduced complement could still be connected and thus lyse the cells.

Since the free complement and the erythrocytes were not affected by the employed antirheumatics concentrations, there was only one possible explanation for the amboceptor inactivation effect. It had to arise indirectly via the inactivation of already connected complement components. For this reason, the erythrocyte-amboceptor complex was treated simultaneously with butazolidine and complement. Hemolysis did not take place with suitable concentrations of complement and butazolidine. Following lavage of the erythrocyte-amboceptor complex treated in this manner, and subsequent addition of complement, hemolysis was inhibited. As shown in chart 2, inhibition was increased in proportion to the increase of complement concentration, used for pretreatment, along with an unchanged concentration of butazolidine. When the erythrocyte-amboceptor complex was treated simultaneously with butazolidine and inactivated complement, hemolysis was not inhibited.

Investigations into this matter have not been concluded yet. However, the present findings can be interpreted that butazolidine does not directly affect the amboceptor, but only indirectly, that is via the

Chart 2. erythrocyte-amboceptor complex pretreated with C' dilution in presence of 0.3 % butazolidine

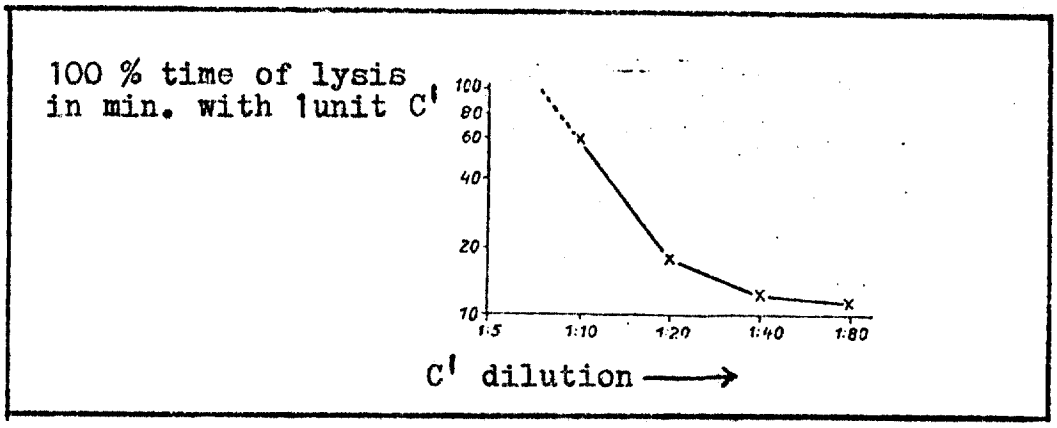
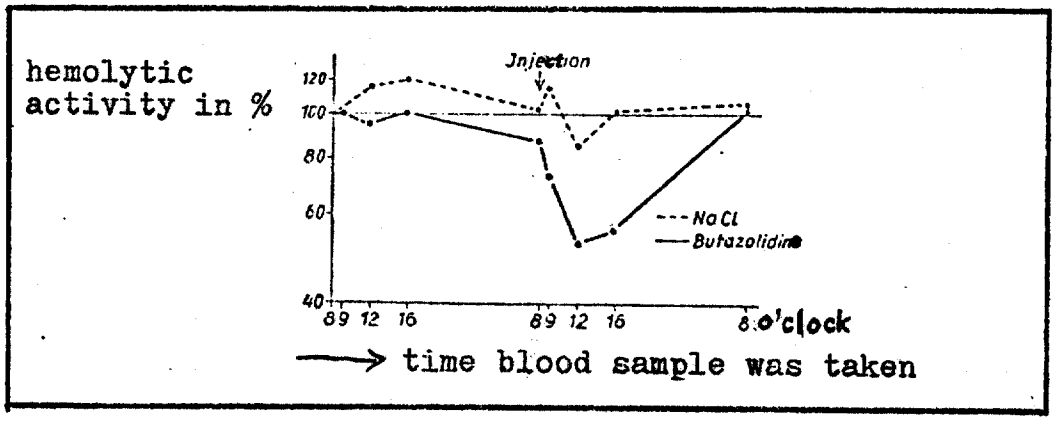


Chart 3. Effect of butazolidine (administered intravenously) on the hemolytic activity of the serum of sensitized rabbits



complement connected to the erythrocyte-amboceptor complex. The complement connected with the complex is inactivated directly and does not permit an additional complement connection.

Based on the results of these in vitro experiments, we resumed the animal experiments in collaboration with ZABORSKI. 12 rabbits were again sensitized against sheep erythrocytes. After the end of the sensitizing period, the not inactivated, i.e. the compliment-containing, serum of the rabbits was examined for its hemolytic activity against photometrically

standardized suspensions of sheep erythrocytes, and we did not, as is done usually, determine the titer of the amboceptor in the inactivated serum. For this purpose, the serum was diluted with the erythrocyte suspension at a ratio of only 1:2. After determining the hemolytic activity of the serum of each rabbit 4 times at different times of the day, 6 rabbits received intravenous injections of butazolidine and the 6 other rabbits sodium chloride as control on the following day. 1, 4, 8 and 24 hours later, the hemolytic activity of the sera was determined photometrically.

Chart 3 shows the results of these tests. The hemolytic activity of the serums is reduced unequivocally by butazolidine, i.e. by about 46 % in comparison to the initial value. Statistically significant are the differences between the average values of the previous day and those of the day of treatment with $p < 0.01$. An effect of this kind was not attained with sodium chloride. It can therefore be demonstrated with a suitable method that butazolidine concentrations attainable in vivo are capable of inhibiting hemolysis caused by complement and amboceptor. With that it becomes probable that the antiallergic action of antirheumatics is caused at least in part by inactivation of the complement connected to the antigen-antibody complex, which may be called an indirect inactivation of the antibody.

In order to now show the relationship between the antiallergic effect of antirheumatics on the one hand and the rheumatic process on the other hand, we refer to the theory of KLINGE. According to that, the rheumatic tissue damage is supposed to arise from an antigen-antibody reaction taking place at the locality of the damage.

Antirheumatics can inhibit the process of the rheumatic tissue damage. Insofar as KLINGE's theory is pertinent, one can suppose that this effect of the antirheumatics is caused not only by their antiphlogistic , but also by their antiallergic action.

jours. Nous avons pu faire une expérience avec de l'hydroxyde de soude chez un Lapin qui présentait une résistance spontanée et exceptionnelle à l'huile de moutarde ; or nous n'avons constaté aucun changement important dans un sens ou dans l'autre, les variations ne dépassant pas un degré et demi.

Il nous semble alors permis d'admettre l'existence d'une relation directe entre l'action anti-inflammatoire des deux composés magnésiens en question et leur capacité de fixer le calcium au moins pour un certain temps. Nous nous proposons de rechercher l'influence du sulfate de magnésie sur les phénomènes inflammatoires ; ce sel appartient au groupe des sels magnésiens à absorption difficile et à élimination rapide, qui amène par ce mécanisme des pertes considérables de calcium. Nous pensons pouvoir ainsi préciser si l'action anti-inflammatoire des deux composés magnésiens qui font l'objet de nos expériences résulte de la [illegible] calcium ou si elle dépend directement de l'action « cy[illegible] » de l'ion magnésium, établie par les recherches [illegible]

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Thiosulfate

[illegible]e pharmacologie de l'Ecole vétérinaire.)

Action de l'hydroxyde colloïdal de magnésie et du thiosulfate de soude sur les globules blancs,

par J. Lebduska et F. Cervinka.

Nous croyons inutile de développer ici l'historique de nos connaissances actuelles sur la phagocytose : citons seulement le nom de Metchnikoff qui a le premier déterminé les circonstances de cette défense cellulaire contre les microbes. Jusqu'aux travaux de Hamburger et de son école, il n'y a pas eu d'études systématiques sur l'influence que peuvent exercer les divers médicaments sur ce phénomène biologique. Cette question a été enrichie en 1915 par la découverte de Delbet (1), importante surtout au point de vue pratique, sur l'action activante du chlorure de magnésium sur la phagocytose. C'est Delbet qui, le premier, conçut l'importance du magnésium dans l'économie animale et l'exposé récent de ses recherches confirme ses vues précédentes. Le magnésium exercerait sur la cellule vivante une action manifeste que cet auteur désigne du terme d'« action cytophylactique » : quant aux globules blancs, le chlorure de magnésium amène une

(1) *Monde médical*, 1929, p. 969. Voir aussi *C. R. de l'Acad. des sc.*, 1915, t. *161*, p. 268.

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Compt. Rend. Soc. Biol., 103, 1930

augmentation de la phagocytose, qui peut atteindre *in vivo* 300 p. 100 du taux normal.

Il nous a paru intéressant de rechercher si une action identique se manifeste avec un autre composé magnésien : l'hydroxyde colloïdal de Mg, dont l'emploi en thérapeutique, sous le nom de « Polysane », a été préconisé surtout par Becka (2) ; nous avons étudié aussi un autre agent chimiothérapique, le thiosulfate de soude, qui a fait, dans ces derniers temps, l'objet de recherches de la part de Kabelik (3). Nous exposerons ici les résultats que nous avons obtenus avec ces deux substances *in vitro*. Nous avons suivi la même technique expérimentale qu'Hamburger et que Delbet. Nous avons utilisé les globules blancs provenant du sang veineux du Cheval. Les prélèvements de sang ont été faits dans des éprouvettes contenant comme anticoagulant 50 c.c. d'une solution isotonique citratée : 0,7 p. 100 de chlorure de sodium et 1,1 p. 100 de citrate de soude ; cette quantité suffisait pour 150 c.c. du sang. Une fois les globules blancs isolés et lavés, nous avons obtenu une suspension épaisse, contenant en majeure partie des polynucléaires. On a distribué dans des tubes 2 c.c. de cette suspension, 2 c.c. des solutions à étudier et enfin 0,25 c.c. d'une culture de *B. coli* en bouillon peptoné, âgée de 24 heures

Expériences	Substance étudiée	Témoin	Concentration de la solution			
			N/10	N/100	N/1.000	N/10.000
I	$Mg(OH)^2$	20	30	49	42	57
	$Na^2S^2O^3$		56	33	42	64
II	$Mg(OH)^2$	90	46	164	210	176
	$Na^2S^2O^3$		70	219	194	207
III	$Mg(OH)^2$	42	29	51	59	60
	$Na^2S^2O^3$		34	54	64	63
IV	$Mg(OH)^2$	25	26	53	54	59
	$Na^2S^2O^3$		54	58	61	53
V	$Mg(OH)^2$	65	—	100	164	140
	$Na^2S^2O^3$		60	144	170	129

au plus. Deux tubes n'ayant reçu aucune addition d'hydroxyde colloïdal de magnésie ou de thiosulfate de soude servaient de témoins. Tous les tubes ont été portés pendant 10 à 20 minutes à l'étuve ; puis, à l'aide de pipettes de Pasteur, on a fait des étalements qu'on colorait au Bleu alcalin de Lœffler. Pour chaque dilution d'hydroxyde colloïdal de magnésie et de thiosulfate de soude (N/10, N/100, N/1.000, N/10.000), nous avons compté 300

(2) *Casopis lekaru ceskych*, 1927, n°s 22 et 30.
(3) *Ceska dermatologie*, t. 5, n°s 3-5.

polynucléaires et nous avons déterminé le nombre des Bactéries qu'ils ont phagocytées. Le tableau ci-joint expose les résultats que nous avons obtenus ; les chiffres indiquent les phagocytoses p. 100 leucocytes.

L'hydroxyde colloïdal de magnésie et le thiosulfate de soude se sont donc révélés comme des stimulants puissants de la phagocytose *in vitro* ; l'augmentation peut atteindre 250 p. 100 par rapport à la normale. L'intensité d'action des deux substances est très voisine ; elle semble être cependant un peu plus marquée pour le thiosulfate de soude. L'activité de la phagocytose a son maximum pour les concentrations des solutions variant de N/1.000 à N/10.000 ; par contre la concentration N/10 peut diminuer ou même supprimer l'activité des polynucléaires.

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Thiosulfate

THE ACTION OF COLLOIDAL MAGNESIUM HYDROXIDE AND SODIUM THIOSULFATE ON WHITE BLOOD CELLS

by J. Lebduska and F. Cervinka

We believe that it would be of little value to discuss the actual knowledge available on phagocytosis; we would only like to mention the name of Metchnikoff who was the first investigator to determine under what circumstances the cellular defense of phagocytosis occurs against bacteria. Until the works of Hamburger and his school there were no systematic studies on the effects of the various drugs on this biological phenomenon of phagocytosis. In 1915 data on this subject was enriched by the discovery of Delbet (1), and his findings were important from a practical point of view; he discovered the activating action of magnesium chloride on phagocytosis. Delbet was the first author to emphasize the importance of magnesium in the animal organism and recent review of his research confirms this viewpoint. Against the living cell, magnesium exerts what this author designated as a "cytophylactic action". As for the white blood cells, magnesium chloride produces an increase in phagocytosis which may reach as high as 30 per cent of the normal phagocytic level.

It appeared that it would be interesting to study if the other magnesium compounds possess a similar effect; we studied the action of colloidal magnesium hydroxide that is known therapeutically as "Polysane" and which has been studied especially by Becka (2). We also studied another chemotherapeutic

(1) Monde Medical, 1929, p. 969. See also C.R. de l'Acad. des. sc., 1915, t. 161, p. 268.

(2) Casopis Lekara Ceskych. 1927, n. 22 and 30.

agent, sodium thiosulfate, which in the past few years has been the subject of the research being conducted by Kabelik (3). We would like to present, at this point, the results that we have obtained in testing these two compounds in vitro. We followed the same experimental technique as that devised by Hamburger and Delbet. We used white cells obtained from the venous blood of the horse. The blood samples were collected in test tubes containing 50 cc of an isotonic citrated solution as anticoagulant; 0.7 per cent of sodium chloride and 1.1 per cent of sodium citrate were used; this quantity was sufficient for 150 cc of blood. Once the white blood cells were separated and washed, we obtained a thick suspension containing mostly polynuclear cells. Two cc of this suspension was poured into the test tubes, along with 2 cc of the solution to be studied and 0.25 cc of a culture of B. coli in peptone broth, cultivated for at least 24 hours. Two test tubes which did not contain any colloidal magnesium hydroxide or sodium thiosulfate were used as controls. All the test tubes were kept in an oven for ten to twenty minutes. Then, by means of Pasteur pipettes, dilutions were made and these were stained with Loeffler's alkaline blue. For each dilution of colloidal magnesium hydroxide and sodium thiosulfate (N/10, N/100, N/1,000, N/10,000) we counted 300 polynuclear cells and we determined the number of bacteria that had been phagocytosed. The adjoining table presents the results obtained; the figures indicate the percentage of phagocytic leukocytes.

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(3) Ceska dermatologie, t. 5, no. 3-5.

Experiment	Substance studied	Control	Concentration of the solution			
			N/10	N/100	N/1,000	N/10,000
I	Mg(OH)	20	30	49	42	57
	Na S O		56	33	42	64
II	Mg(OH)	90	46	164	210	176
	Na S O		70	219	194	207
III	Mg(OH)	42	29	51	59	60
	Na S O		34	54	64	63
IV	Mg(OH)	25	26	53	59	59
	Na S O		50	58	61	53
V	Mg(OH)	65	--	100	164	140
	Na S O		60	144	170	120

Thus, colloidal magnesium hydroxide and sodium thiosulfate were found to be potent stimulants of phagocytosis in vitro; a 250 per cent increase in phagocytosis could be observed as compared to controls. The potency of these compounds was nearly similar. However, it appeared that sodium thiosulfate was a more potent stimulant of phagocytosis than the colloidal mercury compound. Maximum phagocytic activity was observed for concentrations of the solutions ranging from N/1,000 to N/10,000; in contrast, a concentration of N/10 could decrease or even suppress the activity of polynuclear cells.

(INSTITUTE OF PHARMACOLOGY AT THE VETERINARY SCHOOL)

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THE ACTION OF SODIUM THIOSULPHATE ON THE BLOOD * † ‡

By Joseph Litwins, M. D., Linn J. Boyd, M. D., F. A. C. P. and Louis Greenwald, M. D.

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Sodium thiosulphate has been advocated in a wide variety of intoxications and, in particular, poisoning by arsenic[15, 21, 29, 32] and mercury.[13, 21, 29] In lead[6, 7, 34, 35] poisoning the symptoms are said to be relieved and the coproporphyrinuria[5] abolished. Favorable reports have been published on the value of this chemical in poisoning by potassium permanganate,[38] potassium cyanide,[12, 17, 18, 22, 28] carbon monoxide,[23, 30, 47] avertin,[8, 42, 43] chromic acid[36] and the chromates. It has also been tried with alleged benefit in the toxemias of pregnancy[48] and of uremia[41] as well as in yeast and contact dermatitis.[31] While many call the effects praiseworthy, others[46] regard the chemical as devoid of therapeutic merit. However there is one point of unanimity, namely, its freedom from toxic manifestations. Lash[37] claims that 12 grams may be ingested daily with impunity although catharsis and occasional instances of gastric irritation may be observed.

Little is known about the pharmacology of sodium thiosulphate. It is a diuretic[26] and has been employed as a test of renal function.[9, 19] The reliability of this test is said to be on a par with phenolsulphonaphthalein. In rabbits[45] about one-third of the compound is excreted unchanged in the urine, the remainder as ethereal sulphates; if concentrated solutions are employed the relative percentage of excreted ethereal sulphates decreases. The administration of insulin and glucose retards excretion of the chemical.

Some[39, 40] believe that the beneficial effects depend upon increased oxygen carrying capacity and oxygen content of the blood. This attitude is reflected in the introduction of new com-

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pounds such as sodium tetrathionate which raises the sulphur[33] content and prolongs excretion. An unusually rapid sulphur excretion has been considered important in some diseases such as thromboangitis obliterans and scleroderma. The therapeutic merit of sodium thiosulphate and allied chemicals in these diseases has been attributed to removal of sulphur deficit.

In 1932 Lieb[2, 3, 4, 16] suggested sodium thiosulphate empirically for the prevention of postoperative thrombosis. Bancroft[2, 3, 16] and co-workers subjected this idea to a clinical trial and obtained a favorable therapeutic impression. The acknowledged lack of toxicity and the possible therapeutic merit made a study of the effect of this compound on blood morphology and chemistry imperative since it has become increasingly evident that other agents employed for similar purposes have definite limitations. The physiologic heparin sometimes causes cerebral[29] and other hemorrhages;[27] moreover its cost[24] is almost prohibitive. The less expensive dicoumarin[1, 10, 11, 44] can be administered orally but the resultant prolongation of prothrombin time may necessitate transfusions.

Experimental Studies

In vitro: Preliminary experiments indicated that a 1N solution of sodium thiosulphate was not hemolytic. Variable amounts of this concentration were added to 1 cc of blood in a Lee-White tube and the effect on clotting and clot retraction noted. Observations on the blood of 11 patients are summarized in Table I. No clotting occurred with 0.017 grams or more in 1 cc of blood.

Table 1. Clotting time and clot retraction in vitro; variable amounts of 1N sodium thiosulphate and 1 ccm of normal blood of 11 patients. NCT: normal clotting time; m: minutes; h: hours; r: retraction; nr: no retraction; pr: partly clotted; nc: no clotting.

	Gm. .007	.009	.011	.012	.014	.015	.017
	cc. .05	.05	.07	.08	.09	.10	.11
NCT							
12m(nr)	45m(r)	225m(r)	24h(pr)	nc	nc	nc	nc
8m(r)	22m(r)	42m(r)	4h(nr)	18h(nr)	nc	nc	nc
7m(nr)	27m(r)	45m(r)	45m(r)	45m(r)	45m(r)	nc	nc
10m(r)	72m(r)	105m(r)	4h(r)	18h(nr)	nc	nc	nc
7m(r)	24m(r)	37m(r)	45m(r)	75m(nr)	90m(nr)	nc	nc
7m(r)	17m(r)	22m(r)	15h(r)	18h(r)	pr	nc	nc
8m(r)	30m(r)	41m(r)	21h(r)	nc	nc	nc	nc
8m(nr)	45m(r)	1h(nr)	1.5h(nr)	24h(pr)	24h(pr)	nc	nc
10m(nr)	2h(r)	2h(r)	16h(r)	18h(r)	nc	nc	nc
8m(r)	34m(r)	2h(r)	12h(pr)	18h(pr	nc	nc	nc
8m(r)	50m(r)	2h(nr	18h(nr)	18h(pr)	18h(pr)	nc	nc

These studies indicate that in suitable concentration and amount sodium thiosulphate will prevent the clotting of blood *in vitro*. If thromboplastin is added to oxalated blood, there

is no clot. If thromboplastin is added to blood rendered incoaguable by sodium thiosulphate, clotting occurs but the time required is 3-4 times normal; however the quantity of thromboplastin necessary to elicit this effect is so great that the amount of sodium thiosulphate falls below 0.017 grams per cubic centimeter of blood; moreover the clot usually does not retract. If calcium is added to blood rendered incoaguable by sodium thiosulphate, nothing happens, but if it and thromboplastin are added coagulation is immediate just as happens with oxalated blood.

In vivo: It has been customary for surgeons[3, 4] to give 15 grains of sodium thiosulphate once a day for three or four days right after operation in order to prevent postoperative thrombosis. If the *in vitro* observations just reported are applicable *in vivo*, much larger amounts are necessary.

The systemic effects developing immediately after the intravenous injection of sodium thiosulphate depend upon the dose, speed of administration, and other factors. Usually the slow intravenous administration of 200 cc of a 12% solution evokes no sensations. More concentrated solutions by the same route provoke a salty taste in the mouth so regularly that the chemical could be employed to determine circulation time were another method desirable. Many patients experience a feeling of warmth over the entire body, particularly in the abdomen. There may be nausea or, more rarely vomiting from concentrated solutions after 500-1000 grains, too rapid injection, from old solutions, or when the injection is given after fasting.

The slow administration of 1000 grains in 12 hours to 5 patients failed to evoke symptoms or signs. Two volunteers took 3100 and 3800 grains intravenously in a period of two weeks and biweekly studies over a four-month period failed to disclose any changes of blood morphology or blood chemical findings.

a) *Hematologic effects*: A series of 43 convalescent patients were divided into 5 groups. Each member of a group received a single injection of one of the following solutions: 100 or 200 cc of a 6.5% solution, 100 cc of a 12% solution, 50 cc of a 25 or 50% solution. Observations were made on the hemoglobin, erythrocytes, platelets, leucocytes (including differential counts), prothrombin time, bleeding time, clotting time, erythrocytic sedimentation rate, and cell volume before and at various times for 7 days after the injection. With a single exception no significant changes were observed. The exception is a regular prolongation of clotting time when 50 cc of a 50% solution of

sodium thiosulphate is employed. Since the "before and after" figures for the individual case never exceeded the anticipated margin of error only the final averages are given in Table II. The

Table II. Hematology before and at various times after the intravenous injection of 100-200 ccm of 6.5%,100 ccm of 12%,50 ccm of 25-50% sodium thiosulphate(exclusive of clotting time after 50%). Hemoglobin(Hgb) and prothrombin time(PT) in per cent of normal;erythrocytes(Rbc) in millions, platelets(P) in hundreds of thousands,leucocytes(Wbc) in thousands;granulo-(G) and lymphocytes(L) in percent;bleeding time(BT) andclotting time(CT)in minutes; erythrocytic sedimentation rate(ESR) in mm/hour; cell volume(CV).

	Hgb	Rbc	P.	Wbc	G	L	PT	BT	CT	ESR	CV
Before	81	4.2	2.5	8	68	32	94	2.0	6	5	47
2 hour	82	4.2	2.5	9	70	30	100	2.0	5	4	47
1 day	85	4.3	2.3	9	70	30	94	2.0	5	6	47
2 "	83	4.5	2.6	8	71	29	100	2.5	5	7	46
3 "	80	4.1	2.2	8	72	28	99	1.8	8	8	48
4 "	80	4.1	2.5	9	70	30	92	2.0	6	7	47
5 "	78	4.0	2.4	9	72	28	96	2.0	4	6	46
7 "	80	4.1	2.6	9	71	29	91	1.5	6	8	50

effect on clotting after 50% solutions is discussed later and is not included here. The differential counts are also omitted since in no case was there a change and the number of eosinophiles never amounted to more than 2%.

These results suggest that sodium thiosulphate does not disturb the normal clotting mechanism *in vivo* unless rather large amounts of concentrated solutions are employed.

b) *Blood chemical findings*: A similar study was conducted with sodium thiosulphate solutions of equal strengths and determinations of blood calcium, plasma cholesterol and cholesterol

Table III. The average blood chemical findings in 33 patients before and at various time after the intravenous injection of 200 ccm of 6.5% sodium thiosulphate intravenously. The cholesterol esters reported in per cent and others in mgm. percent

	Ca	Chol.	Chol.Esters	Fibr.	Alb.	Glob.
Before	11.4	170	58.2	0.30	4.4	1.5
3 hours	11.5	157	56.3	0.28		
1 day	11.2	158	47.6	0.23		
3 "	11.3	173	53.9	0.47		
4 "	11.3	194	60.0	0.51		
5 "	11.6	157	57.4	0.45	4.6	1.3

Table 4. The serum fibrinogen,serum total proteins(mgm per cent),plasma clotting time(seconds) and blood specific gravity(at 20°C) before and after the intravenous injection of 200 ccm of 6.5% sodium thiosulphate in 45 patients.

	Fibr.	Tot.Prot.	Pl.Clotting	Sp. gr.
Before	0.28	6.0	94	1.0221
After	0.25	5.8	113	1.0230

esters, serum fibrinogen, albumin and globulin. Since all of these results were negative, the average values for 33 patients who received 200 cc of a 6.5% solution alone will be reported (Table III). These figures exemplify the other negative results. The serum fibrinogen and plasma clotting time were studied after the injection of this solution in 20 additional patients and the serum fibrinogen, blood specific gravity and total serum proteins in 25 others (Table IV). Nothing abnormal was found. Variations in the plasma clotting time were great in both directions so that the average result does not reflect the actual situation; it was markedly prolonged in 7, decidedly shortened in 3, and unaffected in 10. The results have made us extremely skeptical of the value of this test.

c) The influence of 50 cc of a 50% solution on clotting time: A 25% solution of sodium thiosulphate did not alter blood morphology or blood chemical findings. Concentrations between 25 and 50% and amounts under 50 cc of a 50% solution have not been studied in detail although subjective symptoms have been observed with less than 50 cc of a 50% solution.

The effect of 50 cc of a 50% solution of sodium thiosulphate on the clotting time of 11 patients is shown in Table V. In all a definite prolongation of clotting time developed 1-3 days after the chemical was injected intravenously. The grade of the alteration may be legitimately compared to that obtained by repeated administration of heparin and seems much more prolonged. Usually the completed clot is normal save in a few cases in which it remained soft. Simultaneous estimations of serum

Table V. The effect of 50 ccm of a 50% solution of sodium thiosulphate(intravenously on the clotting time of 11 patients. * means additional doses of 10 ccm of the chemical.

	Ho	Hu	Ka	De	Ku	Br	Da	Ab	Sm	Ba	Ja
Before	11	8	13	9	11	10	10	9	9	10	9
1 day	12	21	19	10			13*	9			
2 "	15	15	13	21	14	24	18*	14			
3 "					29			15	14	15	20
4 "	16	13	15	21				17	18		
5 "						23	23			29	23
6 "	25		17	19				17		19	20
8 "		17	16	16	13*	20*					18
9 "	15	15			13*					24	
11 "	17	14	16	12					18		
12 "			18		14*		14*				
13 "	17			16					18		
15 "						15	12				
17 "					10*	18			17		
21 "					7		6				

fibrinogen, prothrombin index, and plasma clotting time during the period of prolonged clotting failed to disclose anything of significance; moreover they were not changed by additional doses of 10 cc of the 50% solution.

d) *Specificity*: The necessary concentration and quantity of the solution was so great that a nonspecific effect seemed probable. Nevertheless preliminary study of a few other, equally concentrated preparations failed to elicit a similar disturbance of clotting; the lack of effect following the administration of a 50% sucrose solution and the action of sodium thiosulphate in the same patient is shown in Table VI.

Table VI. Comparsion of the effect of 50% sucrose and 50% sodium thiosulphate on the clotting time(in minutes) of the same patient.

	Before	2 days	4 days	6 days
Sucrose	10	6	11	7
Sod.Thio.	10	13	13	15
	8days	13 days	15 days	20 days
Sucrose	11	7	12	4
Sod.Thio.	19	15	14	12

Discussion

The mode of action of sodium thiosulphate on blood clotting is unknown. It is generally agreed that blood calcium must be reduced to 2.5 mg. percent before calcium deficiency affects coagulation. The calcium in our patients was not altered. If a disturbance of combined versus ionized calcium is invoked, the retarded nature of the action is difficult to comprehend. Moreover variations of plasma proteins are alleged to be responsible for altered proportions of diffusible and non-diffusible calcium. The serum proteins and serum fibrinogen in particular were not changed in our studies. Blood platelets and the prothrombin index were likewise unaltered. The striking delayed appearance of the effect of sodium thiosulphate strongly suggests that the chemical may act via the liver in some unknown manner.

From a clinical standpoint we have given 100 cc of the 6.5% solution intravenously to 100 patients after abdominal operations. This dose was too small by our present standards since the concentration rather than the absolute amount of sodium thiosulphate seems to be an important factor in its influence on the clotting mechanism. It is interesting that no instance of clinically detectable pulmonary embolism occurred in this series

although many of the patients required extensive surgery and had visible varicosities. Three patients in this post-operative group did develop a post-operative thrombophlebitis of the leg vessels which promptly responded to larger doses of the chemical. Twelve other cases of acute thrombophlebitis in non-surgical patients have been greatly benefited by the 50% solution. By response, one means the striking immediate influence on the pain, for decrease of the signs of inflammation is more difficult to evaluate. At present we are employing one dose of 50 cc of a 50% solution, preferably as an intravenous drip or slow injection followed by 10 cc of the same concentration biweekly, and we have gained an impression of its distinct therapeutic value. Moreover a similar relief from pain is often obtained in acute polyarthritis. Some preliminary studies have been initiated with the combination of sodium thiosulphate and the sulfonamide derivatives in subacute bacterial endocarditis although no opinion has been formed concerning the possible efficiency. Passing allusion should be made to one interesting case of a patient who developed a traumatic cellulitis of the leg during the period of prolonged clotting time after sodium thiosulphate; multiple incision and drainage was performed without difficulty from bleeding. The same observation has been made in conjunction with heparin therapy.

Summary

In certain concentrations sodium thiosulphate prolonged clotting *in vivo* and *in vitro*. The delay is definite, protracted and is not accompanied by untoward effects providing fresh solutions are given slowly. The amount necessary to elicit this effect appears to be much larger than previously anticipated.

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204 thiosulfate

ACTION DE L'HYPOSULFITE DE SOUDE SUR LE CANCER AU MÉTHYLCHOLANTHRÈNE CHEZ LE RAT

par J. et Mlle J. Loeper

Le méthylcholanthrène parmi les carbures cancérigènes a toujours particulièrement attiré l'attention des médecins. Il joint en effet à sa grande activité, la propriété d'avoir des rapports étroits avec des constituants importants de l'organisme humain, les acides biliaires. Rappelons que le méthylcholanthrène a été synthétisé par Fieser et Neuman à partir de l'acide cholique, et à partir de l'acide désoxycholique par Wieland et par Cook.

Nous n'avons pas l'intention de passer en revue ici les très nombreux travaux auxquels il a donné lieu.

Son pouvoir cancérigène dépend du lieu d'introduction, de l'animal choisi, de la quantité injectée, enfin du solvant. Tous ces points ont été parfaitement établis et lorsque ces facteurs sont identiques le pourcentage de cancérisation est très voisin d'un expérimentateur à l'autre.

Nous insisterons davantage sur deux considérations qui ont orienté nos recherches. Le méthylcholanthrène comme d'autres carbures cancérigènes s'élimine par la bile ainsi que l'ont montré Peacock et Chalmers (1 et 2) ; Il est par ailleurs certain que le système endocrinien, bien qu'il s'agisse d'un cancer chimique, ne reste pas indifférent au processus de cancérisation, soit qu'on envisage le problème sous l'angle de l'influence des différentes glandes endocrines et de leurs hormones sur la cancérisation ; soit sous l'angle de l'influence du carbure cancérigène sur les différents parenchymes glandulaires.

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Nous avons recherché, depuis deux ans, l'effet de certains dérivés soufrés sur le cancer provoqué par le méthylcholanthrène, en étudiant en particulier ces deux facteurs : l'élimination biliaire et les réactions endocriniennes. Nous voudrions donner ici brièvement nos résultats et les déductions que nous pouvons en tirer.

Le soufre dans le développement des cancers expérimentaux a été assez largement étudié, et son rôle dans la plupart des cas s'est avéré néfaste.

C'est ainsi que White (3) a constaté qu'un régime pauvre en cystine diminue la fréquence du cancer spontané chez la souris et que Crabtree (4) a vu que tout corps capable de fixer des groupes SH inhibait l'action d'un carbure cancérigène. Ainsi pourrait-on expliquer le pouvoir inhibiteur de carbures non cancérigènes qui pour s'éliminer fixent des groupes SH contenus dans la peau.

De même Brunschwig (5) a montré que le thioglycollate et le thiomalate stimulaient la croissance des tumeurs transplantables du rat, alors que l'injection d'inhibiteurs de groupes SH comme l'iodacétate, les aldéhydes maloniques et citraconiques retardaient la croissance de cette même tumeur.

Nous avons étudié deux corps soufrés, l'hyposulfite de soude et l'acide mercaptopyruvique, et dans ces expériences nous avons utilisé des rats blancs de même race, pesant 120 gr. et soumis à un régime alimentaire équilibré et identique.

1° L'acide mercaptopyruvique, dont la synthèse a été faite par J. Parrod, a été employé à la dose de 0 gr. 02 tous les 3 jours en injection chez des rats ayant reçu auparavant 2 mgr. de méthylcholanthrène par voie sous-cutanée. Nous n'avons pas obtenu ainsi de retard dans l'apparition des tumeurs qui nous ont paru de plus augmenter plus rapidement de taille. Nos constatations sont donc en rapport avec les observations des auteurs précités, puisque ce corps contient une fonction SH particulièrement labile.

2° L'hyposulfite de soude par contre nous a donné des résultats beaucoup plus intéressants.

Injecté à la dose de 0 gr. 01 ou 0 gr. 02 suivant les expériences tous les 3 jours, nous avons enregistré un

réel retard dans l'apparition des tumeurs par rapport aux témoins.

Les injections de méthylcholanthrène étaient de 1 mg. 5, 2 mg, 2 mg. 5, suivant les lots d'animaux.

Ainsi nous avons noté :

— Au bout de 2 mois :
67 % de tumeurs chez les témoins,
46 % chez les rats hyposulfités.

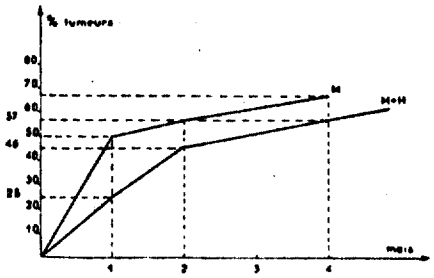
— Au bout de 4 mois :
70 % de tumeurs chez les témoins,
57 % chez les rats hyposulfités.

Voici, le détail de nos observations après 4 mois d'observation.

TABLEAU I

Rats recevant Méthylcholanthrène	Rats recevant Méthylcholanthrène + Hyposulfite	Doses M : Méthylcholanthrène H : Hyposulfite de soude
4 tumeurs sur 9 rats	1 tumeur sur 6 rats	M : 1 mgr. 5 H : 0 gr. 2 tous les 3 jours
5 tumeurs sur 12 rats	2 tumeurs sur 7 rats	M : 1 mgr. 5 H : 0 gr. 01 tous les 3 jours
13 tumeurs sur 13 rats	11 tumeurs sur 15 rats	M : 1 mgr. 5 H : 0 gr. 02 tous les 3 jours
7 tumeurs sur 9 rats	6 tumeurs sur 9 rats	M : 2 mgr. H : 0 gr. 02 tous les 4 jours
10 tumeurs sur 12 rats	10 tumeurs sur 15 rats	M : 2 mgr. 5 H : 0 gr. 2 tous les 6 jours

Comme on peut le remarquer d'après le tableau la distance comprise entre 2 injections d'hyposulfite (3 à 6 jours) ne paraît pas avoir une influence considérable.



Courbes du pourcentage des tumeurs chez les témoins (M) et les rats recevant de l'hyposulfite (H + M).

Nous voyons par contre que pour une quantité identique de méthylcholanthrène il existe des variations dans le pourcentage des tumeurs suivant les expériences ; mais celles-ci sont parallèles tant chez les témoins que chez les rats hyposulfités. Nous ne pouvons affirmer la raison de ces variations, nous signalerons pourtant que nos expériences ont été faites à des périodes différentes de l'année et que nous avons changé légèrement le lieu d'introduction ; le pourcentage des tumeurs étant plus élevé lorsque le méthylcholanthrène était injecté dans le tissu sous-cutané de la paroi thoracique, contre les espaces intercostaux.

Nous ajouterons encore que les tumeurs des rats hyposulfités sont identiques au point de vue histologique à celles des témoins et que la poursuite des injections d'hyposulfite chez un rat présentant une tumeur au début n'empêche pas son développement, que l'injection soit faite à distance ou au contact même de la tumeur.

Par contre, nous avons noté deux différences intéressantes. — Les tumeurs des rats hyposulfités sont moins fréquemment fluorescentes que celles des témoins (47 % des cas au lieu de 75 %). Comme la fluorescence est due à la présence de méthylcholanthrène, il est donc permis de supposer que sa concentralisation locale a diminué.

— Par ailleurs, les injections de méthylcholanthrène en suspension huileuse s'accompagnent souvent d'une réaction nodulaire précoce et passagère et nous avons remarqué que celle-ci était moins fréquente chez les rats recevant de l'hyposulfite. Peut-être cette absence de réaction initiale joue-t-elle un rôle important dans le retard d'apparition ultérieure des tumeurs ?

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Par quel mécanisme l'hyposulfite de soude provoque-t-il un retard dans l'apparition des tumeurs ? Telle est la question que nous avons essayé de résoudre.

I. — **Une première hypothèse** vient immédiatement à l'esprit : il est possible que l'hyposulfite de soude se combine au méthylcholanthrène et diminue ainsi son pouvoir cancérigène. L'on sait, en effet, depuis les travaux de Wood et Fieser (6) qu'il est possible de soufrer des carbures cancérigènes avec du chlorure de soufre ou du sulfure de cyanogène et que ces carbures ne sont plus cancérigènes. Dans notre cas particulier, nous n'avons pu vérifier ce phénomène, mais ce mécanisme nous semble cependant peu vraisemblable puisque l'acide mercaptopyruvique qui présente un soufre labile n'a pas diminué l'action cancérigène du méthylcholanthrène.

II. — Action de l'hyposulfite de soude sur l'élimination biliaire du méthylcholanthrène.

Nous avons surtout recherché si l'hyposulfite de soude ne favorisait pas l'élimination biliaire de cet hydrocarbure. Peacock et Chalmers (1 et 2) en particulier ont bien montré l'importance de la voie biliaire dans l'élimination des carbures cancérigènes. Injectée par voie veineuse, une suspension colloïdale de benzopyrène s'élimine abondamment dès la 15e minute par la bile, qui n'en contient plus que des traces au bout de 2 heures. Cette élimination se fait en grande partie sous forme phénolique (hydroxybenzopyrène) et c'est la raison pour laquelle la bile présente une fluorescence bleuâtre différente de la fluorescence dorée du benzopyrène.

Comme ce même processus peut être invoqué pour d'autres hydrocarbures cancérigènes, nous avons recherché chez le lapin l'influence de l'hyposulfite de soude sur l'élimination biliaire du méthylcholanthrène.

Deux groupes de cinq lapins ont été utilisés.

Après anesthésie et laparotomie, nous plaçions une

fine canule dans le cholédoque pour recueillir la bile, puis nous injections par voie veineuse 0 gr. 20 d'hyposulfite de soude en solution isotonique et quelques instants après, 5 mgr. de méthylcholanthrène par la même voie, en suspension colloïdale suivant le procédé de Boyland (7).

Les témoins recevaient seulement 5 mgr. de méthylcholanthrène. La bile était prélevée pendant 90 minutes et nous mesurions toutes les 20 minutes la quantité de bile évacuée et sa fluorescence.

Dans tous les cas, chez les animaux ayant reçu de l'hyposulfite de soude, le volume biliaire était augmenté ainsi que l'excrétion de méthylcholanthrène.

— soit simplement parce que le volume biliaire était accru, la concentration en méthylcholanthrène étant la même,

— soit, plus souvent, parce que sa concentration était fortement augmentée, surtout dans les deux premiers échantillons.

TABLEAU II

	M	M + H	M	M + H	M	M + H	M	M + H	M	M + H
Echantillon 1	+	+++	+	+++	+	+++	+	++	+	+
2	+	+	++	+++	+	++	++	++	++	++
3	+	+	++	++	+	+	++	+	+	+
4	+	+	+	+	+	+	+	+	+	+

Concentration en méthylcholanthrène dans la bile
(M = Méthylcholanthrène — H = Hyposulfite de soude)

En somme, l'hyposulfite de soude augmente la cholérèse et l'élimination du méthylcholanthrène.

Il nous paraît donc probable que la répétition des injections d'hyposulfite de soude en accroissant d'une façon permanente la cholérèse provoque une diminution plus rapide du méthylcholanthrène au niveau des tissus.

En effet, le mécanisme invoqué après une injection intraveineuse de carbure cancérigène est valable pour une injection sous-cutanée. Là encore, Chalmers et Crowfoot (8) ont montré que l'élimination était avant tout biliaire.

Cette explication cadre bien du reste avec le fait que chez les animaux hyposulfités les tumeurs sont moins souvent fluorescentes.

III. — Action de l'hyposulfite de soude sur les glandes endocrines.

Il est possible encore que l'hyposulfite de soude exerce son action retard par son influence sur le système endocrinien.

Il est évident que les glandes endocrines exercent leur effet favorable ou non dans le développement des cancers d'origine chimique.

C'est ainsi par exemple que Smith (9) et ses collaborateurs ont montré que les hormones gonadotropes accélèrent le développement des tumeurs, que l'hypophysectomie [Moon et collaborateurs (10)] empêche le développement des tumeurs dues au méthylcholanthrène ; que les extraits surrénaux [Arloing et coll. (11), puis Diller et coll. (12)] présentent une action frénatrice sur le cancer greffé.

Pour juger d'une façon exacte l'action de l'hyposulfite sur le système glandulaire nous avons comparé nos résultats avec ceux que l'on obtient par injection de méthylcholanthrène seulement. Nous avons uniquement étudié les *surrénales*, la *thyroïde* et *l'hypophyse*.

a) SURRÉNALE.

Benelli (13), puis Geyer (14), en 1936, notaient une diminution des enclaves lipidiques de la zone fasciculée avec disparition de la zone X chez les animaux atteints de cancer au méthylcholanthrène. Plus récemment, Fleming, Walters et Williams (15) enregistraient une hyperactivité cortico-surrénale dans les mêmes conditions, avec apparition de cellules d'un type particulier aussi bien dans la zone glomérulée que dans la fasciculée.

Dans notre expérimentation, nous avons noté que les rats injectés seulement *avec du méthylcholanthrène* présentaient une hypertrophie modérée des surrénales (12 %), significative du point de vue statistique et portant sur la corticale. Nous n'avons pas retrouvé de cellules d'un type particulier, mais parfois des lésions congestives ou hémorragiques ; d'autre part les enclaves lipidiques de la zone fasciculée ne subissent pas de variations appréciables.

Nous avons recherché des stigmates biologiques d'un hyperfonctionnement surrénal, mais nous n'en avons pas trouvé de preuve formelle. Si la teneur en acide ascorbique de la surrénale s'abaisse de 12 %, par contre l'élimination des 17-cétostéroïdes n'augmente pas sauf au début.

Au contraire, chez les rats hyposulfités l'hyperfonctionnement surrénal est net. Le poids est plus augmenté (19 %), la teneur en acide ascorbique s'abaisse de 28 % et l'élimination des 17-cétostéroïdes est constamment accrue.

b) THYROÏDE.

Les carbures cancérigènes comme le méthylcholanthrène provoquent une hyperplasie thyroïdienne caractérisée par une augmentation de poids, une réduction de la colloïde et le développement d'un épithélium cubique (Geyer et Benelli).

Nous avons retrouvé une hyperplasie considérable de l'ordre de 50 %, d'installation progressive avec forte diminution de la colloïde et assez inconstamment une augmentation de la taille des cellules bordantes.

Par contre, chez les animaux hyposulfités, nous n'avons pas enregistré d'hypertrophie thyroïdienne, bien que l'aspect histologique soit identique au précédent.

c) HYPOPHYSE.

Nous avons noté que sous l'effet du méthylcholanthrène se produit une hypertrophie hypophysaire modérée (14 %), mais valable statistiquement, avec une légère augmentation des cellules basophiles.

Avec l'hyposulfite de soude, l'hypertrophie est un peu plus considérable (18 %) avec une semblable augmentation des cellules basophiles ; quelques-unes d'entre

	Surrénale Poids (moyen)		Acide ascorbique (moyenne)		Thyroïde Poids (moyen)		Hypophyse Poids (moyen)		17 cétostéroïdes (par 10l)		
	N.		N.		N.		N.				
Témoins	17	16 ± 0,48*	7	434 ± 35	7	82 ± 4	9	6 ± 0,2	3 γ	6 γ	11 γ
Méthylcholanthrène	12	18,2 ± 0,77	9	384 ± 17	10	76 ± 8	11	6,9 ± 0,3	12 γ	6 γ	10 γ
Méthylcholanthrène + hyposulfite	11	19,4 ± 0,72	6	330 ± 1	11	52 ± 4	12	7,3 ± 0,3	10 γ	15 γ	20 γ

N. = Nombre de rats.
* = Erreur standard.

elles présentent un aspect d'épuisement (présence de bâtonnets acidophiles dans le cytoplasme).

Nous ne voulons pas discuter ici la signification des modifications de la thyroïde sous l'effet du méthylcholanthrène et de l'hyposulfite de soude, car nous ne pouvons affirmer que la réaction thyroïdienne au carbure cancérigène soit la marque d'un hyperfonctionnement effectif. Par contre, nous insistons sur le fait que l'hyposulfite provoque un *hyperfonctionnement surrénal, vraisemblablement secondaire à une stimulation antéhypophysaire.*

Nous avons signalé des faits analogues au Congrès de Thérapeutique de Rome, avec l'hyposulfite de soude seul, le thiofène et les eaux sulfatées calciques, où nous avons noté une hypertrophie cortico-surrénale, une chute de l'acide ascorbique, une hypertrophie hypophysaire avec quelques cellules basophiles en état d'épuisement.

Comme par ailleurs, Arloing, Morel et Josserand (11), Diller et coll. (12), Smith et coll. (9) ont montré l'action frénatrice des extraits surrénaux sur le cancer greffé, que Driesse et Clay (16) ont obtenu une régression du sarcome de Crocker avec l'ACTH. Il parait donc possible que l'hyperfonctionnement surrénal soit une des causes du retard provoqué par l'hyposulfite de soude dans le développement des tumeurs au méthylcholanthrène.

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En conclusion, l'hyposulfite de soude provoque un retard dans l'apparition des tumeurs dues au méthylcholanthrène chez le rat. A l'origine de cette action nous avons pu mettre en évidence deux facteurs qui ne sont peut-être pas les seuls : une élimination biliaire accrue du carbure cancérigène, un hyperfonctionnement corticosurrénal. Le problème de l'élimination biliaire nous parait particulièrement intéressant. Nous croyons pour notre part qu'il serait utile de rechercher l'effet de substances fortement cholérétiques sur l'élimination des hydrocarbures cancérigènes et leur influence sur les processus de cancérisation secondaire. L'étude de ce problème, qui ne parait pas avoir été envisagée aussi largement que d'autres, aurait peut-être des conséquences thérapeutiques intéressantes.

Travail du Laboratoire de Pathologie expérimentale (Professeur J. LEXÈGRE) *et de la clinique médicale de St-Antoine* (Professeur A. LEMAIRE).

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« Grâce à l'Index Analyticus semestriel et grâce à l'Index placé en tête de chaque numéro, tout article publié dans n'importe quel périodique français ou étranger est rapidement retrouvé. La Semaine des Hôpitaux *a résolu le problème d'être à la fois complète et vite consultée ».*

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thiosulfate

THE ACTION OF SODIUM HYPOSULFITE IN METHYLCHOLANTHRENE INDUCED CANCER IN THE RAT

BY J. and J. Loeper

Among the carcinogenic hydrocarbons, methylcholanthrene was always of particular interest to physicians. This was due to the extensive activity of this compound, its property of having very direct relationships with the important constituents of the human organism, the bile acids. We would like to mention that methylcholanthrene was synthesized by Fieser and Neuman from cholic acid; it was also synthesized by Wieland and Cook from desoxycholic acid.

We do not intend to review at this point the number of studies that have been devoted to this compound.

The carcinogenic ability of methylcholanthrene within the animal system used, depends on the quantity of the compound injected, the site at which it is being introduced, and also on the solvent. All these factors have been well established and when they are all the same, the percentage of tumors produced is very similar from one experiment to another.

Furthermore, we would like to emphasize two considerations that have directed our research. As is true for the other carcinogenic hydrocarbons, methylcholanthrene is eliminated by the bile, as demonstrated by Peacock and Chalmers (1 and 2); it is also certain that although a chemical cancer is being induced, the endocrine system does not remain unresponsive to the process of carcinogenesis, regardless of whether the problem is regarded from the effect of the different endocrine glands and their hormones on the carcinogenesis or from the

effect of the carcinogenic hydrocarbon on the different parenchymal glands.

For the past two years we have studied the effect of certain sulfur derivatives on the cancer induced by methylcholanthrene; we studied two factors in particular: the biliary elimination and the endocrine reactions. We would like to present briefly our results and the conclusions which we have derived from these studies.

The role of sulfur in the development of experimental cancers has been studied quite extensively and in the majority of cases this chemical was found to have negligent effects.

White (3) discovered that a diet poor in cystine decreased the frequency of spontaneous cancers induced in mice; Crabtree (4) demonstrated that all the compounds capable of fixing sulfhydryl groups (SH) inhibit the action of carcinogenic hydrocarbons. Thus, it was possible to explain the inhibitory capacity of noncarcinogenic hydrocarbons which could be eliminated by combining with the SH groups present in the skin.

Moreover, Brunschwig (5) showed that thioglycolate and thiomalate stimulate the growth of transplantable tumors in rats; in contrast injection of inhibitors of SH groups such as iodacetate, malonic aldehydes, citraconic aldehydes, delay the growth of this same type of tumor.

We ourselves have studied two sulfur compounds: sodium hyposulfite and mercaptopyruvic acid. In our experiments we utilized white rats of the same strain, and weighing 120 grams; the animals were fed balanced and yet identical diet.

1) Mercaptopyruvic acid, as synthesized by J. Parrod, was

administered in a dose of 0.02 g every three days in injected form to rats who had previously received two milligrams of methylcholanthrene by the subcutaneous route. We did not obtain a delay in the appearance of the tumors which, in our opinion, appeared to increase more rapidly in size. Our findings are therefore in agreement with the observations of the previously mentioned authors, since these compounds contain a particularly labile SH function.

2) On the other hand, sodium hyposulfite produced much more interesting results.

When injected in a dose of 0.01 or 0.02 g every three days, depending on the experimental group, the compound produced an actual delay in the appearance of the tumors as compared to controls.

The injections of methylcholanthrene were 1.5 mg, 2 mg, and 2.5 mg, depending on the animal lot.

Thus, we observed the following results:

- at the end of two months of treatment:
  - 67% tumors in controls
  - 46% tumors in rats treated with sodium hyposulfite.

at the end of four months:

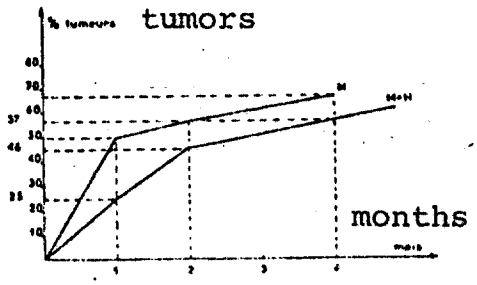
  - 70% tumors in controls
  - 57% tumors in rats treated with sodium hyposulfite.

The following table includes the details observed after four months of the experiment:

TABLE I

Rats receiving methylcholanthrene	Rats receiving methylcholanthrene + sodium hyposulfite	Doses M: methylcholanthrene H: sodium hyposulfite
4 tumors out of 9 rats	1 tumor out of 6 rats	M: 1.5 mg H: 0.2 g every three days
5 tumors out of 12 rats	2 tumors out of 7 rats	M: 1.5 mg H: 0.01 g every three days
13 tumors out of 13 rats	11 tumors out of 15 rats	M: 1.5 mg H: 0.02 g every three days
7 tumors out of 9 rats	6 tumors out of 9 rats	M: 2 mg H; 0.02 g every four days
10 tumors out of 12 rats	10 tumors out of 15 rats	M: 2.5 mg H: 0.2 g every six days

As can be seen from the data in the table the time between the two injections of sodium hyposulfite (three to six days) could not have any significant effect.



Graph showing the percentage of tumors in controls (M) and in rats receiving hyposulfite (H+M)

We do see, however, that for an identical quantity of methylcholanthrene, there are variations in the percentage of tumors produced in the different experimental groups; however, the latter differences appear to be parallel in the controls and in the rats treated with sodium hyposulfite. We were not able to confirm the reason for these differences; however, we would like to mention that our experiments were carried out at different periods of the year and that the site of the injection varied slightly; the percentage of tumors was higher when methylcholanthrene was injected into subcutaneous tissue within the thoracic wall as compared to when it was administered at the intercostal space.

We would like to add that the tumors in rats treated with sodium hyposulfite were identical from the histological point of view to the tumors produced in controls; subsequent injections of sodium hyposulfite in rats already having the onset of tumors did not prevent the neoplasm from developing, regardless of whether the hyposulfite was injected at some distance or in contact with the tumor.

In contrast, we did observe two interesting differences: the tumors of rats treated with sodium hyposulfite were less frequently fluorescent than were the tumors in controls (47% as compared to 75% of the cases). Since the fluorescence is due to the presence of methylcholanthrene, it is conceivable to assume that its local concentration was decreased.

Furthermore, the injections of methylcholanthrene were in the form of an oily suspension and very often were accompanied by an early but transient nodular reaction; we observed that

this reaction was less frequent in rats treated with sodium hyposulfite. Perhaps the absence of this initial nodular reaction plays an important role in delaying the subsequent appearance of the tumors?

What is the mechanism by which sodium hyposulfite delays the onset of tumors? It is this question that we have attempted to answer.

I.- The first hypothesis, comes immediately to mind; it is possible that sodium hyposulfite combines with methylcholanthrene and thus decreases the carcinogenic potency of the latter. In the studies conducted by Wood and Fieser (6), it was shown that it is possible for the sulfur of carcinogenic hydrocarbons to combine with sulfur chloride or with cyanic sulfur and thus, the hydrocarbons are no longer carcinogenic. In our particular case, we were not able to verify this phenomenon; however, this mechanism did not appear to be very plausible since mercaptopyruvic acid which contains a labile sulfur group does not decrease the carcinogenic action of methylcholanthrene.

II.- The effect of sodium hyposulfite on the biliary excretion of methylcholanthrene. We have attempted to discover if sodium hyposulfite favors the biliary excretion of this hydrocarbon. Peacock and Chalmers (1 and 2) were successful in demonstrating the importance of the biliary tract in eliminating carcinogenic hydrocarbons. When injected into a vein, a colloidal suspension of benzpyrene is rapidly eliminated with 15 minutes by the bile and at the end of two hours, only trace amounts of the hydrocarbon are present. The excretion is mostly in the form of a phenol (hydroxybenzopyrene) and it is because of this fact that the

bile had a bluish fluorescence which differs from the gold fluorescence of benzpyrene.

Since these same processes may be involved in the case of other carcinogenic hydrocarbons, we attempted to study the effect of sodium hyposulfite on the biliary excretion of methylcholanthrene in the rabbit.

Two groups of five rabbits each were used.

Following the administration of anesthesia and laparotomy, a fine catheter was placed within the bile duct to collect the bile; then using the intravenous route, 0.20 grams of sodium hyposulfite in the form of an isotonic solution were injected; several seconds later, 5 mg of methylcholanthrene were injected by the same route in the form of a colloidal suspension, according to the process described by Boyland (7).

The control rabbits received only 5 mg of methylcholanthrene. The bile was collected for 90 minutes and then every 20 minutes. The quantity of bile evacuated was measured along with the degree of fluorescence.

In all cases where the animals received sodium hyposulfite, the biliary volume was increased as was the excretion of methylcholanthrene.

- either simply due to the fact that the bile volume was increased, but the concentration of methylcholanthrene was the same;

- or, more often, because the concentration was markedly increased, especially in the first two samples.

TABLE II

Sample	M	M + H	M	M + H	M	M + H	M	M + H	M	M + H
1	+	+++	+	+++	+	+++	+	++	+	+
2	+	+	++	+++	+	++	++	++	++	++
3	+	+	++	++	+	+	++	+	+	+
4	+	+	+	+	+	+	+	+	+	+

Concentration of methylcholanthrene in the bile
(M = methylcholanthrene; H = sodium hyposulfite)

In general, sodium hyposulfite increased choleresis and the elimination of methylcholanthrene.

Thus, it appeared probable that the repetition of the sodium hyposulfite injections, by increasing permanently the choleresis, produced a more rapid decrease of methylcholanthrene at the level of the tissues.

Thus, the mechanism involved following an intravenous injection of carcinogenic hydrocarbon is also valid for a subcutaneous injection. Here again, Chalmers and Crowfoot (8) showed that the excretion is biliary.

This explanation is also in agreement with the fact that in the animals treated with sodium hyposulfite the tumors were less often fluorescent.

III.- The effect of sodium hyposulfite on the endocrine glands

It is possible that sodium hyposulfite exerts its delaying action on tumors by affecting the endocrine system.

It is evident that the endocrine glands exert either a favorable or an unfavorable effect on the development of

chemically induced cancers.

Thus for example, Smith et al. (9) demonstrated that gonadotropic hormones accelerate the development of tumors; Moon et al. (10) showed that hypophysectomy prevented the development of tumors induced by methylcholanthrene; adrenal extracts also had an effect (Arloing et al. (11); then Diller et al. (12) showed that adrenal extracts have an inhibitory effect on transplantable tumors.

In order to determine the action of sodium hyposulfite on the glandular system, we compared the results that we obtained with those resulting from only an injection of methylcholanthrene. We have limited our study to the adrenals, thyroid and pituitary.

a) Adrenals

In 1936, Benelli (13) and then Geyer (14) observed that there was a decrease of the lipid boundaries within the fascicular zone, with a disappearance of zone X in the animals with methylcholanthrene induced cancer. More recently, Fleming, Walters and Williams (15) reported hyperactivity of the adrenal cortex under the same conditions, with the appearance of cells of a particular type both in the glomerular zone and in the fascicular zone.

In our experiments, we observed that the rats receiving only methylcholanthrene injections presented moderate hypertrophy of the adrenals (12%); it was significant from the statistical point of view and involved only the cortical layer. We did not find cells of any one particular type, but sometimes we did observe congestive or hemorrhagic type lesions. Furthermore, the lipid recesses within the fascicular zone did not undergo

any appreciable variations.

We did look for signs of adrenal hyperfunctioning, but we could not find any conclusive proof. The content of ascorbic acid in the adrenals decreased 12%; however, the excretion of 17-ketosteroids did not increase except at the very beginning.

In contrast, in the rats treated with sodium hyposulfite, there was definite adrenal hyperfunctioning. The weight was increased (19 per cent), the ascorbic acid content decreased 28 per cent and the elimination of 17-ketosteroids was constantly higher.

b) Thyroid

Methylcholanthrene and other carcinogenic hydrocarbons produce hyperplasia of the thyroid characterized by an increase in organ weight, a decrease in colloid and the development of cuboidal epithelium (Geyer and Benelli).

We did observe considerable hyperplasia, of the order of 50 per cent, that progressively increased with a marked decrease in colloid content and a quite variable increase in the size of the bordering cells.

On the other hand, the animals treated with sodium hyposulfite, did not manifest any thyroid hypertrophy, although the histological aspects of the thyroid in these animals were identical to that of the previous group.

c) Pituitary

Moderate (14%) pituitary hypertrophy was produced by the administration of methylcholanthrene; however, it was statistically significant; there was a slight increase in the amount of

basophilic cells.

Sodium hyposulfite produced a slight more considerable (18 per cent) hypertrophy of the pituitary with a similar increase in the number of basophilic cells; some of these cells appeared thickened (presence of rodlike acidophilic cells in the cytoplasm).

	Adrenal weight (average)		Ascorbic acid (average)		Thyroid weight (avrg.)		Pituitary weight (average)		17-keto-steroids (per rat)		
1 Témoins	N. 17	16 ± 0,48*	N. 7	434 ± 35	N. 7	52 ± 4	N. 9	6 ± 0,2	3 γ	6 γ	11 γ
Méthylcholanthrène	12	18,[illegible] ± 0,77	9	384 ± 17	10	76 ± 8	11	6,9 ± 0,3	17 γ	6 γ	10 γ
Méthylcholanthrène + hyposulfite	11	19,4 ± 0,72	6	300 ± 1	11	52 ± 4	12	7,3 ± 0,3	10 γ	15 γ	20 γ

1 = Controls

N = number of rats

* = standard error

We do not want to discuss at this point the significance of these changes in the thyroid produced by methylcholanthrene, and sodium hyposulfite, because we cannot verify if these changes were the result of a thyroid reaction to carcinogenic hydrocarbons or the result of hyperfunctioning of the gland. In contrast, we do emphasize that hyposulfite does induce adrenal hyperfunctioning, probably secondary to a stimulation of the pituitary.

We have reported similar findings at the Therapeutic Congress in Rome; sodium hyposulfite, thiophene, sulfated calcium waters, produced hypertrophy of the adrenal cortex, a decrease in the ascorbic acid content, hypertrophy of the pituitary with several

basophilic cells in a thickened state.

Furthermore, such authors as Arloing, Morel and Josserand (11), Diller et al. (12), Smith et al. (9) have demonstrated the inhibitory action of adrenal extracts on transplantable cancers; Driesse and Clay (16) obtained regression of Crocker's sarcoma with ACTH. Therefore, it appears possible that adrenal hyperfunctioning may be one of the causes responsible for the delay in tumor growth produced by hyposulfite in the development of methylcholanthrene induced tumors.

In conclusion: sodium hyposulfite produces a delay in the appearance of methylcholanthrene induced tumors in rats. The reason for this action may be due, possibly to two factors, which, however, may not be the only factors responsible for this delay: an increase in the biliary excretion of carcinogenic hydrocarbons and hyperfunctioning of the adrenal cortical system. The problem of biliary excretion appears to be of particular interest. We believe that it would prove worthwhile to study the effect of strongly choleretic substances on the excretion of carcinogenic hydrocarbons and their effects on the secondary carcinogenesis processes. A study of this problem which has not yet been as broadly studied as certain other aspects, may have interesting therapeutic results.

Laboratory of Experimental Pathology
(Professor J. Lenegre)
and the Medical Clinic of St. Antoine
(Professor A. Lemaire).

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tefois, comme dans le sang de cordon la proportion d'hémoglobine fœtale est en moyenne de 75 pour cent, la quantité totale de fer incorporé reste plus élevée dans l'hémoglobine fœtale que dans l'hémoglobine adulte.

Résumé. — L'incorporation du fer radioactif dans l'hémoglobine du sang de cordon s'effectue de façon hétérogène. La séparation des hémoglobines adulte et fœtale par dénaturation alcaline ménagée montre que la fraction adulte incorpore le fer plus rapidement que la fraction fœtale. L'activité métabolique élevée du sang de cordon *in vitro* par rapport au sang d'adulte n'est donc pas due à la présence d'hémoglobine fœtale (*).

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Thiosulfate

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Action de l'hyposulfite de soude et de l'acide mercaptopyruvique sur le développement des tumeurs au méthylcholanthrène chez le Rat.

par Jacques Loeper et Jacqueline Loeper.

Le rôle du soufre dans le développement des cancers expérimentaux a déjà été largement étudié et dans la plupart des cas, cette action s'est avérée néfaste.

C'est ainsi que J. White (1*) et 2*) a constaté qu'un régime pauvre en cystine diminue la fréquence du cancer spontané chez la Souris, de même H. Grabtree (3*) a montré que tout corps capable de fixer des groupes SH inhibait l'action d'un hydrocarbure cancérigène ; ainsi s'expliquerait le pouvoir inhibiteur de carbures non cancérigènes qui, pour s'éliminer, fixent des groupes SH contenus dans la peau. D'autre part, A. Brunschwig (4*) a vu que le thioglycolate et le thiomalate stimulaient la croissance des tumeurs transplantables du Rat alors que l'injection d'inhibiteurs de groupes SH comme l'iodacétate, les aldéhydes maloniques et citraconiques retardaient la croissance de cette même tumeur. Seul, F. Hammeth (5*) a trouvé que la méthionine retardait l'apparition de tumeurs spontanées chez la Souris encore que les métastases soient apparues ensuite plus rapidement. Nous avons étudié 2 corps soufrés : l'hyposulfite de soude et l'acide mercaptopyruvique.

Dans cette expérience, nous avons utilisé des rats blancs de même

(*) Nous remercions l'Institut national d'Hygiène, et la Caisse nationale de Sécurité sociale de l'aide apportée à ce travail.

(1*) J. White et G. B. Mider, *Journ. Nation. Cancer Institut*, 1941, t. [illegible], p. 95.

(2*) F. B. White et J. White, *Journ. Nation. Cancer Institut*, 1944, t. 5, p. 413.

(3*) H. G. Grabtree, *Cancer research*, 1945, t. 5, p. 346 ; *Cancer research*, 1946, t. 6, p. 553.

(4*) A. Brunchwig, J. Arnold et J. Edgcomb, *Cancer research*, 1946, t. 6, p. 560.

(5*) F. Hammeth (F.), *Growth*, 1941, t. 5, p. 85.

C.R. Soc. Biol., 148, 1954

race pesant 120 g et soumis à un régime alimentaire équilibré et identique. Voici nos résultats :

1°) *L'acide mercaptopyruvique* ne nous a pas donné de résultats intéressants et nos constatations sont en accord avec les observations des auteurs que nous venons de citer puisque ce corps contient une fonction SH. Nous l'avons utilisé à la dose de 0,02 g tous les trois jours en injection chez des rats ayant reçu 2 mg de méthylcholanthrène par voie sous-cutanée. Nous n'avons pas obtenu de retard dans l'apparition des tumeurs qui nous ont paru augmenter plus rapidement de volume.

2°) *Action de l'hyposulfite de soude.* L'hyposulfite de soude était injecté par voie sous-cutanée à la dose de 0,01 g ou 0,02 g tous les 3 ou 6 jours suivant les expériences. Le méthylcholanthrène était injecté par voie sous-cutanée au niveau du flanc droit à la base du thorax à la dose, soit de 1,5 mg, 2 mg, 2,5 mg par animal suivant les cas. Les rats témoins recevaient la même dose de méthylcholanthrène sans hyposulfite. 5 lots d'animaux furent ainsi traités.

Rats recevant Méthylcholanthrène	Rats recevant Méthylcholanthrène + Hyposulfite	Doses
4 tumeurs sur 9 rats	1 tumeur sur 6 rats	M : 1,5 mg H : 0,02 g tous les 3 jours
5 tumeurs sur 12 rats	2 tumeurs sur 7 rats	M : 1,5 mg H : 0,01 g tous les 3 jours
13 tumeurs sur 13 rats	11 tumeurs sur 15 rats	M : 1,5 mg H : 0,02 g tous les 3 jours
7 tumeurs sur 9 rats	6 tumeurs sur 9 rats	M : 2 mg H : 0,02 g tous les 4 jours
10 tumeurs sur 12 rats	10 tumeurs sur 15 rats	M : 2,5 mg H : 0,02 g tous les 6 jours

Dans l'ensemble, nous avons enregistré un réel retard dans l'apparition des tumeurs chez les rats hyposulfités. En comparant nos animaux 4 mois après l'injection initiale de méthylcholanthrène, nous notons : 70 % de tumeurs chez les rats témoins, 57 % des tumeurs chez les rats recevant de l'hyposulfite. Il est à remarquer que les doses de méthylcholanthrène injecté sont fortes, donc que la durée de 4 mois d'attente est longue.

Si nous faisons nos observations au bout de 2 mois, nous notons une différence plus importante entre les rats hyposulfités et les témoins. 67 % de tumeurs chez les témoins, 46 % de tumeurs chez les rats recevant de l'hyposulfite. Le tableau donne le détail de nos observations après 4 mois.

Comme on peut le remarquer, d'après le tableau, la distance comprise entre 2 injections d'hyposulfite (3 à 6 jours) ne paraît pas avoir une influence considérable.

Nous voyons par contre que pour des quantités identiques de méthylcholanthrène existent des variations importantes dans le pourcentage des tumeurs tant chez les témoins que chez les rats hyposulfités ; fait intéressant, ces variations sont du reste assez parallèles dans ces deux lots. Nous ignorons la raison de ces variations, nous mentionnerons pourtant que nos expériences ont été faites à des périodes différentes de l'année et que nous avions varié légèrement le lieu d'injection sous-cutanée du méthylcholanthrène.

Nous avons de plus fait les constatations suivantes : L'aspect des tumeurs des rats ayant reçu en supplément de l'hyposulfite sont identiques au point de vue histologique à celles des témoins ; nous avons pourtant remarqué qu'elles étaient moins fréquemment fluorescentes (47 % de cas au lieu de 75 % chez les témoins). Par ailleurs, la poursuite des injections d'hyposulfite chez un animal présentant une tumeur au début n'inhibe pas son développement que l'injection soit faite à distance ou au contact même de la tumeur.

Nous avons enfin remarqué que les injections de méthylcholanthrène en suspension huileuse s'accompagnaient souvent d'une réaction nodulaire précoce et passagère qui était moins fréquente chez les rats hyposulfités — peut-être cette réaction initiale est-elle importante dans l'évolution intérieure des lésions.

En conclusion, l'hyposulfite de soude, à l'opposé d'autres dérivés soufrés, comme l'acide mercaptopyruvique, provoque un retard dans l'apparition du cancer expérimental au méthylcholanthrène chez le Rat et les tumeurs dans ce cas sont moins fréquemment fluorescentes que chez les témoins. Le point intéressant est évidemment de connaître la cause de ce retard. Nous nous proposons, dans une prochaine note, de discuter le mécanisme de cette action.

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Action de l'yohimbine sur l'hyperkaliémie adrénalinique.

par Jean Delga et René Hazard.

Il est connu depuis 1925 (1) que l'yohimbine inverse chez le Chien l'action hypertensive de l'adrénaline. D'autre part, elle inhibe chez le Lapin la forte action hyperglycémiante de l'hormone (2), tandis que cette inhibition n'est que peu ou pas du tout marquée chez le Chien (3). Enfin, de tout un ensemble de recherches portant sur des

(1) Raymond-Hamet, *C. R. Acad. Sc.*, 1925, t. *180*, p. 2071.
(2) Sten Hanson, *C. R. Soc. Biol.*, 1929, t. *101*, p. 603.
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Comptes Rendus Soc. Biol. 148: 1800-1802 (1954).

THE ACTION OF SODIUM HYPOSULFITE AND MERCAPTOPYRUVIC ACID IN THE DEVELOPMENT OF METHYLCHOLANTHRENE INDUCED TUMORS IN THE RAT

by Jacques Loeper and Jacqueline Loeper

The role of sulfur in the development of experimental cancers has been studied quite extensively and in the majority of cases, this chemical was found to have negligent effects.

Investigator J. White (1, 2) found that a diet poor in cystine decreased the frequency of spontaneous cancers in mice. Moreover, H. Crabtree (3) showed that all compounds capable of fixing SH groups inhibit the action of carcinogenic hydrocarbons; thus, this action would explain the inhibitory action of non-carcinogenic hydrocarbons that are eliminated by combining with the SH groups present in the skin. On the other hand, A. Brunschwig (4) observed that thioglycolate and thiomalate stimulate the growth of transplantable tumors in rats, while such compounds as malonic aldehydes and citraconic aldehydes retard the growth of this same tumor. Only F. Hammeth (5) observed that methionine delays the appearance of spontaneous tumors in mice while, in contrast, metastases appear more rapidly.

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(1) J. White and G. B. Mider, Journ. Nation. Cancer Institut, 1944, t. 2, p. 95.

(2) F. R. White and J. White. Journ. Nation. Cancer Institut, 1944, t. 4, p. 413.

(3) H. G. Crabtree. Cancer research, 1945, t. 5, p. 346; Cancer research, 1946, t. 6, p. 553.

(4) A. Brunchwig, J. Arnold and J. Edgcomb. Cancer research, 1946, t. 6, p. 560.

(5) F. Hammeth, Growth, 1944, t. 5, p. 85.

we studied two sulfuric compounds: sodium hyposulfite and mercaptopyruvic acid.

In our experiments we used white rats of the same strain weighing 120 grams; the animals were fed the same diet. The following results were obtained:

1) Mercaptopyruvic acid did not produce any interesting results and our observations are in agreement with those of other authors whom we have cited since this compound contains an SH function. We used a dose of 0.02 grams every third day in the form of an injection administered to rats who had received 2 mg of methylcholanthrene subcutaneously. We did not observe any delay in the appearance of the tumors which appeared to us to increase more rapidly in volume.

2) The action of sodium hyposulfite. Sodium hyposulfite was injected subcutaneously in a dose of 0.1 grams or 0.02 grams every three or six days in these experiments. Methylcholanthrene was injected subcutaneously at the level of the right side near the base of the thorax in either doses of 1.5 mg, 2 mg, or 2.5 mg per animal. The control rats received the same dose of methylcholanthrene without any hyposulfite. Five lots of animals were treated in this way.

Overall we observed a genuine delay in the appearance of the tumors in the rats treated with hyposulfite. In comparing the animals four months after the initial injection of methylcholanthrene we observed the following: 70 per cent of tumors in the control rats; 57 per cent tumors in the rats receiving sodium hyposulfite. It should be emphasized that the doses of

injected methylcholanthrene were high and the duration of a four month wait quite long.

Rats receiving methylcholanthrene	Rats receiving methylcholanthrene + hyposulfite	Doses
4 tumors out of 9 rats	1 tumor out of 6 rats	M: 1.5 mg H: 0.02 mg every three days
5 tumors out of 12 rats	2 tumors out of 7 rats	M: 1.5 mg H: 0.01 g every three days
13 tumors out of 13 rats	11 tumors out of 15 rats	M: 1.5 mg H: 0.02 g every three days
7 tumors out of 9 rats	6 tumors out of 9 rats	M: 2 mg H: 0.02 g every four days
10 tumors out of 12 rats	10 tumors out of 15 rats	M: 2.5 mg H: 0.02 g every six days

The observations made at the end of two months showed a more significant difference between the rats receiving sodium hyposulfite and control animals. Sixty-seven per cent of the controls had tumors while on forty-six per cent of the rats receiving sodium hyposulfite developed tumors. The table presents the details of our observations after one month of study.

Based on the data presented in the table, it can be seen that the time between the two injections of sodium hyposulfite

(three and six days) did not seem to have any considerable effect.

We would like to emphasize that for identical quantities of methylcholanthrene there were important variations in the percentage of tumors as observed in control animals and in rats treated with sodium hyposulfite. One interesting fact is that the variations remained quite parallel in the two lots of animals. Although we shall ignore the reason for these variations, we would like to mention that our experiments were carried out at different periods of the year and that we varied slightly the location of the subcutaneous injection of methylcholanthrene.

Furthermore, we would like to state the following facts: the aspect of the tumors in rats receiving sodium hyposulfite treatment were identical from the histological point of view to the tumors observed in controls; we would like to note however, that they were frequently less fluorescent (47 per cent in the treated animals as compared to 75 per cent in the controls). Furthermore, injections of sodium hyposulfite administered to animals who had initially presented tumors did not prevent the development of the neoplasms, regardless of whether the injection was made at some distance or in contact with the tumor.

A final fact that we would like to mention is that the injections of methylcholanthrene were an oil suspension and were often accompanied by an early and transient nodular reaction and this reaction was observed less frequently in the rats treated with sodium hyposulfite. Perhaps this initial skin reaction was important in the internal development of the neoplasms.

Conclusion: Sodium hyposulfite, as opposed to other sulfur derivatives, such as mercaptopyruvic acid, produces a delay in the appearance of experimental cancers induced with methylcholanthrene in rats; the tumors of the treated animals were less frequently fluorescent than that of controls. An interesting finding would be to discover the reason for this delay in tumor onset. In our next report we intend to discuss the mechanism of this action.

(Experimental and Comparative Pathological Laboratory:
M. J. Lenegre and the
Medical Clinic of the Saint-Antoine Hospital: M. A. Lemaire).

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The Non-specific Chemotherapy of Malaria

By S. LORANT, M.D.
(*From St. Ann's E.M.S. Hospital, Nottingham*)

THE scarcity of quinine that resulted from the Japanese occupation of Java and other quinine-producing countries together with the increased number of patients infected with malaria, made it highly desirable to explore other avenues of chemotherapy for this infection.

On reflection it was considered that not only organic compounds of simple constitution but some well-known inorganic salts might have valuable effects in these infections.

In spite of various efforts in research, the true nature of the quinine effect on the malarial infections remained, *de facto*, little known. Before the war much discussion arose as to whether quinine acts "directly" or "indirectly," i.e. whether it destroys the parasites wherever it finds them or whether the effect is due "to changes in the tissues" and/or "to mobilization of antibodies"? Many experiments were carried out in which parasite-infected blood was incubated together with antimalarial remedies, and after incubation injected into suitable animals or man, but by such experiments one could never decide whether the injected plasmodia were destroyed *before* the injection or *after* it "as a result of changes in the tissues."

Many intensive chemotherapeutic studies on quinine and other antimalarial remedies were made. These investigations have shown that molecular weight, stereo-configuration, the methoxy-group, the vinyl side-chain, &c., all have a modifying effect on quinine as an antimalarial remedy but only the bridge-hydroxyl between the quinoline and quinuclidine nuclei is absolutely necessary for the antimalarial effect.

Later research revealed effective compounds without the hydroxyl group. Like quinine they contained in their ring-systems elements, such as N, As, S, &c., with changeable valencies. Later still there emerged effective compounds in which the elements with changeable valencies were not built in ring-systems. To-day, it seems that only certain reduction-potential is necessary for the antimalarial effect.

During all these researches it was never claimed that antimalarial effectiveness meant a specific "direct" effect on the parasites themselves.

The existence of an indirect action is proved by the observation that malaria is a self-limiting infection. It is well known that not only do attacks of malaria cease spontaneously, but in many cases an immunity against malaria slowly develops.

As a result of treatment the malarial parasites disappear from the blood, but the reticulo-endothelial system retains them. With the disappearance of the infection from the blood the malarial paroxysms cease. If the parasites stay away long enough from the circulation, there is the possibility that slowly they will disappear from the tissues too, and with it ceases the danger of recrudescence of the infection. If the parasites continue their existence in the tissues, even in very small numbers, they appear from time to time in the circulating blood, and some plasmodia remain under favourable circumstances for years virulent enough to become the source of new exacerbations of the disease.

It is a striking fact that the very first effect of *all* the known antimalarial remedies is to

clear the circulation of the plasmodia, and relegate them to the tissues. How is this effect achieved? As the erythrocytes can move only passively with the circulating blood, it is clear that the tissues must be the agents which *selectively* filter off the erythrocytes. It has been long recognized that aged and physically- and chemically-injured red cells—almost foreign bodies in fact—become retained in the reticulo-endothelial system. The abnormal shape and size of the red blood corpuscles equally promote their retention in the specific tissues.

Red blood cells containing parasites are certainly altered in their physical condition. It is commonplace to assume that such cells are more easily and *selectively* attacked by various agents than other, normal cells. It is also logical to conclude that, if the many different antimalarial remedies cause as their first effect the removal of the plasmodia-containing red cells from the circulating blood, and if all of them have the same first effect, in spite of such different chemical constitutions as that of the quinine, salvarsan, sulphonamides, &c., then this almost unspecific effect has a far-reaching unspecific cause.

The meaning of this reduction becomes evident in the appropriate conclusion that in its wider interpretation *every red cell injuring agent is already a potential antimalarial factor!*

As always in similar cases these factors can become remedies if the pharmacological circumstances are favourable. The essential pharmacological conditions which are requisite for all antimalarial remedies are:—

(1) The possibility of attaining a concentration of the agent in the body which, while injuring the red blood corpuscles containing the plasmodia, will not damage the healthy erythrocytes.

(2) That those concentrations can be kept up for long enough to enable the reticulo-endothelial system to develop its *specific* antimalarial activity.

(3) That the agents should not cause a deterioration to the defence mechanism of the reticulo-endothelial system.

To mention some examples hæmolytic agents such as distilled water, $KClO_3$, phenylhydrazine sodium thiosulphate, &c., are all injurious to the red cells, but not for all of them is it possible to obtain favourable pharmacological conditions.

As a first test of the validity of the above conclusions, the effect of sodium thiosulphate on malaria was tried. Although this compound has disadvantages, i.e. easy decomposition and rapid elimination from the body, it has some definite advantages, and the following consideration is important: the efficiency of this harmless salt would be most useful in the determination of the truth or otherwise of the theory. Sodium thiosulphate has been in use for a long time as a remedy in various pathological conditions. Its use was not confined to malaria-free countries. It is not easy to conceive that had this salt had some effect on malarial infections this fact would not have been observed and duly recorded. Examining the literature, in so far as war conditions permitted, no such records were revealed. Therefore the efficiency of sodium thiosulphate could not have been foreseen except on the grounds of the above-mentioned considerations.

Method

The method of choice for proving the efficiency of a substance on malarial parasites is the treatment of the malaria itself.

Tests of the substance with cultures of the parasites were inconclusive, and owing to the lack of suitable animals the cultures could not be further tested. The motility of certain grass infusoria under the influence of sodium thiosulphate was investigated; it can be stated that the motility of these infusoria was generally diminished and in some cases completely stopped, but the concentrations of sodium thiosulphate used for this purpose were too high in comparison with those which could be obtained in the human blood.

The following two groups of soldiers were excluded from the clinical investigation:

(1) Those who did not wish to be treated with sodium thiosulphate.

(2) Others who were already weakened by their former paroxysms of malaria, or who had in addition to their malarial infection serious complications.

With the remaining cases it was possible to make only short-term observations in order to avoid long periods in hospital. Exceptions could be made only with the very few cases who had to stay in hospital for longer periods.

The malarial cases during the year 1944 came mostly from the Mediterranean theatre of war; the cases in 1945 came almost exclusively from Burma. All patients but one had previously suffered from malaria.

After admission the nursing and care of the patients differed in no respect from that of others

under the routine army treatment. When taking the history of the patients, care was taken to determine the date of commencement, the frequency, and the quality of their previous rigors. If these were quite clear-cut then treatment was immediately initiated on the occurrence of the first rigor. During the paroxysms, temperature and pulse readings were taken at half-hourly intervals. In all cases with more than one paroxysm in the hospital, *before* the sodium thiosulphate treatment, the peaks of temperature increases were carefully observed to exclude unfit cases.

Slides for microscopic investigations were taken as a routine before the treatment, before every change of treatment, and whenever needed for any special observations. In staining as a method of choice, the combined May-Grünwald and Giemsa staining was used. The acidity of the Giemsa stain was kept at 7·4 pH. Schüffner's dots were carefully observed. The number of parasites in the blood was roughly estimated by comparison with the number of erythrocytes. Hæmoglobin estimations, and red and white cell counts were made in special cases only. Due attention was paid to the presence of eosinophilic cells and to the qualitative and quantitative features of the mononuclear cells.

All the cases were of the benign tertian type of malaria.

The sodium thiosulphate, a practically tasteless and easily soluble salt, was dissolved in tap-water and given in the following doses (Table I):

TABLE I.—DOSAGE OF SODIUM THIOSULPHATE

1944	11 cases	Daily 3 times 0·75 gramme.
	6 cases	Daily 3 times 1·5 grammes.
1945	10 cases	Daily 3 times 1·5 grammes.
	15 cases	Daily 3 times 3·0 grammes.

It is well known that the salt is easily decomposed by acids, and even the presence of CO_2 affects it. Therefore the 4·5 per cent. solutions were renewed every week, if precipitation of sulphur occurred the solutions were renewed more often. The remedy was given by mouth.

After the observation of the full effect of sodium thiosulphate treatment, every patient underwent the routine Army treatment for malaria.

The most suitable and reliable signs and symptoms, namely, the paroxysms and the blood changes, were chosen for observation.

Clinical Observation

The main results of the observations are shown in Table II.

TABLE II.—SHOWING RESULTS OF SODIUM THIOSULPHATE TREATMENT.

Year	Number of cases	Responding No.	Responding Per cent.	Refractory No.	Refractory Per cent.
1944	27	19	70·3	8	29·6
1945	52	23	44·2	29	55·7
Total	79	42	53·1	37	46·8

Seventy-nine cases of malaria were treated with sodium thiosulphate, forty-two of them showed a definite response to the treatment; in these cases after the beginning of treatment, the malarial paroxysms ceased immediately and they did not reappear during the whole period of observation. The average observation time amounted to six days for cases of a 24-hourly paroxysm and 6·9 days in cases of a 48-hourly paroxysm.

The curves shown in figs. 1 to 3 illustrate the course of the temperatures and the pulse-rates under the influence of sodium thiosulphate.

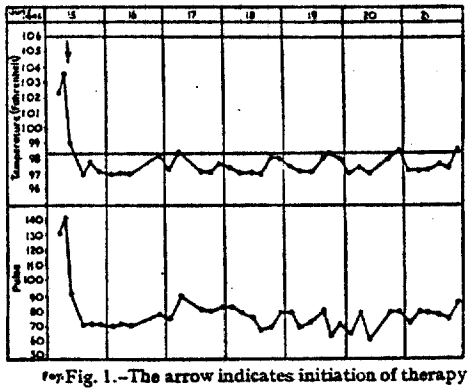

Fig. 1.—The arrow indicates initiation of therapy

The three curves differ from each other only in the numbers of the directly observed paroxysms *before* the start of the sodium thiosulphate treatment. In most of the forty-two cases which responded, two paroxysms were observed before the start of the treatment. Often the first of the two rigors occurred while the patient was being admitted.

In about 150 malarial cases observed in the hospital, spontaneous cessation of the paroxysms occurred in one doubtful case alone. In this case there was on admission a slight rise of temperature and parasites in the blood, and some rhonchi were audible in the lungs. Further febrile rises did not follow, although no anti-malarial treatment was given. There were always a few parasites in the blood, and before discharge the routine anti-malarial treatment was given.

In two of the forty-two cases who reacted well to the treatment, the sodium thiosulphate treatment was stopped on the fifth day; without *immediate* introduction of the quinine treatment. Twelve hours later the rigors started again.

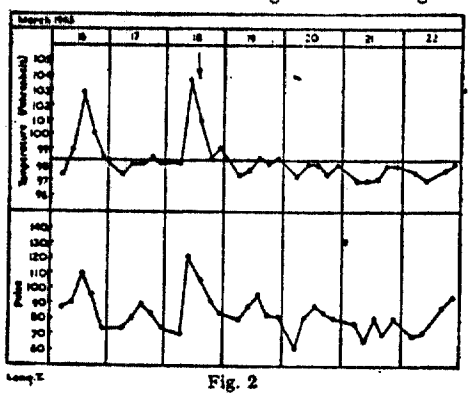

Fig. 2

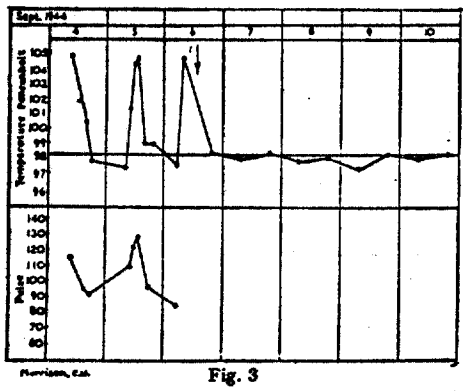

Fig. 3

However, in other cases that responded well, the sodium thiosulphate treatment was kept up for a longer period—e.g. for ten days, *see* fig. 4—and then stopped. These patients remained free of rigors during the time of further observation. In these it was noteworthy that eosinophilic cells reappeared in the blood.

In one of the cases in which the rigors had been arrested a provocative adrenalin injection was given on the fifth day after the cessation of his paroxysms; no rigor followed.

Several of the refractory cases commenced their quinine treatment with superimposed sodium thiosulphate. In these the duration of the last rigor was perceptibly lengthened in comparison with that of the preceding rigors.

In one case a patient on first admission did no react well to sodium thiosulphate. On his

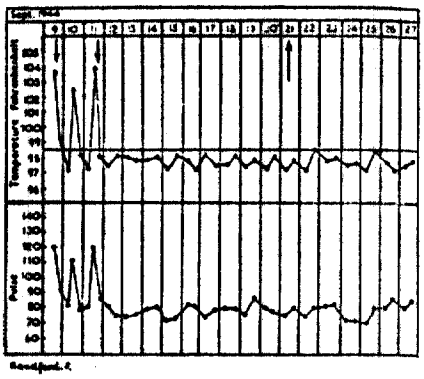

Fig. 4
First dosage 0·75g × 3 daily: second dosage 3·0g × 3 da

second admission, some months later, t reaction was prompt.

In another case, reacting well to sodiu thiosulphate, the patient finished his cu with the routine Army treatment. He return some time later with a fresh attack. Again, reacted well to sodium thiosulphate.

The dose of sodium thiosulphate used 1944 averaged 3·05 g., in 1945 the avera daily dose was 7·2 g. The remedy was us in a thrice daily dosage. No harmful effe was observed.

Results

The clinical observations show with gre certainty that sodium thiosulphate has definite action on the clinical symptoms benign tertian malaria. This may be conclude from the following facts:

(1) In the 79 observed cases, 42, i.e. 51·3 p cent, patients reacted to sodium thiosulpha treatment by the cessation of their malari paroxysms.

(2) In all cases which reacted well the numb of malarial parasites in the blood decreased.

(3) The effect followed immediately after th commencement of the treatment.

Further the controls show:

(1) In all the approx. 150 cases of malari infections observed, in the hospital, the occurred only one case with a possible spo taneous cessation of his paroxysms.

The observed cases had not shown an diminution of the peaks of their temperatu elevations, *before* the start of the sodiu thiosulphate treatment. This circumstance not the rule with spontaneous cessation of th rigors.

The effect of sodium thiosulphate is, in its main points, similar to that of quinine or of the other cinchona derivatives, i.e. the paroxysms cease after the treatment has started. They ceased immediately after the beginning of the therapy, and the parasites vanished from the blood. There are also some minor points of similarity, namely, short treatment with sodium thiosulphate is not sufficient to maintain the freedom from paroxysms; also after longer treatment the sodium thiosulphate can be omitted without immediate recurrence of the paroxysms. It is noteworthy in such cases that the eosinophilic cells reappear in the blood.

The treatment can be administered a second time with good results as repeatedly treated cases show.

The difference in the proportion of responding and refractory cases between the years 1944 and 1945 is obvious from Table I. This difference can have various causes. The dosage of sodium thiosulphate was different in the two years, and so was the time of commencement of medication. In 1944 the average daily dose was 3·05 g., in 1945 the average amounted to 7·2 g. Another important difference between the two is that—as already mentioned—the patients in 1944 came mostly from the Mediterranean theatre of the war but, in 1945, they came almost exclusively from Burma.

Conclusion

The above investigation with sodium thiosulphate strengthens the hypothesis that antimalarial remedies have a non-specific effect at the start.

It is necessary for full proof that investigations with many more substances should be performed, and it is premature to say that, in malaria, quinine has only an indirect effect and that the specific tissues only exert a direct effect. The matter is far more complicated. Nothing is easier and more natural than to assume that the trophozoites in the tissues perish because they are deprived of their natural "living space," i.e. the erythrocytes. In this case the specific tissues, by completing the destruction of the erythrocytes, have only an indirect antimalarial effect. Vice versa, if the *merozoite-containing erythrocytes* burst, the blood is—and very probably the tissue fluids too—the natural element of the released merozoites. It is the task of the tissues to destroy them. It cannot, however, be excluded that quinine, and quinine-like remedies, help in this direction; that is to say, both tissues and remedies may exert a direct effect.

The true nature of the process is unknown but on this probably depends whether organo- or chemotherapy will take the lead in the "direct" therapy of malaria.

There are many more problems concerning malaria which assume a different aspect if the hypothesis made in the introduction proves to be true. To mention one or two, the mystery of the blackwater fever and the problem of the relapses after seemingly successful therapy with any of the known antimalarial remedies.

Finally, there is the practical question: can sodium thiosulphate be used in the future as a remedy for malaria? The observations recorded above indicate that the effect of sodium thiosulphate itself is not certain enough for practical purposes, since treatment with this drug did not achieve 100 per cent. success. Sodium thiosulphate cannot compete with quinine or similar drugs in this respect.[1]

Summary

(1) It was assumed that in malaria the *first* effect of all quinine-like remedies is *a selective injury*, physical or chemical, to the red blood cells.

(2) It is a consequence of this hypothesis that simple inorganic salts such as sodium-thiosulphate may have antimalarial effects.

(3) In a short-term therapeutic experiment, there was in 42 out of 79 malaria cases a prompt response to sodium thiosulphate administration.

(4) The hypothesis which assumed the efficacy of sodium thiosulphate is supported by these results.

I wish to express my thanks to Major-General R. W. D. Leslie for permission to publish the results of these investigations. Also to Professor G. W. Watson of Leeds for his kind interest in the work, and to Dr. Duncan Macmillan, Superintendent of St. Ann's Hospital, for his constant help and great interest.

Further I heartily thank Sisters Hood and Lambert together with their staff for their reliable and thorough help in the care and observation of the patients.

[1] Since this paper was written preliminary investigations have shown that phenyl-hydrazine in small doses was not only effective, but it seems that this compound has some advantages compared with some other antimalarial remedies. Investigations continue.

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Zeitschrift für die gesamte experimentelle Medizin 134, 233–241 (1961)

Aus der Rheumaklinik und dem Institut für physikalische Therapie der Universität Zürich (Direktor: Prof. Dr. A. Böni)

Über die Hautresorption und die Verwertung und Wiederausscheidung von kolloidalem Schwefel und Thiosulfat

Versuche an Kaninchen mit radioaktiv markiertem ^{35}S

Von

Ruth Lotmar

(Eingegangen am 9. Dezember 1960)

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Thiosulfate

In früheren Untersuchungen[1a–c] hatten wir festgestellt, daß radioaktiv markierte *Sulfat*-Ionen aus wäßriger Lösung von der Kaninchenhaut resorbiert werden.

Ein gewisser Anteil des in den Körper gelangten Materials wird innerhalb weniger Tage unverwertet wieder ausgeschieden, während der restliche Teil in die Organe und Gewebe eingebaut wird, vor allem als Chondroitinsulfat. Für menschliche Haut ist eine Resorption von Sulfat-Ionen ebenfalls nachgewiesen (Böni und Mitarbeiter[1], Dirnagl und Mitarbeiter [4a, 4b]).

Auch Hydrogen-*Sulfid* wird von der Haut resorbiert. Neuerdings konnte dies für den Menschen – in Bestätigung älterer Untersuchungen – unter Anwendung der modernen Isotopenmethode auch in quantitativer Weise gezeigt werden (Dirnagl und Mitarbeiter[4c], Hartmann[7]). Dabei ergab sich, daß Sulfidschwefel wesentlich rascher von der Haut aufgenommen wird als Sulfatschwefel. — Die frühere Ansicht, daß nur zweiwertiges Sulfid, nicht aber sechswertiges Sulfat vom Körper verwertbar sei, ist unrichtig. Es hat sich zudem ergeben, daß resorbierter Sulfidschwefel primär jeweils zu Sulfat oxydiert wird (bei percutan aufgenommenem Material geschieht dies zum Teil schon in der Haut (Hartmann[7]) und daß erst von dieser Oxydationsstufe aus der Einbau in organische Schwefelverbindungen erfolgt.

Die Resorption von *elementarem Schwefel* durch die Haut ist ebenfalls schon geprüft worden, in neuester Zeit unter Verwendung von radioaktivem ^{35}S von Geinitz u. Wüst[6*]. Bei diesen Untersuchungen wurde der Schwefel mittels Salben oder in Öl gelöst mit der Haut in Berührung gebracht. Aus dem Nachweis einer Permeabilität für solchen „Salben-Schwefel" darf jedoch nicht ohne weiteres geschlossen werden, daß auch *wäßrig-kolloidal gelöster* oder *suspendierter Schwefel* von der Haut aufgenommen und resorbiert wird. So ist diese balneologisch interessierende Frage heute noch nicht befriedigend beantwortet. — Natürliche Heilwässer enthalten primär allerdings keinen kolloidalen Schwefel. In schwefelwasserstoff-führenden Quellen kann sich jedoch bei Berührung des Thermalwassers mit der Luft oder mit der menschlichen Haut elementarer bzw.

* Ältere Arbeiten siehe[8].

kolloidaler Schwefel durch Oxydation bilden. Es interessierte uns deshalb, ob wäßrig gelöster kolloidaler Schwefel von der Haut überhaupt resorbiert wird, und wenn ja, in welchem Maße. Ferner war zu untersuchen, ob das in den Körper gelangte Material teilweise in organische Verbindungen überführt und zum Aufbau in Organen und Geweben verwertet wird, in ähnlicher Weise, wie dies für Sulfid- und Sulfatschwefel bekannt ist (BOSTRÖM[2], BOSTRÖM u. JORPES[3], DZIEWIATKOWSKI[5], LOTMAR[8a-c], MÜLLER u. v. ERICHSEN[9]). Endlich mußte festgestellt werden, wie sich der verwertete zum unverwertet wieder ausgeschiedenen Anteil verhält, und ob hierin Parallelen zum Sulfatschwefel bestehen. Für die Resorptionsversuche mit kolloidalem Schwefel benutzten wir radioaktiv markiertes „Thiorubrol".

Im Thiorubrol, einem Schwefelbadepräparat der Firma Wolo, Zürich, ist rund 80% des darin enthaltenen Schwefels in kolloidaler Zustandsform vorhanden, während der restliche Teil in Trithioricinolsulfonsäure und 0,2% als Sulfationen enthalten ist. Für die geplanten Versuche stellte uns die Firma Wolo A.G. radioaktiv markiertes Thiorubrol her, wofür wir ihr auch an dieser Stelle unseren besten Dank aussprechen möchten. Da in diesem Muster nur der *kolloidale Schwefel markiert war*, beziehen sich alle unsere Angaben über Resorption und Verwertbarkeit des im Thiorubrol enthaltenen Schwefels allein auf diesen Anteil; das wasserlösliche sulfonierte Trithioricinol, das als Stabilisator für den kolloidalen Schwefel dient, sowie die weiteren Schwefelformen des Präparates enthielten keinen Isotopenschwefel.

Da zur Darstellung von Thiorubrol Natriumthiosulfat benötigt wird, so ergriffen wir die Gelegenheit, das uns zur Verfügung stehende *radioaktive* $Na_2{}^{35}S_2O_3$ ebenfalls auf seine Resorptionsfähigkeit zu prüfen.

Thiosulfatverbindungen sind nicht sehr stabil und zerfallen verhältnismäßig leicht in elementaren Schwefel und Sulfat. Da diese Umwandlung möglicherweise schon in der Haut stattfindet, ergab sich aus entsprechenden Versuchen mit Natriumthiosulfat eine gewisse Ergänzung zu jenen mit Thiorubrol und andererseits zu den früher durchgeführten Versuchen mit Natriumsulfat[8a, 8c].

Methode

Kolloidaler Schwefel. Unverdünntes Thiorubrol enthält rund 52 mg kolloidalen Schwefel pro ml. Da die spezifische Aktivität des markierten Thiorubrols verhältnismäßig schwach war — 1 γ S entsprach 370 cpm —, mußte eine höhere Konzentration angewendet werden, als dies bei Badekuren üblich ist (für welche ein Verdünnungsgrad von 1:3000 empfohlen wird). Die von uns benutzten Lösungen wurden im Verhältnis 1:50 und 1:500 verdünnt. Bei den 4 Versuchstieren mit Verwendung der schwächeren Lösung war eine Bestimmung des resorbierten Schwefels in den Organen allerdings nicht möglich, da ihre Radioaktivität unterhalb der Nachweisbarkeitsgrenze lag. Einzig der im Harn ausgeschiedene Schwefel ließ sich quantitativ erfassen (Tabelle 1, Z. IX). Der Nachweis einer percutanen Resorption von kolloidalem Schwefel konnte hierdurch zwar erbracht werden, die Frage nach der Verwertbarkeit des aufgenommenen Materials blieb jedoch unbeantwortet. Glücklicherweise war dann bei den beiden anderen Thiorubrol-Tieren mit der 10mal stärkeren Lösung eine Schwefelbestimmung in den einzelnen Organen möglich, so daß über die Verwertbarkeit von percutan aufgenommenem kolloidalem Schwefel quantitative Aussagen gemacht werden können.

Natriumthiosulfat. Auch mit Thiosulfat wurden 2 verschiedene Konzentrationen angewendet. In den ersten beiden Versuchen wurde die radioaktive Stammlösung 3mal verdünnt, bei den 2 anderen kam sie unverdünnt zur Anwendung. Die Stammlösung enthielt 0,52 mg S pro ml. Obwohl die schwächere Lösung nur knapp doppelt soviel Schwefel pro ml enthielt wie die schwächere Thiorubrollösung, so war es dank der wesentlich höheren spezifischen Aktivität des Natriumthiosulfates — 1 γ S entsprach 5200 cpm — doch möglich, eine quantitative Bestimmung der Organradioaktivität auszuführen.

Ein *Resorptionsversuch* verlief in folgender Weise: Auf der rasierten Bauchhaut des auf dem Rücken liegenden gefesselten Kaninchens lag ein mehrfacher Gazelappen, der eine Fläche von rund 100 cm² bedeckte und mit 11 bzw. 12 ml der radioaktiven Versuchslösungen getränkt wurde. Bei den Thiorubrolversuchen entsprach dies 11,0 und 1,1 mg, beim Natriumthiosulfat 6,3 und 2,1 mg markiertem Schwefel. Nach Verlauf 1 Std wurde der Gazelappen entfernt, die Haut sorgfältig mit 6 feuchten Wattebäuschen gereinigt und die Kaninchen in ihre Käfige zurückgebracht. An den folgenden 4 Tagen wurde die Radioaktivität im Harn täglich 1mal bestimmt. Danach wurden die Tiere seziert, die verschiedenen Organproben entnommen, getrocknet, gewogen, aufgeschlossen und die Radioaktivität festgestellt (näheres siehe [8c]). Da das Frischgewicht der einzelnen Organe im Verhältnis zum Gesamtgewicht der Versuchstiere bekannt ist ([8c]), ferner das Trockengewicht im Verhältnis zum Frischgewicht, außerdem die Halbwertszeit von ^{35}S, die spezifische Aktivität der verwendeten Lösungen und ihr Schwefelgehalt pro ml, so läßt sich aus den erhaltenen cpm-Werten der Gehalt an verwertetem Schwefel in den einzelnen Organen pro g Trockengewicht oder im ganzen Organ bzw. im gesamten Tier berechnen. Durch Addition des im Harn (und Kot) rasch wieder ausgeschiedenen unverwerteten Schwefels erhält man die im 1 Std-Versuch insgesamt resorbierte Menge*.

Ergebnisse

In den Tabellen 1 und 2 ist das Zahlenmaterial der Thiorubrol- und Natriumthiosulfatversuche zusammengestellt. Als Ergänzung wurden auch die entsprechenden Werte der früheren Sulfatversuche in die beiden Tabellen aufgenommen ([8a, 8c]).

Sowohl *Natriumthiosulfat* als auch der im Badepräparat Thiorubrol enthaltene *kolloidale Schwefel* wird aus wäßriger Lösung von der Kaninchenhaut aufgenommen und resorbiert. Ähnlich wie bei den Sulfattieren (Versuche 1954/1956) erwies sich schon der erste 24-Std-Harn als radioaktiv, ein Beweis also, daß eine gewisse Menge des in Frage stehenden Schwefels percutan aufgenommen worden ist. Für die Sulfattiere konnte seinerzeit gezeigt werden, daß es sich bei dem in den ersten Tagen ausgeschiedenen Schwefel vor allem um den nicht verwerteten Anteil des resorbierten Materials handelt[8a]. Auch bei den jetzt untersuchten Kaninchen war ein ähnlich rascher exponentieller Abfall der Radioaktivität im Harn während der ersten Tage festzustellen. *Der unverwertete Anteil von percutan aufgenommenem kolloidalem Schwefel und von Thiosulfatschwefel*

* Der Schwefelgehalt der nicht untersuchten Organe, wie Darm, Gehirn u. a. wurde pro Tier mit 5% eingesetzt. Der mit dem Kot ausgeschiedene Schwefel kann auf etwa $^1/_3$ des im Harn bestimmten Schwefels geschätzt werden([8c]).

Tabelle 1. *Aufnahme, Verwertung und Wiederausscheidung des Schwefels*

Bindungsart des angebotenen Schwefels	Anzahl der Versuchstiere Datum	Schwefelaufnahme	Schwefelangebot mg	Menge des resorbierten bzw. injizierten Schwefels pro Tier*		Schicksal des in den Körper gelangten Schwefels: Verwerteter Schwefel pro Tier		Unverwerteter Schwefel (im Harn rasch ausgeschieden)**	
				mg × 10^{-3}	in Prozent des angebotenen S	mg × 10^{-3}	in Prozent des aufgenommenen S	in Prozent des aufgenommenen S	in Prozent des angebotenen S
Zeile	Spalte 1	2	3	4	5	6	7	8	9
I	7 1954	Injektion iv, ip, sc	—	12500—22000	—	—	3—7	87—95	—
II	5 1956	Injektion iv, ip	—	6600—8000	—	—	5—15	—	—
— Sulfat —$^{35}SO_4$ III	2 Febr. Mai 1956	perkutan	320	3800/4800	1,2/1,5	300/520	8/11	57/66	0,7/1,0
IV	7 1954	Injektion ip	—	900—2300	—	—	15—45	55—80	—
V	9 1954	perkutan	40	400—1500	1,0—3,7	—	—	—	—
VI	2 Juli 1960	perkutan	6,3	67/76	1,1/1,2	24/21	36/28	49/57	0,5/0,7
—Thiosulfat —$^{35}S_2O_3$ VII	2 Mai 1960	perkutan	2,1	4/5	0,2/0,25	1,9/2	47/40	41/47	0,08/0,1
VIII	2 Juni 1960	perkutan	11,0	50/75	0,45/0,7	36/48	71/64	24/28	0,1/02
Kolloidaler Schwefel —^{35}S IX	4 Mai Juni 1960	perkutan	1,1	Unterhalb Nachweisbarkeitsgrenze		Unterhalb Nachweisbarkeitsgrenze			0,1/0,25

* Summe des in den Organen und im Harn gemessenen und im Kot geschätzten Schwefels.

** Da der im Kot ausgeschiedene Anteil unberücksichtigt ist, so ergänzen sich die Zahlen der Einzeltiere nicht ganz zu 100%.

Tabelle 2. *Verwertung des Schwefels in den einzelnen Organen*

Bindungsart des angebotenen Schwefels	Natriumsulfat 7 Tiere 2,2—2,8 kg	Natriumthiosulfat Tier 1 a 3,3 kg	Natriumthiosulfat Tier 1 b 3,2 kg	Natriumthiosulfat Tier 2 a 3,1 kg	Natriumthiosulfat Tier 2 b 3,3 kg	Kolloidaler Schwefel (Thiorubrol) Tier a 3,2 kg	Kolloidaler Schwefel (Thiorubrol) Tier b 3,5 kg
	1	2		3		4	
Menge des aufgenommenen Schwefels pro Tier: mg × 10^{-3}	3800—8000	4	5	67	76	50	75
In die Organe eingebauter Schwefel pro g Trockengewicht: mg × 10^{-6}	Mittelwerte						
Haut	800	1,5	3,0	75	70	52	46
Muskel	700	1,7	1,3	12	4	30	52
Knochen	300	1,3	1,0	4	4	19	30
Knorpel	2600	7	11	27	18	67	70
Leber	2700	3	7	36	37	57	52
Lunge	3100	14	11	21	27	67	48
Niere	2500	10	12	41	25	99	57
Milz	3200	8	12	38	16	57	38
Pro Organ verwerteter Schwefel in Prozent der pro Tier verwerteten Menge:	Min Max.						
Haut	19—31	18	32	*67*	*75*	32	21
Muskel	24—44 *70—86*	31 82	23 78	17 91	7 90	28 88	38 93
Knochen	13—25	33	23	7	8	28	*31*
Übrige Organe	14—30	18	22	9	10	12	7

scheint somit in entsprechender Weise und ebenso rasch wieder ausgeschieden zu werden wie nach Resorption von Sulfatschwefel.

Von großem Interesse ist die Frage, wieviel Prozent des im Gazelappen angebotenen Thiorubrol- und Thiosulfatschwefels im Laufe des 1stündigen Versuches zur Resorption kommt. Für Sulfatschwefel hatte sich seinerzeit bei 11 Tieren eine Resorptionsquote von 1—3,7% ergeben (Tabelle 1, Sp. 5, Z. III, V). Ergänzend läßt sich aus bisher unveröffentlichten Versuchsreihen mit nahezu 200 weiteren Fällen (und einem Angebot von 32 mg S) schätzen, daß in rund 30 Fällen weniger als 1% und umgekehrt vereinzelt nahezu 5% des angebotenen Schwefels resorbiert worden war. Die Resorptionsquote weist beim Sulfatschwefel somit individuell erhebliche Unterschiede auf. — Bei den jetzt untersuchten Tieren wurde aus den beiden Thiosulfatlösungen 0,2—1,2% und aus der Thiorubrollösung 0,45 und 0,7% der angebotenen Menge resorbiert (Tabelle 1, Sp. 5, Z. VI—VIII). Die Resorptionsquote ist im Durchschnitt also etwas niedriger als bei den Sulfattieren. In Anbetracht der bei diesen festgestellten starken individuellen Streuungen ist es aber durchaus denkbar, daß weitere Thiorubrol- und Thiosulfatversuchstiere auch entsprechend etwas höhere Quoten gezeigt hätten. Als Ergebnis kann jedenfalls festgehalten werden, daß *in der Resorbierbarkeit von Thiosulfatschwefel, von kolloidalem Schwefel und von Sulfatschwefel aus wäßriger Lösung weitgehende Übereinstimmung herrscht.* Mit rund 1% bzw. etwas mehr oder weniger liegt die Resorptionsquote der genannten Schwefelarten im Vergleich zu Sulfidschwefel[4c] insgesamt verhältnismäßig niedrig.

Der Einbau des aufgenommenen Schwefels in die einzelnen Organe, d. h. seine Verwertung, erfolgt bei kolloidalem Schwefel und Thiosulfat ebenfalls nach ähnlichen Prinzipien wie beim Sulfatschwefel. Bei den Sulfattieren hatte sich gezeigt, daß in den Organen mit geringerem Chondroitinsulfatgehalt, wie enthaarte Haut, Muskel und Knochen, pro g Trockengewicht deutlich weniger radioaktiver Schwefel eingebaut wird als im Knorpel und in den inneren Organen (Tabelle 2, Sp. 1). Da andererseits Haut, Muskel und Knochen nahezu 80% des Körpergewichtes bei Kaninchen ausmachen, so ergibt sich bei Berechnung des Gesamtschwefelgehaltes pro Tier doch ein starkes Überwiegen des verwerteten Schwefels in diesen 3 Gewebegruppen; auf alle anderen Organe und Gewebe entfallen nur 14—30% (Tabelle 2, Sp. 1 unten).

Wie verhalten sich nun in dieser Hinsicht die untersuchten Thiosulfat- und Thiorubroltiere? Betrachten wir zuerst die Versuche mit Thiosulfat. Absolut hatten die *Thiosulfattiere* als Folge des geringeren Angebotes wesentlich weniger Schwefel aufgenommen als die vergleichbaren Sulfattiere (Tabelle 2, Sp. 1—3). Erwartungsgemäß ergab sich deshalb bei ihnen ein insgesamt niedrigerer Schwefelgehalt, doch war auch hier in den inneren Organen und im Knorpel pro g Trockengewicht wesentlich mehr

Schwefel eingebaut als in Haut, Muskel und Knochen (Tabelle 2, Sp. 2/3). Ganz aus dem Rahmen fallen dabei allerdings die überraschend hohen Hautwerte, welche die beiden im Monat Juli untersuchten Tiere 2a und 2b ergeben hatten (Tabelle 1, Z. VI und Tabelle 2, Sp. 3). Die Erklärung liegt in Folgendem: Als Einzige unter allen früher und jetzt untersuchten Kaninchen standen diese beiden Tiere zufälligerweise mitten im sommerlichen Pelzwechsel. Alle anderen Versuchstiere trugen zur Zeit der Untersuchung noch das Winterkleid oder standen dicht vor der Haarerneuerung. Da für das Keratin der neuen Haare Schwefel benötigt wird[8b], herrscht zur Zeit des Pelzwechsels in der Haut ein besonders intensiver Stoffwechsel. So scheint es naheliegend, die hohe Hautradioaktivität der Tiere 2a und 2b diesem Umstand zuzuschreiben und nicht etwa einem spezifischen Verhalten der Thiosulfatbindungsform des aufgenommenen Schwefels; dies umso mehr, als die Thiosulfattiere 1a und 1b, die noch vor dem Pelzwechsel im Monat Mai zur Untersuchung gekommen waren, ganz normale Hautwerte aufwiesen (Tabelle 2, Sp. 2). *Percutan resorbierter Thiosulfatschwefel wird somit in den verschiedenen Organen und Geweben in ähnlicher Weise zum Aufbau verwertet, wie sich für Sulfatschwefel ergeben hatte*[8c].

Auch bei Betrachtung der *prozentualen Verteilung* des eingebauten Schwefels innerhalb des gesamten Organismus, ergibt sich eine gute Übereinstimmung mit den für die Sulfattiere erhaltenen Mittelwerten, speziell bei Vergleich mit den Tieren 1a und 1b (Tabelle 2, Sp. 1 und 2 unten). Der Gesamtschwefelgehalt von enthaarter Haut, Muskel und Knochen entspricht bei ihnen mit 78 und 82% durchaus der Norm, einzig der Wert für Knochen liegt beim einen Tier mit 33% etwas über dem Maximum der Sulfattiere. Die beiden im Pelzwechsel stehenden Tiere 2a und 2b können nicht verglichen werden, da die auf 67 und 75% erhöhten Hautwerte logischerweise ein prozentuales Absinken aller übrigen Werte zur Folge hatten (Tabelle 2, Sp. 3 unten).

Für *Thiorubrol* wurden Schwefelbestimmungen in den einzelnen Organen und Geweben nur bei 2 Versuchstieren gemacht; bei den 4 anderen Tieren lag der Schwefelgehalt der Organe unterhalb der Nachweisbarkeitsgrenze (Tabelle 1, Z. IX). Die für Tier a und b erhaltenen Werte zeigen im Vergleich zu den übrigen Organen eine verhältnismäßig hohe Einlagerung in Haut, Muskel und Knochen (Tabelle 2, Sp. 4). Aus der prozentualen Verteilung im gesamten Organismus (Sp. 4 unten) ergibt sich jedoch, daß die Werte für Haut, Muskel und Knochen wohl etwas hoch sind, insgesamt aber noch der Norm bzw. dem Maximum der für die Sulfattiere erhaltenen Zahlen entsprechen oder sie doch nur unwesentlich überschreiten. Einzig die 34% der Knochen von Tier b liegen deutlich über dem Maximalwert der Sulfattiere. Abgesehen von dieser Ausnahme, auf die jedoch im Hinblick auf die immer wieder festgestellten starken

individuellen Streuungen kein allzu großes Gewicht gelegt werden soll, hat sich somit auch für die *Verwertung von percutan aufgenommenem kolloidalem Schwefel eine normale Verteilung innerhalb der verschiedenen Organe und Gewebe ergeben.*

Im weiteren war noch zu untersuchen, wie sich der verwertete zum unverwerteten Anteil im Vergleich zum insgesamt resorbierten Schwefel verhält. Bei den Sulfattieren hatte sich ergeben, daß der verwertete Anteil prozentual umso niedriger war, je mehr Material in den Körper aufgenommen wurde. Diese Gesetzmäßigkeit scheint auch bei der Verwertung von percutan resorbiertem Natriumthiosulfat und kolloidalem Schwefel gültig zu sein. Bei einer 1. Gruppe von Sulfattieren, denen 3800 bis 22000 γ Schwefel injiziert worden war, wurden 3—15% in die Organe eingebaut und bis zu 95% im Harn als unverwerteter Anteil rasch wieder ausgeschieden. Eine 2. Gruppe mit 900—2300 γ einverleibtem Sulfatschwefel verwertete 15—45%, durchschnittlich also etwas mehr, und schied maximal 80% unverwertet wieder aus. Bei den Thiosulfat- und Thiorubroltieren, die wesentlich weniger Schwefel aufgenommen hatten, 4—76 γ, war der verwertete Anteil mit 28—71% im Mittel nochmals höher, und der im Harn unverwertet wieder ausgeschiedene Anteil mit maximal 57% entsprechend niedriger (Tabelle 1, Sp. 4, 7, 8, Z. I—VIII).

Wie bei den Sulfattieren ergaben sich auch hier starke individuelle Streuungen der Einzeltiere. Die für Natriumsulfat gemachte Beobachtung, wonach der *Prozentanteil des zum Aufbau in den Organen verwerteten Schwefels in gewissem Grade abhängig ist von der Menge des aufgenommenen Materials, gilt somit auch für Thiosulfat und kolloidalen Schwefel.*

Zusammenfassung

1. Der im Badepräparat Thiorubrol enthaltene kolloidale Schwefel (—^{35}S), wie auch Schwefel in Thiosulfatbindung (—$^{35}S_2O_3$) werden aus wäßriger Lösung percutan von der Kaninchenhaut resorbiert.

2. Die Resorptionsquote liegt mit $\lesseqgtr 1\%$ im Rahmen jener von Sulfatschwefel mit $< 1\%$—$< 5\%$.

3. Der unverwertete Anteil des resorbierten kolloidalen Schwefels und des Thiosulfatschwefels wird innerhalb weniger Tage im Harn (und Kot) rasch wieder ausgeschieden.

4. Auch in den Organen und Geweben wird der kolloidale Schwefel und der Thiosulfatschwefel nach gleichen Prinzipien zum Aufbau verwertet wie der Sulfatschwefel. Auf das Gramm Trockengewicht bezogen, wird in Haut, Muskel und Knochen weniger Schwefel eingebaut als in Knorpel und in die inneren Organe. Der abnorm hohe Schwefelgehalt in der Haut von 2 Thiosulfattieren hängt vermutlich mit ihrem Pelzwechsel zusammen. Der prozentuale Anteil des verwerteten Schwefels ist bis zu einem gewissen Grade abhängig von der Menge des resorbierten Materials.

5. Die unter 2—4 zusammengestellten Ergebnisse zeigen, daß in der Resorbierbarkeit, in der Verwertung und in der Wiederausscheidung von kolloidalem Schwefel, Thiosulfatschwefel und Sulfatschwefel weitgehende Übereinstimmung herrscht. Es ist deshalb zu vermuten, daß die beiden erstgenannten Schwefelarten vor ihrer Verwertung im Körper ebenfalls in Sulfatschwefel übergeführt werden in ähnlicher Weise, wie dies für Sulfidschwefel bekannt ist.

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LOS thiosulfat

CUTANEOUS ABSORPTION, UTILIZATION AND SUBSEQUENT EXCRETION OF COLLOIDAL SULFUR AND THIOSULFATE

Experiments on rabbits with radioactive-labeled ^{35}S

We determined in earlier experiments[8a-c] that SULFATE ions with radioactive tracers from aqueous solutions are absorbed by the skin of rabbits.

A certain portion of the material entering the body is excreted unutilized within a few days, while the remainder is assimilated by organs and tissues, primarily as chondroitin sulfate. The absorption of sulfate ions by the human skin has been demonstrated as well (BÖNI and collegues[1], DIRNAGL and collegues[4a,4b]).

Hydrogen SULFIDE is also absorbed by the skin. This could recently be demonstrated for humans in quantitative terms as well - confirming previous investigations - by using the modern isotope method (DIRNAGL and collegues[1c], HARTMANN[7]). It was shown in this way that sulfide sulfur is absorbed by the skin considerably faster than sulfate sulfur. - The recent assumption that only bivalent sulfide, but not hexavalent sulfate, is utilized by the body is incorrect. Furthermore, it has been shown that absorbed sulfide sulfur is primarily oxydized to sulfate (in material absorbed cutaneously, this already happens in part in the skin(HARTMANN[7])) amd that the assemblage into organic sulfur compounds only follows after this oxydation phase.

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The cutaneous absorption of ELEMENTARY SULFUR has also been investigated, in most recent times by GEINETZ and WÜST[6*] by employment of radioactive ^{35}S. In these experiments, sulfur was brought into contact with the skin by way of ointments or dissolved in oil. However, the confirmation of permeability of such "ointment-sulfur" should not lead to the conclusion that SULFUR DISSOLVED IN EMULSION COLLOIDS or SUSPENDED SULFUR is accepted and absorbed by the skin as well. This question, of interst for balneology, has not yet been answered satisfactorily. - Natural medicinal waters do not primarily contain colloidal sulfur. In springs containing sulfur hydride, however, elementary or colloidal sulfur may be formed by oxydation when the thermal water comes in contact with air or human skin. We were therefore interested in the question whether colloidal sulfur dissolved in water is absorbed by the skin at all, and if, to what extent. Furthermore, we wanted to investigate whether the material, after entering the body, is transformed into organic compounds and utilized for the metabolism in organs and tissues in a way similar to that known in sulfide and sulfate sulfur (BOSTRÖM[2], BOSTRÖM and JORPES[3], DZIEWIATKOWSKI[5], LOTHMAR[8a-c], MÜLLER and V. ERICHSEN[9]). Finally, we had to determine the ratio of the portion which is utilized to the one which is excreted unutilized, and whether parallels to sulfate sulfur exist. For the absorption experiments with colloidal sulfur we used "thiorubrol" with radioactive tracers.

Thiorubrol, a sulfur bath preparation by the firm Wolo, Zürich, contains ca. 80 % of the ingredient sulfur in colloidal form, the remainder as trithioricinolsulfic acid and 0.2 % as sulfation. For the projected

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* earlier reports on experiments see [6].

experiments the firm Wolo A.G. prepared radioactive-labeled thiorubrol, and for this we would like to express our gratitude here. Since only the COLLOIDAL SULFUR WAS LABELED in this sample, all our statements on absorption and utilization of the sulfur contained in thiorubrol refer solely to this portion; the watersoluble sulfonized trithioricinol, which serves as stabilizor for the colloidal sulfur, as well as the additional sulfur forms of the preparation did not contain isotope sulfur.

Since sodium thiosulfate is required for the preparation of thiorubrol, we took this opportunity to also examine the absorptivity of the available RADIOACTIVE $Na_2{}^{35}S_2O_3$.

Thiosulfate compounds are not very stable and decompose relatively easy into elementary sulfur and sulfate. Since this transformation may possibly take place in the skin already, corresponding experiments with sodium thiosulfate represented a certain supplementation to those with thiorubrol and also to the previously conducted experiments with sodium thiosulfate[8a,8c].

METHOD

COLLOIDAL SULFUR. Undiluted thiorubrol contains ca. 52 mg of colloidal sulfur per ml. Since the specific activity of the labeled thiorubrol was relatively low - 1γ S corresponded to 370 cpm - , we had to use a higher concentration than that commonly used for mineral bath therapy (for which a dilution ratio of 1:3000 is recommended). The solutions we used were diluted in the ratio of 1:50 and 1:500. With the 4 experimental animals in which we used the milder solution, a determination of the absorbed sulfur in the organs was not possible, however, since its radioactivity was below the limit of demonstrability. Only the sulfur excreted in the urine could be determined in terms of quantity (table 1, row IX).

Cutaneous absorption of colloidal sulfur could be demonstrated by this, but the question of whether the absorbed material was utilized remained unanswered. Fortunately, a determination of sulfur in the individual organs was possible in the other two thiorubrol animals with the 10 times stronger solution, so that quantitative statements on the utilization of cutaneously absorbed colloidal sulfur could be made.

SODUIM THIOSULFATE. 2 different concentrations were also used with thiosulfate. In the first two experiments, the radioactive initial solution was diluted 3 times, in the other two it was used undiluted. The initial solution contained 0.52 mg S per ml. Although the weaker solution contained only scarcely double the amount of sulfur per ml as the weaker thiorubrol solution, it was possible, due to the substantially higher specific activity of sodium thiosulfate - 1 γ S corresponded to 5200 cpm - to measure the quantity of the organ radioactvity.

An ABSORPTION TEST was conducted in the following manner: The abdominal skin of the rabbit was shaved, it was restrained and laid on its back with a multilayered piece of gauze on the abdominal skin covering an area of ca. 100 cm^2, which was impregnated with 11 and 12 ml respectivel of the radioactive test solutions. In the thiorubrol experiments, this corresponded to 11.0 and 1.1 mg; in the sodium thiosulfate, to 6.3 and 2.1 mg of labeled sulfur. After the course of 1 hour, the gauze was removed, the skin was carefully cleaned with 6 moist cotton balls and the rabbits were returned to their cages. On the following 4 days the radioactivity in the urine was determined once a day. Thereafter the animals were dissected, the various organ samples were removed, dried, weighed, disintegrated and their radioactivity determined (for details see [8c]). Since the fresh weight of the individual organs in proportion to the total weight of the experimental animals is known ([8c]), further the dry weight

in proportion to the fresh weight, furthermore the half-life time of ^{35}S, the specific activity of the employed solutions and their sulfur content per ml, one can calculate from the derived cpm values the content of utilized sulfur in the individual organs per g of dry weight or in the entire organ or the entire animal. The total amount absorbed during the 1 hour experiment is obtained by adding the unutilized sulfur excreted within a short time in the urine (and feces) *.

RESULTS

In tables 1 and 2 the data of the thiorubrol and sodium thiosulfate experiments have been compiled. As supplementation, the corresponding values of earlier sulfate experiments have been incorporated in both tables ([8a,8c]).

SODIUM THIOSULFATE as well as COLLOIDAL SULFUR contained in the mineral bath preparation thiorubrol is received and absorbed from aqueous solution by the skin of rabbits. Similar to the sulfate animals (experiments of 1954/1956), already the first 24 hour urine proved radioactive, a demonstration that a certain amount of the sulfur in question has been absorbed cutaneously. It has been shown previously that in the sulfate animals, the sulfur excreted during the first days was primarily the unutilized portion of the absorbed material [8a]. In the rabbits examined by us, a similarly rapid exponential decrease of radioactivity in the urine was observed during the first days. THE UNUTILIZED PORTION OF CUTANEOUSLY ABSORBED

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* The sulfur content of organs not examined, such as intestines, brain etc., was set at 5 % per animal. The sulfur excreted in feces can be estimated at 1/3 of the sulfur determined in the urine ([8c]).

Table 1. ABSORPTION, UTILIZATION AND SUBSEQUENT EXCRETION OF SULFUR

type of bonding of applied sulfur		number of experimental animals - date	type of application of sulfur	amount of sulfur applied - in mg	amount of sulfur absorbed & injected respectively per animal		destination of sulfur absorbed by the body			
							utilized sulfur per animal		unutilized sulfur (rapidly excreted in urine)**	
					mg $\times 10^{-3}$	in percentage of applied sulfur	mg $\times 10^{-3}$	in percentage of absorbed S	in percentage of absorbed S	in percentage of applied S
	ROW	COLUMN 1	2	3	4	5	6	7	8	9
	I	7 1954	Injektion iv, ip, sc	--	12500 -22000			3 -7	87—95	—
	II	5 1956	Injektion iv, ip	--	6600 --8000			5 -15	—	—
— Sulfate —$^{35}SO_4$	III	2 Febr. May 1956	percutan	320	3800/4800	1,2/1,5	300/520	8/11	57/66	0,7/1,0
	IV	7 1954	Injektion ip	--	900 -2300	--	---	15—45	55—80	—
	V	9 1954	percutan	40	400 -1500	1,0—3,7	--	—	—	—
	VI	2 July 1960	percutan	0,3	67/76	1,1/1,2	24/21	36/28	49/57	0,5/0,7
—Thiosulfate —$^{35}S_2O_3$	VII	2 May 1960	percutan	2,1	4/5	0,2/0,25	1,9/2	47/40	41/47	0,08/0,1
	VIII	2 June 1960	percutan	11,0	50/75	0,45/0,7	36/48	71/64	24/28	0,1/02
Colloidal SULFUR —^{35}S	IX	4 May June 1960	percutan	1,1	below limit of demonstrability					0,1/0,25

*Sum of sulfur demonstrated in organs and urine and estimated in feces

**Since the portion excreted in feces has not been taken into account, the amounts in the INDIVIDUAL ANIMALS DO NOT ADD UP TO 100 %. (row 7,8, column III, VI, VIII)

Table 2. UTILIZATION OF SULFUR IN THE INDIVIDUAL ORGANS

type of bonding of applied sulfur	sodium sulfate 7 animals 2.2–2.8 kg	sodium thiosulfate				colloidal sulfur (thiorubrol)	
		animal 1a 3.3 kg	animal 1b 3.2 kg	animal 2a 3.1 kg	animal 2b 3.3 kg	animal a 3.2 kg	animal b 3.5 kg
	1	2		3		4	
amount of absorbed sulfur per animal: mg × 10^{-3}	3800—8000	4	5	67	76	50	75
sulfur assimilated into organs per g of dry weight: mg × 10^{-6}							
skin	800	1,5	3,0	75	70	52	46
muscels	700	1,7	1,3	12	4	30	52
bones	300	1,3	1,0	4	4	19	30
cartilage	2600	7	11	27	18	67	70
liver	2700	3	7	36	37	57	52
lungs	3100	14	11	21	27	67	48
kidney	2500	10	12	41	25	99	57
spleen	3200	8	12	38	16	57	38
sulfur utilized per organ in percentage of amount utilized per animal:	Min Max.						
skin	19—31	18	32	67	75	32	21
muscels	24 -44 } 70 -86	31 } 82	23 } 78	17 } 91	7 } 90	28 } 88	38 } 93
bones	13—25	33	23	7	8	28	31
remaining organs	14 -30	18	22	9	10	12	7

COLLOIDAL SULFUR AND THIOSULFATE SULFUR THEREFORE SEEMS TO BE EXCRETED IN A CORRESPONDING MANNER AND WITH THE SAME RAPIDITY AS AFTER ABSORPTION OF SULFATE SULFUR.

The percentage of absorption of thiorubrol- and thiosulfate-sulfur applied by gauze during the course of the 1 hour experiment is a question of great interest. In previous experiments with 11 animals, the absorption ratio of sulfate sulfur was demonstrated to be 1 - 3.7 % (table 1, column 5, row III, V). It can be estimated on the evidence of a hitherto unpublished series of experiments with nearly 200 additional cases (and an application of 32 mg S), that in ca. 30 cases less than 1 % and, inversely, in isolated cases almost 5 % of the applied sulfur had been absorbed. We see that the absorption ratio of sulfate sulfur shows substantial individual differences. - In the animals examined by us, the absorption ratio of the applied amount was 0.2-1.2 % for the two thiosulfate solutions and 0.45 and 0.7 % for the thiorubrol solution (table 1, column 5, row VI-VIII). The average absorption ratio is consequently slightly lower than in the sulfate animals. In view of the great individual differences observed in these, it is quite probable that additional experiments with thiorubrol and thiosulfate animals could have shown slightly higher ratios. The following results can be stated without doubt: THE ABSORPTIVITY OF THIOSULFATE SULFUR, COLLOIDAL SULFUR AND SULFATE SULFUR FROM AQUEOUS SOLUTION IS LARGELY CONGRUENT. The combined absorption ratio of the referred types of sulfur in comparison to sulfide sulfur[4c] is relatively low with about 1 % or slightly more or less respectively.

The assimilation of absorbed sulfur into individual organs, i.e. its utilization, follows in similar principles for colloidal sulfur and thiosulfate as for sulfate sulfur. It was demonstrated in the sulfate

animals that distinctly less radioactive sulfur per g of dry weight is assimilated by organs with low chondroitin sulfate content, such as depilated skin, muscles and bones, than by cartilage and by the inner organs (table 2, column 1). Since, on the other hand, skin, muscles and bones comprise nearly 80 % of the body weight of rabbits, the calculation of the total sulfur content per animal reveals a considerable excess of the absorbed sulfur in these 3 groups of tissue; the portion in all other organs and tissues is only 14-30 % (table 2, column 1 bottom).

How do the examined thiosulfate and thiorubrol animals react in this respect? Let us first consider the tests with thiosulfate. The THIOSULFATE ANIMALS had absolutely absorbed considerably less sulfur than the comparable sulfate animals, since less was applied (table 2, column 1-3). As was to be expected, the total sulfur content was lower in these, but even here considerably more sulfur per g of dry weight was assimilated by the inner organs and by cartilage than by skin, muscles and bones (table 2, column 2/3). The surprisingly high skin values in the animals 2a and 2b examined in the month of July are completely incongruent (table 1, row VI and table 2, column 3). This can be explained in the following way: These two animals, as the only ones among all previously and presently examined animals, happened to be in the middle of the summer fur change. All other test animals still had their winter fur or were close to molting at the time of the test. Since sulfur is required for the keratin of the new hair[8b], an especially intensive metabolism takes place at the time of molt. It therefore seems obvious that the high skin radioactivity of animals 2a and 2b is due to this circumstance and not toaspecific reaction of the type of thiosulfate bonding of the absorbed sulfur; all the more since the thiosulfate animals 1a and 1b, which were examined before molt in the month of May,

showed completely normal skin values (table 2, column 2). CUTANEOUSLY ABSORBED THIOSULFATE SULFUR IS THEREFORE METABOLIZED IN THE VARIOUS ORGANS AND TISSUES IN A MANNER SIMILAR TO THAT DEMONSTRATED FOR SULFATE SULFUR[8c].

When considering the PERCENTILE DISTRIBUTION of the absorbed sulfur within the entire organism, one can see there, too, a good concurrence with the average values obtained for the sulfate animals, especially when comparing the animals 1a and 1b (table 2, column 1 and 2 bottom). 78 and 82 % values for the total sulfur content in depilated skin, muscles and bones concur with normal values, only in one animal the value for bones is slightly above the maximum for sulfate animals with 33 %. The two molting animals 2a and 2b cannot be compared, since the logical consequence of the increased skin values of 67 and 75 % was a percentage decrease of all remaining values (table 2, column 3 bottom).

For THIORUBROL, sulfur determinations in the individual organs and tissues were only made in 2 test animals; in the 4 other animals the sulfur content in the organs was below the limit of demonstrability (table 1, row IX). The values obtained in animals a and b show a relatively high assimilation by skin, muscles and bones in comparison to the remaining organs (table 2, column 4). The percentage ratio of the entire organism reveals, however, that the values for skin, muscles and bones are slightly increased, but in total concur with the normal or maximum values of the sulfate animals, or exceed these only to a minor degree. Only the 40 % for the bones in animal b is clearly above the maximum value in the sulfate animals. Outside of this exception, which should not be considered too important, however, in view of the repeatedly observed substantial individual differences, it has been demonstrated that the UTILIZATION OF CUTANEOUSLY ABSORBED COLLOIDAL SULFUR IS DISTRI-

BUTED NORMALLY AMONG THE VARIOUS ORGANS AND TISSUES.

Finally, we had to determine the ratio of the utilized portion to the unutilized one in comparison to the total amount of sulfur absorbed. In the sulfate animals we observed that the percentage of the utilized portion was the lower the more material was absorbed by the body. This conformity seems to apply to the utilization of cutaneously absorbed sodium thiosulfate and colloidal sulfur as well. In a first group of sulfate animals who had been injected with 3800 to 22000 γ of sulfur, 3 to 15 % was assimilated by the organs and up to 95 % was rapidly excreted in the urine as the unutilized portion. A second group, with 900 to 2300 γ of sulfur injected, utilized 15 to 45 %, a slightly higher average value, and excreted a maximum of 80 % as unutilized. In the thiosulfate and thiorubrol animals, who had absorbed considerably less sulfur, i.e. 4 to 76 γ the utilized portion, with an average of 28 to 71 %, was still higher, and the unutilized portion excreted in the urine was accordingly lower with a maximum value of 57 % (table 1, column 4,7,8, row I - VIII).

As in the sulfate animals, considerable individual differences were observed here, too. The observation made with sodium sulfate, that the PERCENTILE PORTION OF SULFUR METABOLIZED BY THE ORGANS IS CONTINGENT TO A CERTAIN DEGREE ON THE AMOUNT OF MATERIAL ABSORBED, THEREFORE ALSO APPLIES TO THIOSULFATE AND COLLOIDAL SULFUR.

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undankbare Aufgabe ist, und sie sollen Anregung geben für weitere Bemühungen in dieser Richtung, die besonders von Bedeutung sein müssen für den praktischen Arzt, der bisher dieser Krankheit ziemlich tatenlos gegenüberstehen mußte.

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Thiosulfate

Behandlung der multiplen Sklerose mit Natriumthiosulfat[1])

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Obwohl der *Schwefel* bei der Behandlung der multiplen Sklerose in Form von *Fibrolysin, Sulfosin, Sulfrogel* usw. schon seit langer Zeit eine Rolle spielt, führte uns nicht der Gedanke einer Weiterentwicklung der Schwefeltherapie, sondern eine zufällige Beobachtung zu unseren Versuchen mit *Natriumthiosulfat.*

Im Mai 1934 begannen wir auf der Klinik ein 22jähriges, seit drei Jahren an multipler Sklerose leidendes Mädchen mit *Neosilbersalvarsan* zu behandeln. Nach der dritten Injektion trat, bei unverändertem Befund des Nervensystems, eine sehr ausgedehnte Dermatitis (an beiden Unterschenkeln, an der Streckseite der Ober- und Unterarme) auf, weshalb wir der Kranken intravenöse Einspritzungen von *Natriumthiosulfat* in Dosen von 0,60, 1,00 1,50 g gaben. Bereits nach den ersten 4 Injektionen stellte sich eine überraschende Besserung ein: Der Gang der Kranken, die bei der Aufnahme nur sich an den Betten festhaltend oder mit Unterstützung gehen konnte, besserte sich dermaßen, daß sie sich allein, ohne Unterstützung in die in einem anderen Gebäude befindliche Kapelle begeben konnte. Daraufhin kam uns der Gedanke, daß an dieser Besserung auch das *Natriumthiosulfat* beteiligt sein könnte. Wir setzten deshalb die Injektionen auch nach dem Verschwinden der Dermatitis fort, und zwar in Dosen von 1,50 g täglich. Darauf besserte sich der Zustand der Kranken weiter, ihr anfänglich sehr spastischer und ataktischer Gang wurde leichter und sicherer und nach der 25. Injektion unternahm sie auch ohne Begleitung längere Spaziergänge. Nystagmus und Intentionszittern verschwanden, die Hypertonie der unteren Extremitäten und die mit dem Kniehackenversuch nachweisbare Ataxie nahmen ab, das BABINSKIsche Zeichen verschwand beiderseits, die schlechte Schrift wurde völlig normal, die Sehschärfe nahm zu: rechts von 25 auf 60 Oxyoptrien (von $^5/_{12}$ auf $^5/_5$), links von 40 auf 60 Oxyoptrien (von $^5/_8$ auf $^5/_5$).

Auf Grund dieser Erfahrung schien ein Versuch mit *Natriumthiosulfatinjektionen*, und zwar ohne *Silbersalvarsan*, auch bei anderen Kranken lohnend zu sein. Mit Rücksicht auf die bei der multiplen Sklerose häufig vorkommenden Spontanremissionen war zur Beurteilung der therapeutischen Wirkung des Mittels ein großes Krankenmaterial benötigt. An diesen Arbeiten nahm LAUBÁL, der inzwischen aus der Klinik ausgeschieden war, nicht mehr teil.

Ich konnte innerhalb von 2 Jahren 40 Kranke regelmäßig behandeln und dieselben wiederholt untersuchen. In einer Kur gab ich meistens 50 Injektionen,

[1]) Vorgetragen in der Psychiatrisch-neurologischen Sektion des Budapester Kgl. Ärztevereins am 22. V. 1936.

in einigen Fällen, in denen die Besserung stetige Fortschritte machte und ich dadurch den Eindruck gewann, daß von der Fortsetzung der Kur noch weitere Besserungen zu erwarten waren, gab ich auch 70 Injektionen, worauf sich der Zustand der Kranken tatsächlich noch weiter besserte. Ich gab am ersten Tage 3 ccm, am zweiten 5 ccm, am dritten 7,5 ccm, am vierten 10 ccm einer *20%igen Natriumthiosulfatlösung*. Am fünften Tage wurde eine Pause eingeschaltet und dann 3 Tage lang je 10 ccm gegeben, darauf folgten nach einer eintägigen Pause wieder 3 Injektionstage und so fort. Bei ambulanten Kranken begnügte ich mich in mehreren Fällen mit nur an jedem zweiten Tag gegebenen Injektionen, um den Kranken das übermäßige Gehen zu ersparen.

Da ein ausführlicher Bericht über 40 Krankheitsgeschichten an dieser Stelle nicht möglich ist, fasse ich meine Erfahrungen zusammen. Von den 40 Kranken waren 18 männlichen und 22 weiblichen Geschlechtes. Sie standen im Lebensalter zwischen 13 und 48 Jahren (die Krankheit hatte jedoch in keinem einzigen Falle nach dem 40. Lebensjahre begonnen). Unter diesen 40 Fällen besserten sich erheblich 18 = 45%, mäßig 8 = 20%, in geringem Grade 12 = 30%, gar nicht 2 = 5%.

Marburg fordert mit vollem Recht von den therapeutischen Mitteilungen, daß die behandelten Fälle in 4 Gruppen geteilt werden, wobei eine stationäre Form, eine chronisch progressive Form mit kaum merkbaren Remissionen, eine remittierende bzw. intermittierende Form und eine akute Form zu unterscheiden wäre. Unter meinen Kranken konnten in die stationäre Gruppe kein einziger, in die chronisch progressive Gruppe 24, in die remittierende Gruppe 12, in die akute Gruppe 4 Fälle eingereiht werden.

Von den 24 chronisch-progressiven Fällen besserten sich 10 erheblich, 6 mäßig, 7 wenig und 1 Fall gar nicht. Von den 12 remittierenden Fällen besserten sich 5 sehr erheblich, 2 mäßig, 5 wenig. Von den 4 akuten Fällen besserten sich 3 weitgehend und 1 Fall gar nicht.

Vergleichen wir die chronisch-progressive und die remittierende Gruppe, so sehen wir, daß das prozentuelle Verhältnis der erheblich gebesserten Fälle das gleiche ist (42%), was an und für sich schon für die Wirksamkeit der Therapie spricht.

Wie der Heilerfolg sich je nach der Dauer des Bestehens der Krankheit gestaltete, ist aus nachstehender Tabelle ersichtlich.

Dauer der Krankheit	Zahl der Fälle	Erheblich gebessert	Mäßig gebessert	Wenig gebessert	Nicht gebessert
0—3 Jahre	19	13	3	2	1
3—5 „	8	3	2	3	—
5—17 „	13	2	3	7	1
Zusammen	40	18	8	12	2

Aus dieser Zusammenstellung geht hervor, daß die weniger als 3 Jahre bestehenden Fälle am besten reagierten und die seit 5—17 Jahren bestehenden am schlechtesten.

Unter den behandelten Kranken lagen 22 in der Klinik, 3 im Spital der Barmherzigen Brüder, 3 im St.-Imre-Spital zu Sashalom, 1 im Bethesda-Spital, 1 im Rotkreuz-Spital, 3 daheim, während 7 ambulant behandelt und untersucht wurden. Den Herren Chefärzten Dr. Karl v. Pákozdy, Dr. Eugen Józsa, Dr. László v.

Csek-Szombathy und Dr. László Borsos möchte ich auch an dieser Stelle meinen besten Dank für die Liebenswürdigkeit aussprechen, mit der sie mir die Beobachtung ihrer Kranken ermöglichten.

Um nun einen Einblick über die Art und Weise der Besserung zu geben, berichte ich nachstehend über die Änderungen der einzelnen Symptome.

Die Sehschärfe konnte ich bei 26 Kranken wiederholt untersuchen lassen. Unter diesen hatten 9 eine verminderte Sehschärfe, die sich bei 7 Kranken gebessert hat. Die Besserung war in mehreren Fällen sehr erheblich, so stieg in einem Falle der Visus von 25 Oxyoptrien auf 60 Oxyoptrien (von $^{5}/_{12}$ auf $^{5}/_{5}$) an, in einem anderen Falle von 15 auf 60 Oxyoptrien (von $^{5}/_{20}$ auf $^{5}/_{5}$). Bei einem Kranken besserte sich der Visus nicht, die Sehschärfe betrug jedoch in diesem Falle auf beiden Augen 50 Oxyoptrien ($^{5}/_{6}$) und war vielleicht auch vor der Erkrankung nicht besser. Eine Verschlechterung des Visus habe ich nur in einem sehr schweren akuten Falle beobachtet, auf welchen ich später noch besonders eingehen werde. Eine Augenmuskellähmung bestand in 9 Fällen und wurde in 4 Fällen behoben. Nystagmus fand ich in 28 Fällen und sah ihn in 8 Fällen wieder verschwinden. Beim Stehen mit geschlossenen Augen und aneinander geschlossenen Füßen verloren vor der Kur 20 Kranke völlig das Gleichgewicht. 10 von diesen konnten nach der Kur stehen, sie schwankten jedoch, 2 schwankten nicht. Vor der Kur schwankten 9, von diesen schwankten 4 nach der Kur nicht mehr.

In 28 Fällen bestand eine Schwäche der oberen Extremitäten; diese verschwand in 17 Fällen, besserte sich in 7 Fällen und blieb in 4 Fällen unbeeinflußt. Intentionszittern war in 30 Fällen vorhanden, hörte in 13 Fällen auf, wurde in 10 Fällen schwächer und blieb in 7 Fällen unverändert. In ähnlicher Weise besserte sich bei einem großen Teil der Fälle die Schrift und die Fingerfertigkeit.

Eine Hypertonie der unteren Extremitäten wurde in 35 Fällen beobachtet. Dieselbe hörte in 11 Fällen auf, nahm in 22 Fällen ab und änderte sich in 2 Fällen nicht. Das aktive Heben der ausgestreckten Extremitäten in liegender Stellung war in 35 Fällen eingeschränkt, wurde während der Kur in 11 Fällen völlig normal, besserte sich in 20 Fällen und blieb in 3 Fällen unverändert, in einem Falle ist eine Verschlechterung eingetreten.

Der Gang war in 39 Fällen gestört, unter diesen konnten 4 nicht einmal mit Unterstützung gehen. Nach der Behandlung konnte von diesen einer ohne Unterstützung, 2 mit Unterstützung gehen, während ein seit 17 Jahren Kranker auch nach der Kur nicht gehen konnte. Nur mit Unterstützung bzw. sich an den Möbelstücken festhaltend konnten 14 Kranke gehen. Von diesen konnten nach der Kur 10 ohne Unterstützung bzw. ohne Festhalten gehen: einer ganz einwandfrei, unverändert gingen 3 und einer konnte auch mit Unterstützung nicht gehen. Allein gehen, wenn auch spastisch bzw. ataktisch vermochten 21 Kranke; unter diesen war nach der Kur der Gang von 7 Kranken normal, er besserte sich bei 13, bei einem jedoch nicht. Beim Kniehackenversuch zeigte sich in 14 Fällen eine hochgradige Ataxie, dieselbe verschwand in 2 Fällen, nahm in 9 Fällen ab, besserte sich aber in 3 Fällen nicht. Eine geringere Ataxie war mit Hilfe des Kniehackenversuches in 17 Fällen nachweisbar; diese verschwand in 8 Fällen, besserte sich in 9 Fällen.

Ein beiderseits positiver Babinski war in 30 Fällen nachweisbar; er verschwand während der Behandlung in 6 Fällen beiderseits, in 6 Fällen auf der einen Seite. Einseitiges Babinskizeichen beobachtete ich in 4 Fällen, es verschwand in 2 Fällen. Beiderseitiger Oppenheimreflex war in 16 Fällen vorhanden, er verschwand in 6 Fällen beiderseits, auf der einen Seite in 3 Fällen. Einseitiger Oppenheim war in 6 Fällen auslösbar und verschwand in allen 6 Fällen. Beiderseitigen Patellarklonus fand ich in 8 Fällen, derselbe hörte in 3 Fällen auf beiden Seiten, in einem Falle nur auf der einen Seite auf. Einseitigen Patellarklonus beobachtete ich in 5 Fällen, dessen Verschwinden in 3 Fällen. Beiderseitiger Fußklonus war in 23 Fällen vorhanden, hörte in 3 Fällen auf beiden Seiten, in einem Falle nur auf der einen Seite auf. Der in 6 Fällen vorhandene einseitige Fußklonus verschwand in 3 Fällen. Bauchdeckenreflexe fehlten in 38 Fällen. Diese kehrten in 11 Fällen wieder zurück, darunter in 3 Fällen sämtliche Reflexe nach vorherigem Fehlen.

Miktionsstörungen bestanden in 15 Fällen. Sie hörten in 9 Fällen auf, nahmen in 1 Falle ab und blieben in 5 Fällen unverändert.

Störungen der Oberflächenempfindung waren in 17 Fällen nachweisbar und verschwanden in 7 Fällen, besserten sich in 2 Fällen. Störungen der Bewegungs- und Lageempfindung waren in 9 Fällen anwesend, hörten in 6 Fällen auf, verminderten sich in 2 Fällen, blieben in einem Falle unverändert. Paraesthesien wurden in 13 von 24 Fällen behoben.

Obwohl die Behandlung bereits in den ersten Fällen erfolgreich zu sein versprach, war es doch fraglich, ob die eingetretenen Besserungen nicht einfache Spontanremissionen waren. Mit der Zunahme der Zahl der Fälle schwand diese Besorgnis allmählich, die Besserung trat nämlich konsequent am Beginn der Kur ein. Es schien daher nicht wahrscheinlich, daß alle 38 gebesserten Fälle gerade zur Zeit des Beginnes der Behandlung zufällig in eine Spontanremission gelangt wären, um so weniger, als der Zustand der Kranken sich zum großen Teil gerade vor dem Beginn der Kur allmählich verschlechtert hatte und die deutliche Progression genau mit dem Einsetzen der Behandlung von einer stetig zunehmenden Besserung abgelöst wurde. Für die Wirksamkeit der Kur sprach außerdem auch der Umstand, daß die Besserung sich sozusagen regelmäßig schon während der ersten 10 Injektionen meldete. Ich habe auch erwogen, ob die Besserung nicht dem Liegen, der Ruhe zuzuschreiben wäre? Dem widersprach jedoch entschieden der Umstand, daß der Zustand mehrerer Kranken während des wochenlangen Liegens vor der Kur sich ständig verschlechtert hatte und die Besserung gleichzeitig mit dem Beginn der Kur rasch eintrat. So geschah es auch bei einem 13. Jahre alten Mädchen, das vor dem Beginn der Behandlung 7 Wochen lang im Bett lag und dessen Zustand sich während dieser Zeit dermaßen verschlechterte, daß sie selbst mit Unterstützung weder gehen noch stehen konnte, ja nicht einmal fähig war, im Bette aufzusitzen. Nach der Beendigung der Kur konnte sie die Klinik allein, ohne Unterstützung gehend, verlassen. Ich habe auch ambulante Kranke behandelt, bei denen also ein dauerndes Liegen als Heilfaktor nicht in Betracht kommen konnte und deren Zustand sich dennoch gebessert hatte.

Erwähnenswert ist noch der Fall eines 19jährigen jungen Mannes, bei dem starker Kopfschmerz, Schwindelgefühl, fast täglich wiederkehrendes Erbrechen, Stauungspapille sowie eine von Zeit zu Zeit anfallsweise auftretende Paraesthesie auf der rechten Körperhälfte den Verdacht eines Hirntumors erweckte. Nach einer *Wismut-Kur* besserte sich sein Zustand einigermaßen, insofern, als das Erbrechen seltener auftrat und die Störung der Tiefensensibilität im rechten Handgelenk nachließ; dagegen blieben Kopfschmerzen, Schwindelgefühl, Stauungspapille, Schwäche, Ungeschicklichkeit und Astereognose der rechten Hand, vollständiges Fehlen der Bauchdeckenreflexe, ataktischer Gang, Rombergsches Zeichen unbeeinflußt. Da begannen wir die *Natriumthiosulfatkur*, während welcher nicht nur die Kopfschmerzen, das Schwindelgefühl und das Erbrechen aufhörten, sondern auch die Stauungspapille, die Störung der Tiefensensibilität, der ataktische Gang und das Rombergsche Zeichen verschwanden; es kehrten auch sämtliche Bauchdeckenreflexe zurück. Nur der Nygstagmus, die Stereoagnosie, die geringe Schwäche und Ungeschicklichkeit der rechten Hand blieben unverändert, diese Symptome bestanden jedoch schon seit 5 Jahren und haben bereits vor Jahren den Kranken zum Erlernen des Schreibens mit der linken Hand gezwungen.

Ich muß nun noch die beiden Kranken eingehender besprechen, die in meiner obigen Zusammenstellung als nicht gebessert angeführt sind. Der eine von diesen war bereits seit 10 Jahren krank und litt an sehr schweren Lähmungen der oberen und unteren Extremitäten mit ausgedehnten Sensibilitätsstörungen. Sein Fall gehörte in die chronisch-progressive Gruppe. Er erhielt insgesamt 25 Injektionen. Als Erfolg der Behandlung konnte ich in seinem Zustande nur infofern eine Veränderung beobachten, als sein Nystagmus verschwand und die mit dem Kniehackenversuch nachweisbare Ataxie abnahm. Diese Besserung war so geringfügig, daß ich den Fall nicht einmal in die Gruppe der wenig gebesserten einreihen konnte. Hier erblicke ich den Grund des Ausbleibens einer belangreichen Besserung in dem seit langer Zeit schwerem Zustande des Kranken.

In dem zweiten nicht gebesserten Falle handelte es sich um ein 21jähriges Mädchen; es war dies ein akuter Fall mit stürmischer Progression, die auch nach 18 Injektionen weiter fortschritt, weshalb die Injektionen eingestellt wurden. 32 Tage nach dem Aussetzen der Injektionen starb die schlafsüchtige, gänzlich unbeholfene Kranke an den Folgen eines Sigmavolvulus. Die Sektion deckte im Gehirn und Rückenmark ungewöhnlich ausgedehnte schwere Veränderungen auf. Im Großhirn waren auf jeder Schnittfläche durchschnittlich 50 Plaques sichtbar. Sowohl bei der makroskopischen, wie auch bei der mikroskopischen Untersuchung erwiesen sich mehr als 90 % der Plaques als frische Veränderungen. In diesem Falle bestand also ein sich ungewöhnlich stürmisch ausbreitender Prozeß, und die Wirkungslosigkeit der Therapie kann vielleicht auf diesen Umstand zurückgeführt werden.

Auf Grund meiner mit *Natriumthiosulfat* bei multipler Sklerose gemachten günstigen Erfahrungen halte ich für möglich, daß die von Horn bei 8 Kranken ausgeführte *kombinierte Elektrokollargol-Osmon-Natriumthiosulfat-Therapie* ihre Wirksamkeit hauptsächlich dem *Natriumthiosulfat* verdankt, welches der Autor zwar nur aus dem Grunde gegeben hat, um durch die Reduktion des *Silbers* die flüchtige Wirkung des *Elektrokollargols* dauernd zu gestalten. Für diese meine Auffassung spricht auch jener Fall von Horn, in welchem eine reine *Elektrokollargolkur* unwirksam war, dagegen die nachfolgende *Elektrokollargolkur* in Kombination mit *Natriumthiosulfat* eine wesentliche Besserung brachte.

Es liegt mir fern, behaupten zu wollen, daß mit der *Natriumthiosulfatkur* die multiple Sklerose geheilt oder auch nur endgültig zum Stillstand gebracht wird; ich beobachtete nämlich bei 12 früher behandelten Kranken in 6 Fällen ein Rezidiv. Wurden dann innerhalb einiger Tage nach dem Eintritt der Verschlechterung die Kranken wieder in Behandlung genommen, konnte ich erfahren, daß der Rückfall aufgehalten wurde und wieder eine Besserung auftrat. Wurde der Kranke jedoch erst eine längere Zeit nach dem Eintritt des Rezidivs in Behandlung genommen, war das Ergebnis weit ungünstiger. Im allgemeinen waren die Erfolge der Kuren nach Rezidiven immer ungünstiger als bei der ersten Kur. Es scheint mir deshalb empfehlenswert, zwischen den einzelnen Kuren nie eine längere Pause als 1 Monat einzuschalten. Eine Pause von einigen Wochen ist indessen meistens auch aus dem Grunde notwendig, um den von den 50 Injektionen stark in Anspruch genommenen Venen eine Ruhe zu gönnen. Nach meinem Ermessen sollen im Falle einer allmählich fortschreitenden Besserung 3—4 Kuren mit je 50 Injektionen, mit Pausen von einem Monat durchgeführt werden. Ein endgültiges Urteil über die Zahl der Kuren konnte ich mir jedoch noch nicht bilden, da auch der seit längerer Zeit behandelte Kranke erst seit 2 Jahren beobachtet wird.

Ich muß noch bemerken, daß die Kur völlig gefahrlos ist, daß keinerlei schädliche Nebenwirkungen auftreten, daß die Kranken die Injektionen gut ertragen. Es klagten nur 2 Kranke zeitweise unmittelbar nach der Injektion über Brechreiz, was aber wohl auf ihre Aufregung zurückzuführen war.

Meine Ansicht kann ich darin zusammenfassen, daß die Anwendung der *Natriumthiosulfatkur* der Mühe wert ist, da sie wirksamer zu sein scheint als die anderen Behandlungsmethoden, ferner weil sie gänzlich ungefährlich ist und die Kranken nicht der Gefahr von Verschlechterungen aussetzt, wie es — leider — bei den verschiedenen Fiebertherapien oft und in bedeutendem Grade vorkommt.

Zusammenfassung: Bei der Behandlung von 40 an multipler Sklerose leidenden Kranken mit *intravenösen Einspritzungen einer 20%igen Natriumthiosulfatlösung* beobachtete ich bei 38 eine Besserung des Krankheitsbildes; die Besserung war bei 18 Kranken sehr erheblich, bei 8 Kranken mäßig, bei 12 Kranken gering. Die Kur ist frei von schädlichen Nebenwirkungen.

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Übersichten

Erfahrungen mit einer Schwerstbeschädigtenabteilung[1])

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Aus dem Krankenhause Bergmannstrost in Halle (Saale)
(Direktor: Prof. Dr. Joh. Volkmann)

Es ist noch nicht sehr lange her, seitdem dank der ständigen Bemühungen von Lohmar, dem Verwaltungsdirektor des Verbandes der Deutschen gewerblichen Berufsgenossenschaften, Schwerstbeschädigten-, Amputierten- und Schulungsabteilungen errichtet wurden. Sie gehen auf Versuche zurück, die schon im Kriege mit der Zusammenlegung bestimmter Gruppen von Verletzten mit Schädel-, Kieferschüssen usw. gemacht wurden. Meines Wissens sind dann später die ersten regelrecht aufgezogenen derartigen Abteilungen die in Münster, meiner früheren Wirkungsstätte, Oberhausen und Koblenz gewesen, während im Krankenhaus Bergmannsheil I in Bochum, Magdeburg (Heilanstalt Prof. Blencke), Breslau (Wenzel-Hanke-Krankenhaus), Halle (Bergmannstrost), Berlin-Dahlem (Oskar-Helene-Heim) und Hohenlychen ähnliche Abteilungen folgten. Ich möchte Ihnen nun einiges über meine Erfahrungen auf diesem Gebiete mitteilen, einem Gebiete, das in Ihrer Vereinigung, soviel ich weiß, noch nicht besprochen worden ist und an dem ja Vertreter der Versicherungsträger wie Ärzte in gleicher Weise interessiert sind.

Über die Berechtigung des Bestehens einer solchen Abteilung ist verschiedentlichst gestritten worden. Aber ich trete unbedingt für sie ein; nicht deshalb, weil ich selbst eine solche Abteilung habe, sondern obwohl ich in meinem früheren Krankenhause eine Zeitlang auch zu den Leidtragenden gehörte, denen die Frischamputierten über kurz oder lang weggenommen wurden. Man kann sich aber im allgemeinen tatsächlich nicht für einige wenige Amputierte oder Schwerverletzte den ganzen großen Apparat leisten, der auf einer solchen besonderen Abteilung sozusagen von selbst in Kraft tritt und sich selbsttätig abwickelt. Und selbst wenn man sich wohl das eine oder andere Mal die besondere Mühe macht, so kann man sich doch nicht jeden Tag zu derselben Zeit und Stunde mit derselben Ruhe und Muße diesen Dingen widmen. Dazu braucht man eben geschulte Hilfskräfte.

Es muß deshalb eine solche Abteilung einem Krankenhause angeschlossen sein, das an sich schon hinreichend groß ist und auch von sich aus über eine

[1]) Nach einem Vortrag bei der Tagung der Schlesischen Vereinigung berufsgenossenschaftlicher Verwaltungen in Neusalz a. d. O. am 25. April 1936.

Fortschritte der Therapie 12: 738-743 (1936).

THERAPY OF MULTIPLE SCLEROSIS WITH SODIUM THIOSULFATE[1)]

Although SULFUR has been employed for a long time in the treatment of multiple sclerosis in the form of FIBROLYSINE, SULFOSINE, SULFROGEL etc., we were led to experiments with SODIUM THIOSULFATE not by the intention to further develop sulfur therapy, but by an incidental observation.

In May of 1934, we began treating in our clinic a 22 year old woman, who had been suffering from multiple sclerosis for three years, with NEOSILVERSALVERSAN. After the third injection the findings of the nervous system remained unchanged, but a very extensive dermatitis appeared (on both lower legs, on the inner sides of upper and lower arms). We therefore gave the patient intravenous injections of SODIUM THIOSULFATE in doses of 0.6, 1.0, 1.5 g. Already after the first 4 injections, we observed a surprising improvement: On admission, the patient could only walk holding on to beds or with support; her gait now improved to such an extent that she was able to walk alone, without support, to the chapel which was located in another building. We theorized that the SODIUM THIOSULFATE was involved in this improvement and we therefore continued the injections even after the dermatitis had subsided, in daily doses of 1.5 g. The patient's condition continued to improve, her initially very spastic and ataxic walk became more effortless and secure, and after the 25th injection she took longer walks without attendants.

1) Lecture of 5/22/1936 in the psychiatric-neurologic section of the Royal Medical Association of Budapest

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Fortschritte der Therapie, vol. 12, Dec. 1936

Nystagmus and intention tremor disappeared, hypertonia of the lower extremities and ataxia demonstrable by the knee jerk reflex subsided, Babinski's reflex on both sides regressed, her poor handwriting became completely normal, vision improved: in the right eye from 25 to 60 oxyopters (from 5/12 to 5/5), in the left eye from 40 to 60 oxyopters (from 5/8 to 5/5).

On the strength of this experience, trial injections with SODIUM THIOSULFATE without SILVERSALVERSAN seemed to be worthwhile in other patients as well. Because of the frequency of spontaneous remissions in multiple sclerosis, a large sample group of patients was needed in order to assess the therapeutic effectiveness of this agent. LAUBÁL did not participate in these experiments since he had meanwhile left the hospital.

In the course of 2 years, I was able to treat 40 patients on a regular basis and to examine these repeatedly. I usually gave 50 injections during the course of a treatment. In some cases, which improved progressively and which gave me the impression that continuation of the treatment might bring about further improvement, I gave as much as 70 injections, which did indeed result in further improvement of the patients' condition. I administered a 20% SOLUTION of SODIUM THIOSULFATE, 3ccm on the first day, 5 ccm on the second day, 7.5 ccm on the third day, 10 ccm on the fourth day. On the fifth day we interrupted therapy for a day and then we gave 10 ccm on three consecutive days. After a one day interval, injections were given for three more days and so forth. Several of the ambulatory patients were given injections only on alternate days in order to spare these patients excessive walking.

Since a detailed report on 40 case histories is not possible here, I shall summarize my experiences. Of the 40 patients, 18 were male and 22 were female. Their ages ranged between 13 and 48 yaers (there was not a single case, however, in which the disease had started after the age

of 40). Among these 40 cases, improvement was as follows: significant - 18 (45 % of cases), moderate - 8 (20 % of cases), minor - 12 (30 % of cases), no improvement - 2 (5 % of cases).

MARBURG is fully justified in requesting that, in therapeutic reports the cases treated be divided into 4 groups, differentiating between chronic form, chronic progressive form with minimal remission, remitting or intermittent form and acute form. Among my patients, there were none in the chronic group, 24 in the chronic progressive group, 12 in the intermittent group, 4 in the acute group.

Of the 24 chronic progressive cases, 10 improved significantly, 6 moderately, 7 minimally and 1 not at all. Of the 12 intermittent cases, 5 improved significantly, 2 moderately, 5 minimally. Of the 4 acute cases, 3 improved considerably and 1 not at all.

In comparing the chronic progressive cases with the intermittent ones, we see that the percentage of significantly improved cases is the same in both (42 %), which in itself speaks for the effectiveness of the treatment.

The following table illustrates the success of the treatment in reference to the duration of the disease.

duration of disease	number of cases	significant improvement	moderate improvement	minimal improvement	no improvement
0-3 years	19	13	3	2	1
3-5 years	8	3	2	3	-
5-17 years	13	2	3	7	1
tal	40	18	8	12	2

This table shows that cases with a disease duration of less than 3 years have the most successful results and those with a 5-17 year duration have the least successful results.

Of the patients treated, 22 were hospitalized in our clinic, 3 in the Barmherzigen Brüder Hospital, 3 in the St.-Imre-Hospital in Sashalom, 1 in the Bethesda-Hospital, 1 in the Rotkreuz-Hospital, 3 were at home and 7 were treated and examined on an ambulatory basis. I would like to express my gratitude to Drs. KARL V. PAKOZDY, EUGEN JOZSA, LASZLO V. CZEH-SZOMBATHY and LASZLO BORSOS for their kindness of allowing me the observation of their patients.

In order to give an insight into the type of improvement, I shall describe the changes of the individual symptoms.

The vision of 26 patients was examined repeatedly. 9 of these had impaired vision, which was improved in 7 patients. In several cases the improvement was very substantial: In 1 case vision increased from 24 oxyopters to 60 oxyopters (from 5/12 to 5/5), in another from 15 to 60 oxyopters (from 5/20 to 5/5). In one patient vision did not improve, in this case, however, vision in both eyes was 50 oxyopters (5/6) and was probably not better before the onset of the disease. I observed a deterioration of vision in only one very severe, acute case which I shall describe later in detail. Paralysis of ocular muscels was present in 9 cases and was alleviated in 4. Nystagmus was observed in 28 cases and subsided in 8. Before treatment, 20 patients completely lost their equilibrium when standing with eyes and feet closed. After treatment, 10 of these were able to stand, although they were unstable, with the exception of 2 cases. Before treatment, 9 were unstable, 4 of these were stable after treatment.

Weakness of the upper extremities was present in 28 cases; this

subsided in 17 cases, improved in 7 cases and remained unchanged in 4 cases. Intention tremor was observed in 30 cases, subsided in 13 cases, was improved in 10 cases and remained unchanged in 7 cases. Hand writing and finger motoricity improved in a similar fashion in a great number of cases.

Hypertonia in the lower extremities was observed in 35 cases. This receded in 11 cases, decreased in 22 and did not change in 2 cases. Active lifting of extended extremities in the supine position was impaired in 35 cases, became completely normal during treatment in 11 cases, improved in 20 cases and remained unchanged in 3 cases; in 1 case this deteriorated.

Gait was impaired in 39 cases, 4 of these could not even walk with support. After treatment, 1 of these was able to walk without support, 2 with support, while 1 patient who has had the disease for 17 years was not able to walk even after treatment. 14 patients were able to walk only with support or holding on to furniture. After treatment, 10 of these were able to walk without support or holding on: 1 completely alone, 3 walked without change and 1 could not walk even with support. 21 patients were able to walk alone, although in a spastic or ataxic manner; after treatment, the gait of 7 of these patients was normal, improved in 13 and did not change in 1. The knee jerk reflex revealed severe ataxia in 14 cases, this receded in 2 cases, decreased in 9 cases, but did not improve in 3 cases. A milder form of ataxia could be demonstrated by the knee jerk reflex in 17 cases; this subsided in 8 cases, improved in 9 cases.

A bilateral positive Babinski could be demonstrated in 30 cases; during treatment it receded in 6 cases, in 6 cases on one side only. Unilateral positive Babinski was observed in 4 cases, it subsided in 2 cases. Bilateral Oppenheim's reflex was present in 16 cases, in 6 cases it receded in both sides, in 3 cases on one side. Unilateral Oppenheim

was confirmed in 6 cases and subsided in all of these. Bilateral patellar clonus was found in 8 cases, it subsided in 3 cases on both sides, in 1 case on one side only. Unilateral patellar clonus was observed in 5 cases, it subsided in 3 cases. Bilateral foot clonus was present in 23 cases, subsided on both sides in 3 cases and on one side in 1 case. Unilateral foot clonus, present in 6 cases, subsided in 3 cases. Reflexes of the abdominal wall were absent in 38 cases. These returned in 11 cases, in 3 out of these, all reflexes which had previously been absent, returned.

Difficulty in micturition was present in 15 cases. This subsided in 9 cases, decreased in 1 case and remained unchanged in 5 cases.

Disturbances of cutaneous sensation were confirmed in 17 cases, subsided in 7 and improved in 2 cases. Disturbances of motor and position sense were present in 9 cases, receded in 6 and decreased in 2 cases and remained unchanged in 1 case. Paresthesias were alleviated in 13 out of 24 cases.

Although the treatment promised to be successful after the first few cases, it was still questionable whether the improvements were not simply spontaneous remissions. This concern lessened as the number of cases increased, since improvement consistently occurred with the beginning of treatment. It therefore did not seem probable that all 38 improved cases went into remission exactly at the time of onset of treatment; all the less since, in a large number of cases, the patients' conditions had gradually deteriorated before the beginning of treatment and this obvious progression was relieved by a steady improvement just at the onset of treatment. The fact that improvement regularly set in during the first 10 injections already, is another indication of the effectiveness of the treatment. I have also considered the question whether the improvement might not be due to bed rest and quiescence. However, this was contradicted by the fact that the condition of several patients had deteriorated steadily

during prolonged confinement to bed before treatment and that improvement rapidly became apparent, synchronous with the beginning of treatment. This was also the case with a 13 year old girl, who had been confined to bed for 7 weeks before treatment and whose condition during this time deteriorated to such an extent that she could not even walk or stand with support or sit up in bed. After termination of treatment, she could leave the clinic walking alone, without support. I have also treated ambulatory patients; in these, prolonged bed rest could not have been a factor affecting the healing process, and yet their condition improved.

The case of a 19 year old man is worth mentioning. His severe headaches, dizziness,, almost daily recurring vomiting, choked disk and paresthesia on the right side which exacerbated periodically, seemed to indicate a brain tumor. After treatment with BISMUTH, his condition improved somewhat, to the extent that vomiting occurred less often and impairment of deep sensation in the right wrist diminished; however, headaches, dizziness, choked disk, weakness, stumbling, astereognosis of the right hand, complete absence of reflexes in the abdominal wall, ataxic gait and Romberg's sign remained unchanged. We then initiated treatment with SODIUM THIOSULFATE. Not only headaches, dizziness and vomiting, but also choked disk, impairment of deep sensation, ataxic gait and Romberg's sign receded; all reflexes in the abdominal wall returned as well. Only nystagmus, astereognosis, minor weakness and ineffectiveness of the right hand remained unchanged. These symptoms, however, had been present for 5 years and had forced the patient years ago to write with his left hand.

I will now discuss in detail the two patients who were described as not improved in the above summary of cases. One of these had been ill for 10 years and suffered from severe paralyses of the upper and lower

extremities with extensive impairment of sensation. His case belonged into the chronic progressive group. He received a total of 25 injections. The only improvement I could observe was subsiding of the nystagmus and diminished ataxia demonstrable by the knee jerk reflex. The improvement was so minor that I could not even list his case in the group with minimal improvement. I see the failure to improve in the fact that the patient's condition had been severe for a prolonged period. The second patient who did not improve was a 21 year old woman. It was an acute case with rapid progression, which continued even after 18 injections. We therefore discontinued the injections. 32 days after termination of injections, the hypersomniac, totally helpless patient succumbed to a volvulus of the sigmoid colon. Autopsy revealed severe changes of unusual extensiveness in the brain and the spinal cord. In the cerebrum, there was an average of 50 plaques on each section. During macroscopic as well as microscopic examinations, more than 90 % of the plaques proved to be recent changes. The disease was progressing with unusual rapidity in this case, and this circumstance may be responsible for the failure of the treatment.

Based on my favorable experiences with SODIUM THIOSULFATE in the treatment of multiple sclerosis, I think that the main factor in effectiveness of COMBINED ELECTROCOLLARGOL-OSMON-SODIUM THIOSULFATE THERAPY of patients with multiple sclerosis, as conducted by HORN on 8 patients, may possibly be the SODIUM THIOSULFATE. The author, however, gave this agent only for the purpose of reducing the SILVER and thereby maintaining the transient effect of ELECTROCOLLARGOLKUR My opinion is also supported by the case of one of HORN's patients in whom TREATMENT alone was ineffective, but who showed significant improvement after subsequent ELECTROCOLLARGOLKUR TREATMENT in combination with SODIUM THIOSULFATE.

I do not intend to claim that treatment with SODIUM THIOSULFATE can cure or definitely arrest multiple sclerosis; because in 6 out of 12 previously treated patients I observed a recurrence. When treatment was reinitiated within a few days after onset of exacerbation, I was able to ascertain that the relapse was arrested and improvement reappeared. If, however, the patient was not treated soon after onset of recurrence, the results were less favorable. In general, the results of treatment after recurrence were always less favorable than after the initial treatment. I therefore do not recommend an interval of more than 1 month between each treatment. However, an interval of a few weeks is necessary in most cases in order to let the veins recover from the severe strain of the 50 injections. In my experience, 3 to 4 treatments of 50 injections each with intervals of 1 month should be given in cases of gradually progressing improvement. I could not make a final decision on the number of treatments, since even the patient who had been treated for a longer period of time has not been observed for more than 2 years.

In addition, I would like to mention that the treatment is completely harmless, that no injurious side effects whatsoever occur, that the patients tolerate the injections well. Only 2 patients occasionally complained of vomiturition immediately after the injection, which may have been caused by excitement.

To summarize my opinion, treatment with SODIUM THIOSULFATE is worth the effort since this seems to be more effective than the other forms of treatment; furthermore, it is completely harmless and does not subject the patients to the danger of exacerbation, which - unfortunately - occurs frequently and to a substantial degree with the various fever therapies.

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mosomes found in the KB cells was absent in the third subline and some of its cells were endoreduplicated. A hypothesis of genomic selection is discussed.

We are grateful to Dr. Guy Cousineau for assistance in carrying out the PPLO examination on cell cultures.

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On the Synthesis of Taurine from Sulfate by the Chick: I. Influential Dietary Factors.* (31588)

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The sulfate requirements of many animals are met through the degradation of the sulfur amino acids. Some investigations have indicated that young chicks can utilize dietary sulfate in the synthesis of taurine. Lowe and Roberts(1) reported that within 36 minutes after administration of sulfate-S^{35} to a chick embryo, labeled taurine could be detected. Sulfate-S^{35} was not found in cystine, cysteine or methionine in the chick embryo(1), and we have found little or no S^{35} in cystine of the tissues examined in the chick after sulfate dosing(2). Recent reports have shown the universal presence of taurine in practically all tissues and have shown that the functions of taurine in the different tissues were diversified and essential(3,4,5,6,7,8). In view of these reports, the conversion of sulfate to taurine in the animal becomes increasingly significant and of interest.

The over-all purpose of the investigation reported in this manuscript was to study the utilization of sulfate-S^{35} in the chick, specifically the synthesis of taurine from sulfate as it is influenced by the nutritional state of the animal.

Experimental. One-day-old White Leghorn cockerels were maintained on purified basal diets(9) with supplements for a period of time, usually 14 days, as specified in each trial. Groups of 4 or 5 chicks were then orally dosed with 20 microcuries of carrier-free $H_2S^{35}O_4$ by means of a one ml pipette inserted through the mouth into the crop and emptied by gravity flow. The C^{14}-labeled compounds were administered by subcutaneous injection.

Blood, bile and liver samples were taken at various times after dosing. The blood from each group was pooled, allowed to coagulate, then centrifuged. The serum was assayed for total and protein bound S^{35} activity.

*Data in this paper are from a thesis submitted by the senior author in partial fulfillment of the requirements for the degree of Master of Science. West Virginia Univ. Agri. Exp. Station Scientific Paper 904. This research was supported in part by grant GB-406 of Nat. Science Foundation.

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TABLE I. Incorporation of Orally Dosed Sulfate-S^{35} into Taurine in the Various Tissues of 14-Day-Old Chick Fed a Purified Basal Diet.

Tissue	Hr after dose	Taurine-S^{35}, cpm/g tissue	Total taurine, μg/g tissue	S.A., cpm/μg
Heart	1	900	100.0	9.00
	2	2,500	125.0	20.00
	3	4,700	100.0	47.00
	4	19,400	121.5	159.67
	6	17,400	121.5	143.21
Spleen	1	300	47.3	6.34
	2	750	59.9	12.52
	3	2,800	56.3	50.62
	4	6,550	45.0	145.55
	6	12,850	56.3	228.24
Kidney	1	462	57.5	8.03
	2	1,122	43.8	25.62
	3	1,584	54.5	29.06
	4	7,260	51.3	141.52
	6	7,260	44.5	163.15
Liver	1	1,100	58.7	18.74
	2	2,750	65.0	42.31
	3	5,600	63.3	88.47
	4	13,100	54.0	242.59
	6	27,150	54.0	502.77

The bile fluid was chromatographed in butanol which had been equilibrated with acetic acid (1:1, 1-butanol:3% acetic acid) on Whatman No. 4 paper that had previously been dipped in 70% acetic acid solution and dried. Liver homogenates (20%) were prepared with distilled water and the deproteinized supernatant was chromatographed on Whatman No. 4 paper in an 80% aqueous phenol solvent system. Other tissues were handled in a manner similar to that of liver. The radioactivity of the chromatograms was counted in a liquid scintillator using a PPO: dimethyl-POPOP:toluene (5 g:.1 g:liter) scintillation solution. Total taurine concentrations of the liver and other tissues were determined by the method of Pentz *et al*(10).

Results and discussion. The extent of sulfate-S^{35} incorporation into the taurine of the various tissues in the chick was determined. The taurine-S^{35} concentration of liver, heart, kidney, and spleen was measured (Table I). *In vitro* studies with chick liver enzymes resulted in the production of labeled compounds similar to those found in the chick body indicating that intestinal microbial synthesis was not an interfering factor. The highest concentration of total taurine occurred in the heart. The results indicated that sulfate-S^{35} was incorporated into taurine-S^{35} in all of the tissues analyzed as early as one hour after the dose and the concentrations continued to increase with time. From these data, it appears that each organ tested is equipped with the necessary enzymes for conversion of sulfate-sulfur into taurine. The taurine-S^{35} of the heart was higher than that of either the spleen or kidney but the high total taurine concentration of the heart caused the specific activities of these tissues to be similar. The taurine-S^{35} of the liver was higher than that of the other tissues, especially at the 6-hour interval, and consequently liver taurine had the highest specific activities of the above tissues.

Methionine supplementation of a diet low in methionine and other sulfur amino acids was associated with enhanced growth and increased incorporation of sulfate-S^{35} into liver taurine (Table II). Methionine can be utilized to fulfill sulfur requirements and hence could exert a "sparing action" on the utilization of inorganic sulfur to supply these needs. Thus, more sulfate-S^{35} would be available for the synthesis of taurine with a subsequent increase in taurine-S^{35} concentrations.

Investigators have known for some time that methionine sulfur is used in the synthesis of cysteine. A low organic sulfur diet sup-

TABLE II. Influence of Methionine Supplementation to the Purified Basal Diet on Incorporation of Orally Dosed Sulfate-S^{35} into Taurine of Chick Liver.

Supplement to basal diet*	Avg wt, g/chick	Hr after $S^{35}O_4$ dose 1	2	3	4	6
		(Net cpm taurine-S^{35}/μg taurine/g liver)				
None	92.0	9.4	21.2	44.3	121.3	344.0
Methionine (0.3%)	120.0	248.8	299.6	263.8	35.6	19.9

* The basal diet used in these studies was the simplified ration of Martin and Patrick(8) minus methionine.

TABLE III. Influence of Cysteine HCl Supplementation to the Purified Basal Diet on Incorporation of Orally Dosed Sulfate-S^{35} into Taurine of Chick Liver.

Supplement to basal diet	Hr after $S^{35}O_4$ dose 1	2	3	4	6
	(Net cpm taurine-S^{35}/μg taurine/g liver)				
None	26.4	60.0	105.0	105.0	112.2
Cysteine HCl*	118.5	181.1	210.3	251.0	257.5

* Supplement equimolar to 0.1% taurine.

TABLE IV. Influence of Taurine Supplementation to the Purified Basal Diet on Incorporation of Orally Dosed Sulfate-S^{35} into Liver Taurine in the Chick.

Supplement to basal diet	Hr after $S^{35}O_4$ dose 1	2	3	4	6
	(Net cpm taurine-S^{35}/g liver)				
None	1730	1200	1800	3900	3700
Taurine (0.1%)	550	1700	2250	2100	2500
	(Net cpm taurine-S^{35}/μg taurine/g liver)				
None	26.4	60.0	105.0	105.0	112.2
Taurine (0.1%)	11.3	21.8	33.8	37.2	41.9

plemented with cysteine enhanced growth as well as the utilization of sulfate-S^{35} in the synthesis of taurine. Liver taurine radioactivity increased, total taurine concentration decreased, and thus the specific activities increased when cysteine was added (Table III). When methionine and cysteine were both supplemented, total taurine concentration of the liver was not repressed as it was when cysteine was added alone. The lowered total taurine concentrations may indicate repression of key enzymes in the organic pathway of taurine formation. Pasternak *et al* (11) working on the control of sulfate reduction in bacteria reported that when *E. coli* or *B. subtilis* are grown on cyst(e)ine instead of sulfate, the ability of extracts to synthesize PAPS is repressed. They found that in low concentrations, cysteine repressed sulfate activation by repressing the enzymes adenosine triphosphate sulfate-adenyltransferase and adenosine triphosphate adenyl-sulfate 3-phosphotransferase. With slightly altered PAPS formation and markedly repressed taurine concentration, the specific activity of liver taurine-S^{35} would still be higher. Mason *et al* (12) have suggested that taurine can be used to synthesize cysteine in the hen. Thus, in the presence of optimal concentrations of cysteine, feedback inhibition of phosphoadenosine phosphosulfate formation is conceivable. The possibility of enhanced phosphoadenosinephosphosulfatase activity due to enzyme activation must also be considered.

Preformed taurine supplemented to the chick ration effected a decreased incorporation of sulfate-S^{35} into taurine-S^{35} (Table IV). Some incorporation still occurred despite the presence of adequate amounts of preformed taurine in the diet, although dietary taurine reduced the need for synthesis in the animal. The presence of labeled intermediates in the liver homogenates similar to those obtained during *in vitro* studies with purified liver enzymes diminished the possibility of simple ion exchange of $S^{35}O_4$ and the sulfur of taurine.

Supplementation of a low-methionine diet with serine, glycine, ethanolamine or sodium isethionate enhanced sulfate-S^{35} incorporation into liver taurine (Table V). The presence of supplementary serine or glycine in the diet resulted in increased sulfate utilization for taurine synthesis when compared to animals on the non-supplemented ration. Supplementation with ethanolamine was associated with a rapid incorporation of S^{35} into taurine. The data of Table V indicate a 2-to 3-fold increase of taurine-S^{35} specific activity over that of the non-supplemented chicks up to one hour after dosing. This could indicate a sparing action for serine by ethanolamine at this early time.

TABLE V. Influence of Amino Acid Supplementation to the Purified Basal Diet on the Specific Activities of Liver Taurine of Chicks.

Supplement to basal diet*	Hr after $S^{35}O_4$ dose .5	1	1.5	2	4	6
	(Net cpm taurine-S^{35}/μg taurine/g liver)					
None	10.9	8.3	11.6	8.4	20.8	30.8
Serine	4.0	16.3	26.0	36.3	50.6	45.1
Glycine	5.5	6.9	10.5	17.5	59.1	100.6
Na isethionate	6.3	10.6	34.9	29.8	61.3	69.4
Ethanolamine	16.9	27.5	13.7	13.2	27.7	22.7

* Supplements equimolar to 0.1% taurine.

TABLE VI. Influence of Sulfur Anion Supplementation to the Chick Purified Diet on Incorporation of Orally Dosed Sulfate-S^{35} into Liver Taurine and Bile Taurocholate of Chicks.

Supplement to basal diet	Hr after $S^{35}O_4$ dose 1	2	3	4	6
	(Net cpm taurine-S^{35}/g liver)				
None	1700	2200	5100	5300	5400
Thiosulfate (0.3%)	4700	2300	4300	7900	21400
Sulfate (0.3%)	500	1500	2600	6600	7500
Sulfite (0.3%)	2200	4400	2100	6400	5700
	(Net cpm taurocholate-S^{35}/ml bile)				
None	16,720	19,760	24,252	43,860	65,760
Thiosulfate (0.3%)	13,200	22,016	64,680	61,408	23,716
Sulfate (0.3%)	3,748	34,048	21,980	70,080	130,080
Sulfite (0.3%)	10,640	26,800	32,832	41,400	52,728

Other trials have indicated a graded increase in incorporation of sulfate-S^{35} with increased supplementation of glycine to the ration. The interconversion between glycine and serine must be considered as these results indicate that these amino acids play a prominent role in the sulfate to taurine reactions.

Supplementation of the purified sulfur deficient diet with the sodium salts of thiosulfate, sulfate or sulfite effected increased taurine-S^{35} concentrations in the liver as well as increased taurocholate-S^{35} activity in the bile (Table VI). Sodium sulfate supplementation increased the bile volume, enhanced chick growth, and increased sulfate-S^{35} incorporation into taurine. Gordon and Sizer (13) indicated that the chick can satisfy part of its total sulfur requirement with inorganic sulfate. This was verified by our observation that supplementation at 0.7% of the diet enhanced the sulfate-S^{35} to taurine reactions. Machlin (14) previously reported optimal growth in chicks with sulfate supplementation of 0.5% of the diet. This supplementation appears to provide a sulfate pool of optimal substrate level for the enzymes involved in sulfate metabolism without producing a level where repression might occur. The total taurine of chick liver increased significantly with supplemental sulfate to the basal diet (unpublished data). The reduction of a S^{35}-labeled pool due to added sulfate to the diet does not necessarily detract from an increased synthesis of taurine-S^{35} since the more optimal substrate level may enhance total utilization, and also the formation of more taurine.

Various C^{14}-labeled compounds were subcutaneously injected into chicks and incorporation of radioactivity into liver taurine was measured. The labeled carbon of β-alanine-1-C^{14} was not used in the synthesis of taurine. Incorporation into taurine occurred from the carbons of cysteine, alpha alanine, pyruvate, and ethanolamine, but the greatest incorporation occurred when serine-3-C^{14}, serine-C^{14}-UL, and glycine-1-C^{14} were injected (Table VII). Whenever unlabeled glycine was supplemented to the diet, the serine-3-C^{14} appeared to be utilized at a more rapid rate in the synthesis of taurine (Table VIII). These results indicated that serine and glycine were closely related to the re-

TABLE VII. Specific Activity of Liver Taurine After Subcutaneous Injection of Various C^{14}-Labeled Compounds into Chicks Fed Purified Basal Diets.

Compound injected	Hr after injection .5	1	2	3	4
	(Net cpm taurine-C^{14}/μg taurine/g liver)				
Basal diet					
Serine-3-C^{14}	22.95	29.06	23.33	—*	16.67
Serine-C^{14}-UL	—*	—*	42.02	—	48.32
Serine-1-C^{14}	.00	.00	.00	—	.00
β-Alanine-1-C^{14}	3.20	.00	.00	—	.00
L-Alanine-C^{14}-UL	27.31	27.30	5.81	—	2.53
Ethanolamine-1-2-C^{14}	5.92	7.86	12.50	—	19.41
Cysteine-3-C^{14}	7.20	13.40	12.10	13.80	18.40
Casein diet† and 1.0% glycine					
Serine-3-C^{14}	48.18	30.19	2.66	—*	3.33
Serine-3-C^{14}‡	8.07	4.78	14.69	—	76.70
Glycine-1-C^{14}	9.77	10.79	11.80	3.33	—*
Pyruvate-2-C^{14}	4.29	7.78	4.21	1.63	—

* Samples not obtained at these times.
† Basal diet with protein supplied as isolated soybean protein (5%) plus casein (20%) and 62% cerelose.
‡ No glycine added.

TABLE VIII. Influence of Glycine Supplementation to the Purified Diet on Incorporation of Injected Serine-3-C^{14} into Liver Taurine of 17-Day-Old Chicks.

Supplement to casein diet	Hr after dose	Taurine-C^{14}, cpm/g liver	Total taurine, μg/g liver	S.A., cpm/μg
None	.5	250	50.8	4.92
	1	300	62.8	4.78
	2	550	37.5	14.69
	4	3950	51.5	76.70
Glycine (1.0%)	.5	2650	55.0	48.18
	1	1700	56.3	30.19
	2	150	56.3	2.66
	4	200	60.0	3.33

actions leading to the synthesis of taurine and completely agreed with the results obtained from the dietary studies utilizing sulfate-S^{35}. Perhaps the carbon moiety of taurine is a compound that originates from glycine or serine. In a study designed to determine which carbons of serine were utilized most readily in the synthesis of taurine, incorporation of the 3-carbon was evident in the liver taurine as early as one hour after injection and little or no radioactivity from the 1-carbon of serine was found in liver taurine (Table VII). Good incorporation of the carbons of uniformly labeled alanine-C^{14} was observed at the 0.5 and 1 hour intervals while the carbons of ethanolamine-1-2-C^{14} were more evident after 2 and 4 hours.

The chromatographic analysis of labeled compounds in the liver of the chicks after administration of the radioisotopes indicated several peaks of radioactivity. From time studies, possible pathways of taurine synthesis from sulfate might include cysteinesulfinic acid, isethionic acid, hypotaurine, or mercaptoethylamine all of which have tentatively been identified chromatographically. Little or no activity was observed at areas comparable to cystine, cysteine, or methionine even though the chick does convert C^{14}-cysteine into taurine (Table VII).

Summary. Many tissues in the chick are equipped with the enzymes necessary for conversion of sulfate-S^{35} into taurine. Supplementation of a low sulfur amino acid diet with serine, glycine, ethanolamine, sodium isethionate, methionine, or cysteine enhanced sulfate-S^{35} incorporation into liver taurine. Addition of the sodium salts of thiosulfate, sulfate, or sulfite to the ration increased liver taurine-S^{35} and bile taurocholate-S^{35}. The

optimum level of sodium sulfate supplementation was 0.7% of the diet. Incorporation into taurine of labeled carbons occurred when cysteine-3-C^{14}, alanine-C^{14}-UL, ethanolamine-1-2-C^{14}, serine-3-C^{14}, serine-C^{14}-UL, and glycine-1-C^{14} were administered.

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Reversal of Contact-Inhibition in Primary Amnion Cultures by Hydrocortisone.* (31589)

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Surface contact by diploid cells in primary cultures results in certain alterations which in aggregate are given the term "contact-inhibition." Physical changes noted early after cells come into contact include cessation of motion of the undulating cytoplasmic membrane, decreased cellular motility and later, cementation of the intercellular membranes(1). Biological changes associated with these physical phenomena are decreased mitotic activity(2) and decreases in protein, RNA and DNA metabolism with disappearance of free ribosomes from the cytoplasm(3).

Confluent monolayers of primary human amnion cells are relatively stable in cellular density over long periods of maintenance *in vitro* under conditions where incubation is provided at 37°C in stationary racks and culture medium is replaced every 48-72 hours. That replication is present in such monolayers is difficult to determine, the mitotic index approaching 0.1% or less(4).

Reversal of contact-inhibition and growth induction were observed in amnion monolayers when hydrocortisone was added to the culture medium.

Material and methods. Amnion cultures. Cells were obtained throughout from fresh, clean amniotic membranes using versene 0.1% and trypsin 0.25%, explanted into stationary roller tubes or 3 oz prescription bottles in Eagle's minimal essential medium (EMEM) with 20% mammalian serum (10% human, 10% bovine) and incubated at 37°C. These primary cultures became confluent after periods of 5-7 days, at which time maintenance medium was added, containing 10% equine serum. In early experiments, media were completely replaced every 72-96 hours; in later experiments, every 48 hours.

Cell and nuclei counts. Initially, cells were enumerated in suspensions prepared by treating the monolayers first with 0.1% versene and then with 0.2% trypsin, which rendered

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EFFECTS OF CYANIDE ANTIDOTES AND INANITION ON ACUTE LETHAL TOXICITY OF MALONONITRILE IN MICE

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BY

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HYDEN and HARTELIUS (1948) have introduced the use of malononitrile in treatment of mental disease in man, and terminated toxic symptoms following malononitrile therapy by administration of Na thiosulfate. Recent interest in use of less toxic, more stable analogues of malononitrile is evidenced by a report of clinical trial in France of succinic acid dinitrile, in a Foreign Letter to the *Jour. Amer. Med. Assoc.* (1950). HEYMANS and MASOIN (1897) demonstrated that Na thiosulfate is an efficient antidote to malononitrile: „ Une expérience classique pour démontrer l'action antitoxique préventive et curative de l'hyposulfite de soude vis-à-vis du nitrile malonique consiste à prendre trois lapins (chiens, rats ou souris), auxquels on injecte par voie hypodermique ou intraveineuse une dose 1-9 fois mortelle de nitrile malonique, soit 5 à 55 mgr. par kilogramme; le premier lapin sert de témoin; le deuxième reçoit immédiatement 5 cc. par exemple d'une solution d'hyposulfite de soude à 10 %; le troisième reçoit cette même dose lorsque le nitrile a développé la plénitude de son action, soit à la période de paralysie complète. Le premier meurt, le deuxième demeure normal et le troisième redevient normal après dix minutes en moyenne." The mechanism of toxic action of malononitrile is considered by HEYMANS and MASOIN (1897) to be similar to that of cyanide intoxication, with 1 mol of malononitrile yielding 1 mol of cyanide ion under conditions in vivo.

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RENTZ (1930) has reviewed the various agents effective against cyanide poisoning, and has intensively studied detoxication of malononitrile in rabbits and frogs by dihydroxyacetone.

In view of controversial opinions as to the clinical usefulness of various cyanide antagonists in cyanide poisoning, it was of interest to test several of these agents comparatively against the acute lethal toxicity of malononitrile in Swiss-W mice. Further, since malononitrile may be used in patients prepared by fasting as for insulin- or electro-shock therapy, a study of effects of inanition was suggested, particularly since we observed (PARKER et al., 1950) that mice pretreated with malononitrile during fasting before administration of alloxan were more susceptible to toxic effects of malononitrile than were fed mice.

EXPERIMENTAL

Malononitrile was obtained through the courtesy of Dr Edward HENDERSON from the Schering Corporation, Bloomfield, N. J. The material was shipped in the form of 700 mg. ampules packed in solid CO_2, and was kept in the laboratory at approximately 4°C. Solutions for intraperitoneal injection were made immediately before use, following Dr. Henderson's recommendations. Dextrose and Na thiosulfate solutions were prepared from reagent grade chemicals. The $NaNO_2$ used was of practical grade, while glutathione, methylene blue and *dl*-methionine were of the highest purity grade supplied by Eastman Kodak Company. 2,3-Dimercaptopropanol was obtained as the 10 % solution in peanut oil, containing 20 % benzylbenzoate, supplied as „BAL in Oil Ampules 10 %" by Hynson, Westcott & Dunning, Baltimore, Maryland, and was diluted immediately before use with olive oil U. S. P. XIII. The Na formaldehydesulfoxylate used was of medicinal grade supplied by Merck and Company, Rahway, N. J. All injections of malononitrile were made intraperitoneally; all other agents were given intramuscularly approximately 5 minutes before injection of malononitrile. In mice pretreated with both $NaNO_2$ and $Na_2S_2O_3$, injections of these solutions were made separately into muscles of opposite hind legs.

Approximately 600 adult white mice, all obtained from the same source, were used to estimate the LD-50 of malononitrile and the value of the several antidotes. These mice were healthy during the time they were maintained in the laboratory before use, and were not fasted before receiving the drugs.

Results are noted in table I. The LD-50 of malononitrile, as estimated by the use of log-probit paper by the method of MILLER and TAINTER (1944), is 12.75 ± 0.39 mg./kg. Intramuscular administration of 1 g./kg. of Na thiosulfate 5 minutes before malononitrile decreases the toxicity about 8-fold. Protection with 1.5 g./kg. of Na thiosulfate is not significantly greater. $NaNO_2$, 50 mg./kg., similarly injected, decreases toxicity of malononitrile only slightly > 2-fold. Simultaneous treatment with 50 mg. kg. of $NaNO_2$ and 1 g./kg. of Na thiosulfate, or with 100 mg. kg. of $NaNO_2$ and 1.5 g./kg. of Na thiosulfate, does not increase the prophylactic effect of these doses of Na thiosulfate and may decrease it; with injection of 100 mg./kg. of malononitrile, twice as many deaths occurred among 30 mice pretreated with 100 mg./kg. $NaNO_2$ and 1.5 g./kg. of Na thiosulfate as did among 30 mice pretreated with this dose of Na thiosulfate alone. CHEN et al. (1934) reported potentiation of the antidotal action in cyanide poisoning of $NaNO_2$ by $Na_2S_2O_3$, but HUG (1933) cites contrary results obtained by LUDUENA.

None of the other possible antagonists tested showed sufficient antidotal efficacy to warrant extensive testing of these substances. All were used in doses considered well below the lethal level, with the exception of methylene blue which was given in doses within the toxic range found by ANDERSON et al. (1934). HANZLIK and RICHARDSON (1934) consider methylene blue, in intravenous doses of 10-20 mg./kg., to be the antidote of first choice in treatment of cyanide poisoning in man, in which condition thiosulfate alone is „ practically inefficient ", in their opinion. Dimercaptopropanol was tolerated by all of 5 mice treated with 5 mg./kg. Survival of all animals treated with low doses of Na thiosulfate, $NaNO_2$, and glutathione indicates tolerance of these antidotal doses (table I).

SZANTO and FELSENFELD (1949) found malononitrile toxic to mice at levels of 6 mg./kg. HEYMANS and MASOIN found the lethal range for mice to be about 8-9 mg. kg. PARKER et al. (1950), using the 3 mg./kg. intraperitoneal dose of malononitrile found effective by SZANTO and FELSENFELD against experimental poliomyelitis in mice, noted in mice fasted 20 hours that 1 of 5 mice succumbed to this dose of malononitrile after 2 similar injections has been given 14 and 19 hours previously, and that all showed markedly increased symptoms of toxicity. Toxicity was estimated in 164 mice fasted 4-44 hours, and compared with that in 280 mice not fasted before receiving malononitrile.

Results are summarized in table II. Inanition apparently broadens the dose-effect curve of malononitrile without greatly affecting the LD_{50}. While none of 40 normal mice died following treatment with 7 mg./kg.

TABLE I

Effects of Cyanide Antidotes on Acute Lethal Toxicity of Malononitrile in Mice

Antidote	Dose mg./kg. (intramusc.)	Dose of Malononitrile, mg./kg. (intraperitoneal)														
		4	5	6	7	8	10	12	15	20	25	30	75	100	125	150
None	—	0/5	0/5	0/15	0/15	11/60	17/60	11/30	32/60	28/30						
$Na_2S_2O_3.5H_2O$	1000								0/3			0/10	0/30	15/30		
	1500									0/3				11/30		
$NaNO_2$	50									0/5	3/10	19/30	5/5			
$NaNO_2$ plus $Na_2S_2O_3.5H_2O$	50												1/10	6/10	10/10	
	1000															
$NaNO_2$ plus $Na_2S_2O_3.5H_2O$	100											0/5	2/5	22/30	5/5	5/5
	1500															
Dimercapto-propanol	5							4/5	3/5			5/5				
Glutathione	1500							0/5				5/5				
dl-Methionine	1000							3/5	1/5			4/5				
Methylthionine choride	200						1/3									
	500								2/3							
Na formaldehyde sulfoxylate	1000						1/3									
Dextrose	10000						1/3									

or less, 19 of 88 mice (21.6 %) fasted for various periods died after treatment with 4-7 mg./kg., and 10/20 (50 %) after 7 mg./kg. At higher dosage levels, the differences are not as significant: 8 mg./kg. killed 18.3 % of 60 normal mice and 30 % of 60 fasted mice, while 10 mg./kg. killed 28.3 % of 60 normal mice and 46.4 % of 56 fasted mice.

TABLE II

Effect of Inanition on Toxicity of Malononitrile in Mice

Dose mg. kg.	Time of Fasting, Hours					
	0	4	8	10	24	44
4	0/5	0/5	0/5	1/5	0/5	0/5
5	0/5	1/5	0/5	1/5	0/5	
6	0/15	2/5	1/5	1/5	1/4	1/4
7	0/15	2/5	3/5	0/5	5/5	
8	11/60	1/20	4/10	2/10	9/15*	2/5
10	17/60	3/20	4/11	5/10	14/15*	
12	11/30					
15	32/60					
20	28/30					

* See text under Discussion.

Fasting produces relatively much more rapid changes in mice than in larger mammals, probably due to their relatively higher metabolic rate. This is evidenced, for example, by the more rapid development of susceptibility to diabetogenic effects of alloxan after short periods of fasting in mice than in larger mammals. Interpretation of results in table II in terms of clinical application should not be made in regard to the actual periods of fasting. It is of particular interest that a fast of 44 hours, rendering mice hypothermic and moribund, has little effect on toxicity of malononitrile.

DISCUSSION

Detoxication of cyanide may be effected by agents furnishing S, by agents forming methemoglobin, by carbohydrate metabolites or agents yielding these, and by -SH compounds. RENTZ (1930) emphasizes the importance of treating with complete redox systems rather than with single H-donors or acceptors. VOEGTLIN et al. (1926) suggest that Na thiosulfate increases the formation of thiocyanate from cyanide, while

the sulfhydril compounds facilitate formation of cyanate without loss of their sulfur. RENTZ (1930) considers the possibility of detoxication of cyanide by carbohydrate metabolites through formation of cyanohydrins, for example, dihydroxyacetonecyanohydrin. HUG (1933) notes that several detoxication mechanisms may be of significance in the prophylaxis of cyanide poisoning, but believes, despite the high efficacy of Na thiosulfate or tetrathionate as prophylactics, that only those agents forming methemoglobin are appreciably active as antidotes after the cyanide effects are well established; of these, nitrites are more effective than methylene blue.

If these concepts are applied to malononitrile poisoning, which may be considered equivalent to effects of slow administration of cyanide, it would appear Na thiosulfate should be more effective than agents forming methemoglobin since the toxic effects of malononitrile develop slowly after the initial symptoms. Under these conditions, formation of thiocyanate is a more efficient means of detoxifying malononitrile than is the formation of cyanate, cyanohydrins or cyanomethemoglobin, or the substitution of redox systems to replace temporarily the cyanide-poisoned cytochrome oxidase system. The present results support this view. However, HYDEN and HARTELIUS (1948) consider that the effect of malononitrile on nucleoprotein metabolism of neurons is a result of slow liberation of cyanide, but were unable to affect nucleic acid content of neurons by slow injection of HCN.

Results with fasted mice are more difficult to interpret. Loss of weight during a short fast, of 4-8 hours, probably does not involve the mass of living tissues to a significant degree, representing rather a loss of weight in alimentary contents and body stores of glycogen and fat. An expected apparent decrease in toxicity was not found. Table II shows instead a considerably increased toxicity at the lower dosage range; only with doses of 8-10 mg./kg. is there an indication of reduced toxicity in mice fasted 4 hours. Acidosis consequent to fasting might accelerate liberation of cyanide by malononitrile, but results reported by RENTZ (1930) minimize the importance of pH changes, within the range compatible with life, in regard to toxicity of malononitrile. With longer periods of fasting, an increase in toxicity is noted, most marked in mice fasted 24 hours and unexpectedly less in mice fasted 44 hours.

Five of 5 mice fasted 24 hours before treatment with malononitrile died at each of the 7, 8, and 10 mg./kg. levels. Eight and 10 mg./kg. doses were given to each of 2 groups of 10 mice after a 24 hour fast, during which these mice were treated with 8 and 10 mg./kg. of malononitrile, respectively, after the first 4 hours of fasting. No exhaustion of the

detoxifying mechanism was noted, since mortality from treatment after 24 hours of fasting was only 4/10 at 8 mg./kg. and 9/10 at 10 mg./kg. doses, significantly less than in those receiving only one dose of malononitrile after 24 hours of fasting. The combined results are noted in table II.

Summary

The LD50 of malononitrile given intraperitoneally to white mice is 12.75 ± 0.39 mg./kg. Intramuscular injection of 1 g./kg. of $Na_2S_2O_3 \cdot 5H_2O$, 5 minutes before administration of malononitrile, increases the LD50 approximately 8-fold. Protection with 1.5 g./kg. of Na thiosulfate is not appreciably greater than with 1 g./kg. Injection of 50 mg./kg. $NaNO_2$ given similarly, decreases toxicity of malononitrile only slightly over 2-fold. Combinations of these two antidotes are not more effective than Na thiosulfate alone. Dimercaptopropanol, glutathione, *dl*-methionine, methylene blue, Na formaldehydesulfoxylate and dextrose are not effective antagonists to malononitrile. Fasting for 4-10 hours broadens the toxicity curve without changing the LD50 to any considerable extent. Fasting for 24 hours appreciably increases the toxicity of malononitrile, but further fasting, to 44 hours, has little effect on toxicity despite the moribund appearance of mice fasted so drastically.

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MECHANISM OF THE PROLONGED ADRENERGIC BLOCKADE PRODUCED BY HALOALKYLAMINES (1) (2) (3)

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BY

MARK NICKERSON

(Received for publication 1-2-1962).

One of the characteristics first noted to differentiate Dibenamine and related haloalkylamines from other adrenergic blocking agents was its prolonged action (21). From the beginning, this was attributed to some relatively stable combination with adrenergic receptors. A major factor suggesting this interpretation was the similarity of the reactive haloalkylamine grouping of Dibenamine to those of the nitrogen mustards, which were known to react with a variety of groupings found in biological systems to form relatively stable covalent bonds (5, 6). More recently, Dibenamine and phenoxybenzamine (Dibenzyline) also have been shown to react with sulfhydryl, amino and carboxyl groups in biological material (14). This mechanism of action also provided an adequate explanation for the effectiveness of the blockade produced by these agents against very large doses of a sympathomimetic amine.

Several more recent studies have demonstrated that the haloalkylamine blocking agents react with the same α-receptors in smooth muscle as do the catecholamines (7, 18). However, the stable drug-receptor bond prevents the mass-action equilibrium of agonist, antagonist and receptor which is characteristic of a classical competitive antagonist. This mecha-

(1) This paper is dedicated to Professor C. HEYMANS on the occasion of his 70th birthday.

(2) Supported by grants-in-aid from the Medical Research Council of Canada and the Manitoba Heart Foundation.

(3) Preliminary reports of portions of this work were presented at the 1954 and 1956 meetings of the Federation of American Societies for Experimental Biology.

nism of action, which may be referred to as "nonequilibrium" (20), assumes a relatively stable combination of the antagonist with specific receptors, and appears to be compatible with all of the data currently available on the characteristics of the adrenergic blockade produced by the haloalkylamines.

An alternative explanation for the prolonged action of these agents, based on their accumulation in and slow release from fat depots was proposed by BRODIE and associates (1, 2) on the basis of tissue analyses following the injection of large doses of Dibenamine or Dibenzyline. Although this mechanism does not provide a basis for explaining other distinctive features of the pharmacology of these agents, it has been accepted in a number of recent textbooks of pharmacology (3, 10, 16, 25). Because agents of the Dibenamine series frequently are used in studies of the specificity and other properties of adrenergic receptors on the assumption that they form relatively stable bonds with these receptors, it appeared desirable to reinvestigate the mechanism of their prolonged blockade. In the experiments reported below, an attempt has been made to assess the roles of stable bonding to receptors and of accumulation in fat or other depots in determining the duration of action of Dibenzyline.

METHODS

This report includes three separate series of experiments: 1) studies of the alteration by thiosulfate of the blockade produced by Dibenzyline in intact rats, 2) studies of the rate of dissipation of the blockade produced by a short exposure of rabbit aorta strips to Dibenzyline *in vitro*, and 3) studies of the persistence and tissue distribution of doses of Dibenzyline-C^{14} in the range usually used to produce adrenergic blockade in animals.

1. *Blockade in intact rats.* Male rats of a Sprague-Dawley strain weighing 320 to 380 g were injected intramuscularly with 3.0 mg kg of Dibenzyline, preceded and/or followed by subcutaneous administration of sodium thiosulfate in an initial dose of 2.0 g/kg, followed by 1.0 g/kg/hour, as a 10 % solution in water. This dose was found to maintain a blood level of at least 100 mg %, which consistently and effectively antagonized the adrenergic blocking action of Dibenzyline. At various times following the administration of Dibenzyline, the animals were anesthetized with pentobarbital sodium, a carotid artery was cannulated and blood pressure was recorded with a small-bore mercury manometer. Heparin (5.0 mg/kg) was injected intravenously as anticoagulant, and

progressively increasing doses of adrenaline hyrochloride (1.0 to 1000 μg/kg) were injected via a femoral vein to determine the extent of the adrenergic blockade. The maximum dose which produced a response in which the depressor was greater than the pressor component was taken as the "maximum dose reversed".

2. *Blockade in rabbit aorta strips in vitro.* Spiral strips of rabbit aorta were cut as described by FURCHGOTT and BHADRAKOM (9) and suspended in a 10 ml bath containing Krebs-bicarbonate solution with 0.1 % glucose, aerated with 95 % O_2–5 % CO_2. Contractions were recorded by means of isotonic levers. After equilibration for about 1 1/2 hours, control responses to a concentration of 0.1 μg/ml of adrenaline were recorded. Half of each group of four strips was then exposed to a concentration of 0.05 μg/ml of Dibenzyline for 5 minutes, and responses to concentrations of 0.1 and 1.0 μg/ml of adrenaline and to 0.1 μg/ml of angiotensin were determined at intervals thereafter for about six hours. The strips were then placed in a Petri dish on filter paper moistened with the Krebs-bicarbonate solution and stored at about 6° C until the next day, when equilibration and testing were repeated. This procedure could be continued for a total of about 5 days. The strips were treated with Dibenzyline only on the first day. Parallel "control" strips were exposed to 100 μg/ml of piperoxan (Benodaine) for 5 minutes and tested and stored in the same manner as those treated with Dibenzyline, except that exposure to the short-acting adrenergic blocking agent was repeated daily.

3. *Distribution of Dibenzyline-C^{14} in mice.* Dibenzyline-C^{14} was dissolved in 95 % ethanol to make a stock solution, which was diluted about 1 : 10 with 0.9 % NaCl solution and injected rapidly intravenously into 25 to 30 g Swiss-strain albino mice. The animals were sacrificed at various intervals after injection. In some experiments the whole animal except the tail was homogenized and duplicate aliquots taken for analysis, in others the brain, liver, kidneys and samples of skeletal muscle and peritoneal and perirenal fat were taken for determinations of radioactivity. Tails were analyzed separately to check the completeness of the intravenous injection. In some experiments the animal was placed in a dessicator through which a slow stream of air was passed. The air was then drawn through $Ba(OH)_2$ to trap CO_2. Radioactivity in the $BaCO_3$ formed was counted in the same manner as that prepared from the tissue samples.

Tissue samples were dried to constant weight and then oxidized in H_2SO_4-H_3PO_4-$K_2Cr_2O_7$ with heating. The CO_2 evolved was drawn

through a $Ba(OH)_2$ trap and the $BaCO_3$ formed was deposited directly on a filter paper-containing planchet by suction filtration. Samples were counted at infinite thickness in a windowless Q-gas flow counter (Tracerlab). The results are expressed as counts/mg of tissue calculated on the basis of both wet and dry weights.

Results

1. *Blockade in intact rats.* The results of this series of experiments are summarized in Fig. 1. Intramuscular injection of 3.0 mg/kg of Dibenzyline produced a complete blockade of pressor responses to all doses of adrenaline up to 1000 μg/kg, the maximum tested, at all time intervals between 2 and 23 hours after administration (Group I).

In Group II, administration of sodium thiosulfate was begun 2 hours before the injection of Dibenzyline and continued through the period

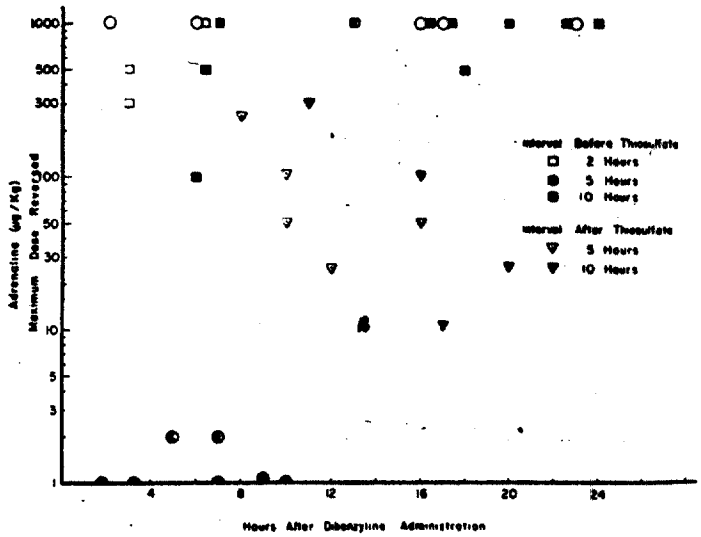

FIGURE 1

Magnitude of adrenergic blockade produced by 3.0 mg/kg of Dibenzyline injected intramuscularly. Group I (open circles) Dibenzyline alone; Group II (solid circles) thiosulfate administration started 2 hours before Dibenzyline and continued throughout the experiment; Group III (squares) thiosulfate administration started at various intervals after Dibenzyline and continued during the remainder of the experiment; Group IV (triangles) thiosulfate administration started 2 hours before Dibenzyline and discontinued 5 or 10 hours before test of blockade.

of challenge with adrenaline. The total duration of thiosulfate administration varied up to 12 hours, the maximum period the animals could be kept in sufficiently good condition to allow reliable tests of their pressor responses to adrenaline. This treatment almost completely prevented the development of the Dibenzyline blockade. Partial reversal of pressor responses to 2.0 μg/kg of adrenaline was seen in 2 of 7 animals and of responses to 1.0 μg/kg in one. There was no definite indication of adrenergic blockade in the remainder. The results with this group demonstrated clearly that the levels of thiosulfate attained were adequate to prevent blockade by the dose of Dibenzyline employed.

In Group III, thiosulfate administration was initiated at various times from 1 to 18 hours after injection of the Dibenzyline, and continued through the adrenaline test period — 2, 5 or 10 hours later. As can be seen in Fig. 1, thiosulfate administered on this schedule had little effect on the blockade, with the possible exception of experiments in which administration was started 1 hour after the intramuscular Dibenzyline.

In the final series of animals (Group IV) thiosulfate injections were started 2 hours prior to the Dibenzyline, as in Group II, and continued for 4 to 10 hours after administration of the blocking agent. Tests with adrenaline were carried out 5 or 10 hours after the last injection of thiosulfate, when the blood levels were always less than 20 and usually below 10 mg %. The degree of blockade produced on this schedule clearly was less than that produced in the controls (Group I) or in animals to whom thiosulfate was not administered until 2 or more hours after the Dibenzyline (Group III). However, a very significant degree of blockade, well above that produced in any animal receiving thiosulfate before and throughout the period of Dibenzyline action, was produced in all members of this group.

2. *Blockade in rabbit aorta strips in vitro.* Treatment with either 0.05 μg/ml of Dibenzyline or 100 μg/ml of piperoxan for 5 minutes almost completely eliminated responses to the standard test dose of adrenaline, the initial blockade produced by piperoxan being slightly more complete (FIG. 2). However, subsequent responses were very different. The responses of strips treated with piperoxan returned to essentially the same magnitude as the control contractions within 15 minutes, too rapidly to be shown accurately in this figure. The control responses of these strips on subsequent days decreased progressively and were about 50 % of the initial value after 96 hours. Responses to the repeated daily blockade and the subsequent recovery remained essentially the same as on the first day.

In contrast, the responses of strips exposed to Dibenzyline increased only slightly during the 6-hour period of observation after blockade, and remained markedly depressed throughout the experimental period of about 100 hours without further exposure to the blocking agent. Responses to 0.1 μg/ml of angiotensin, tested at intervals during each day were not depressed by the Dibenzyline, but declined over the total period of the experiments at about the same rate as did the daily control responses of the piperoxan-treated strips to adrenaline.

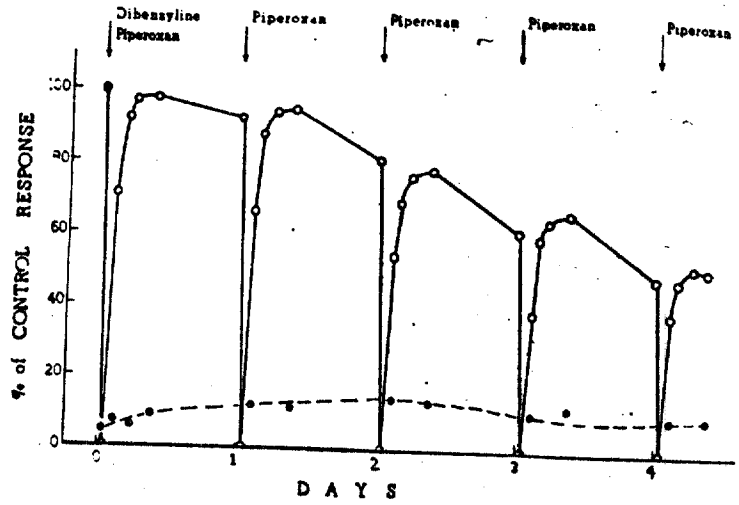

FIGURE 2

Responses of rabbit aorta strips to 0.1 μg/ml of adrenaline before and after 5-minute exposure to 0.05 μg/ml of Dibenzyline (solid circles) or 100 μg/ml of piperoxan (open circles). All responses are plotted as percent of control responses of the same strip prior to contact with any blocking agent. Strips were exposed to Dibenzyline only once, to piperoxan 5 times at 24-hour intervals. Each point is the average of responses of 6 strips.

The magnitude of the responses of Dibenzyline-treated strips after the first day is the resultant of two factors : 1) dissipation of the blockade and 2) progressive deterioration of the preparation, as reflected in the progressively lower responses to angiotensin and the lower daily "control" responses of the strips treated with piperoxan. In an attempt to separate these two factors, responses of Dibenzyline-treated strips have been plotted in Fig. 3 as a percentage of the "control" responses of the piperoxan-treated strips on the same day. This figure shows responses to the standard concentration of 0.1 μg/ml and to 1.0 μg/ml of adrenaline,

both plotted against the same control values. Responses to 1.0 μg/ml were larger and could be measured more accurately. Consequently, the curve for this concentration probably reflects more accurately the rate of dissipation of the blockade. Various factors in the experimental procedure, particularly the intermittent storage of the strips at a low temperature, may have modified the rate of dissipation of the blockade. However, it obviously is very low, not over 10 % per 24 hours under these experimental conditions.

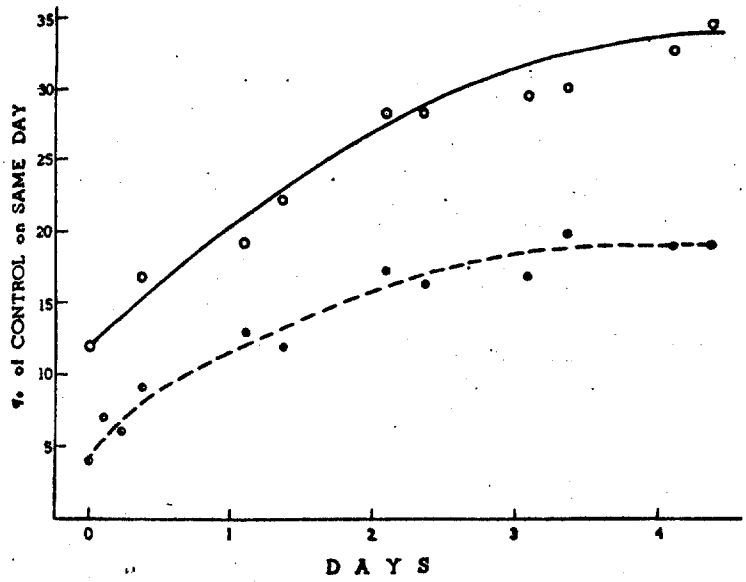

FIGURE 3

Responses of rabbit aorta strips to 0.1 (solid circles) and to 1.0 μg/ml (open circles) of adrenaline at various intervals after 5-minute exposure to 0.05 μg/ml of Dibenzyline. All responses are plotted as percentage of "control" responses of piperoxan-treated strips on the same day. (See text and Figure 2.) Each point is the average of responses of 6 strips.

3. *Distribution of Dibenzyline-C^{14} in mice.* Fig. 4 illustrates the time-course of the dissipation of total-body radioactivity in mice following the intravenous administrations of 10 mg/kg of Dibenzyline-C^{14}. Activity declined very rapidly during the first 24 hours, and at the end of this period less than 15 % of the initial dose remained. About two-thirds of the radioactivity disappearing from the body during this period was found in the urine. It is probable that much of the remainder was

excreted in the bile. A pentobarbital-anesthtized dog with cannulated ureters and bile duct excreted 43 % and 12 % of the total administered radioactivity (5.0 mg kg Dibenzyline-C^{14}) in the urine and bile, respectively, within 9 hours. No radioactivity could be detected in expired CO_2. After the first 24 hours and through the 7th day, the latest that significant radioactivity was detected, the activity fell much more slowly.

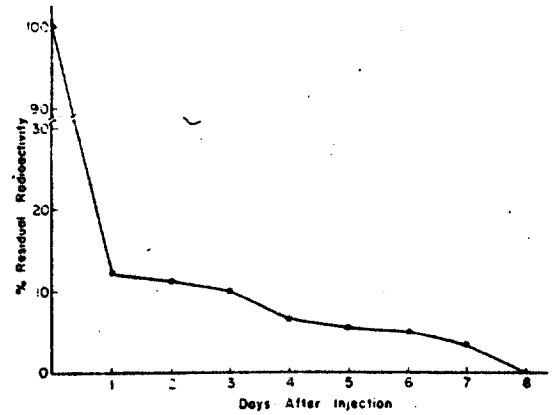

FIGURE 4

Total-body radioactivity of mice at daily intervals after intravenous injection of 5.0 mg/kg of Dibenzyline-C^{14}. All points are plotted as percentage of total injected radioactivity. Each point is the average of duplicate determinations on each of 2 mice.

Data on the relative amounts of radioactivity in fat and in several other tissues and organs are shown in Table I, calculated on the basis of both wet and dry weights. On the basis of dry weight, fat had the lowest concentration up to 4 hours after injection and at all periods its content was among the lowest of the tissues and organs studied. On the basis of wet weight, the radioactivity of fat was the highest of the samples studied at 4 and at 24 hours. However, the content was not remarkably greater than that of several other organs.

DISCUSSION

The objective of the experiments reported above was to evaluate the relative importance of binding to specialized receptors and of accumulation in and slow release from fat depots in determining the duration of the adrenergic blockade produced by Dibenzyline, and presumably

TABLE I

Distribution of Radioactivity in Mice Receiving 5.0 mg/kg of Dibenzyline-C^{14} Intravenously

No. Animals	4	5	4	2	2	4	5	4	2	2
Time After Injection	20 min.	1 hr.	4 hr.	12 hr.	24 hr.	20 min.	1 hr.	4 hr.	12 hr	24 hr
	CPM/mg wet wt.					CPM/mg dry wt.				
Kidney	23.2	6.9	4.1	3.9	3.7	100.7	30.0	17.8	16.8	16.2
Liver	20.3	9.7	5.1	4.4	2.6	65.4	31.2	15.8	14.2	8.5
Brain	4.4	2.2	1.6	0.9	1.1	20.8	10.1	7.1	3.9	4.9
Skeletal Muscle	3.1	2.4	1.5	0.5	0.8	12.8	10.1	6.3	2.2	3.4
Fat	2.9	5.1	6.4	3.0	4.4	3.3	5.8	7.3	3.4	4.6
Blood	4.0	2.4	2.6	1.1	0.6	27.6	17.2	18.8	7.6	4.6

also that produced by other haloalkylamines. The participation of accumulation in and release from fat depots was deduced from correlations between concentrations in fat and adrenergic blockade (1, 2). In both of these studies large doses of the blocking agent (30 to 60 and 13 to 25 mg/kg of Dibenamine and Dibenzyline, respectively) were administered intravenously. These substances have low aqueous solubilities, and it is quite understandable that a relatively high percentage of such doses should localize in the lipid phase. However, little evidence of selective accumulation of radioactivity in fat was found in the present studies with 5.0 mg/kg, the upper limit of the dose range usually employed to produce adrenergic blockade in animal experiments. Once present in fat depots, active drug probably could contribute to maintenance of the blockade, and such a process cannot be ruled out as contributing to the development of a partial adrenergic blockade after cessation of thiosulfate administration in the rats of Group IV reported above. However, these results could be explained also on the basis of a local depot of precipitated drug in the area of intramuscular injection.

It is particularly interesting to note that the radioactivity in skeletal muscle and liver persisted for a considerable period of time, whereas BRODIE *et al.* (2) reported that extractable Dibenzyline in these tissues fell to very low levels within one hour after intravenous injection. This agent readily alkylates many groupings found in tissues, and it is probable that much of the radioactivity represented drug which had reacted in this manner. It is unlikely that the readily diffusible dealkylated metabolite of Dibenzyline (N-phenoxyisopropyl-N-benzylamine) contributed much to the persistent radioactivity.

In line with their concept that the prolonged action of haloalkylamine adrenergic blocking agents is due to the slow release of unchanged drug from fat depots, AXELROD *et al.* (1) and BRODIE *et al.* (2) rejected the concept that reactive intermediate ethyleneiminium ions are involved in the production of the blockade or, in fact, are formed *in vivo*. This interpretation was based primarily on their failure to detect in either tissues or urine the hydrolysis products which would be formed by the reaction of these intermediates with water. However, it is not surprising that most of the ethyleneiminium should react with substances other than water *in vivo*. Dibenamine and Dibenzyline have been shown to react much more readily with several biologically important groupings than with water *in vitro*, approaching 100 % preferential reaction with cysteine (14). Several workers have presented convincing indirect evidence for the participation of ethyleneiminium ions in the production of adrenergic blockade (4, 12, 13), and GRAHAM (11) has demonstrated

that isolated ethyleneiminium derivatives of several Dibenamine congeners may be even more potent than the parent compounds in producing the prolonged adrenergic blockade characteristic of the haloalkylamines. These experiments bear directly on the role of fat accumulation, because the highly water soluble iminium forms would not localize in fat in appreciable quantities.

Irrespective of whether haloalkylamine present in fat depots after the administration of large doses may play some role in prolonging the blockade, it is most pertinent to enquire whether accumulation in fat is essential to the production of a persistent blockade.

The above observations on alteration of the Dibenzyline blockade by thiosulfate strongly suggest that continued release of active drug is unnecessary for maintenance of a prolonged, stable blockade. The results obtained with animals in Group II indicate that the blood levels of thiosulfate attained in these experiments were adequate to inhibit the Dibenzyline blockade almost completely when present during the entire period of drug action. However, when the blockade was established prior to thiosulfate aeministration, it persisted essentially unaltered for at least 10 hours, although the thiosulfate would have inactivated any drug released from fat depots before it could have reached and combined with adrenergic receptors of the vascular smooth muscle. Only when the thiosulfate was administered within one hour of the Dibenzyline, before the drug administered intramuscularly had produced its full effect, was there any appreciable reduction in the magnitude of the blockade. These results are entirely compatible with previous scattered observations on the alteration of adrenergic blockade by thiosulfate in several species and after the administration of several different haloalkylamines (12, 15, 17, 22, 23).

The most convincing evidence of the adequacy of some relatively stable reaction with tissues to explain the long duration of the adrenergic blockade produced by Dibenzyline was obtained from the experiments on segments of rabbit aorta *in vitro*. Exposure for 5 minutes to a low concentration of Dibenzyline produced a blockade which was not completely dissipated 5 days later. In this preparation there was little fat available to accumulate Dibenzyline during the short period of exposure, and any drug not firmly bound to tissue should have been washed out early in the experiment, as was the case with piperoxan. Each strip was exposed to an average of about 40 changes of bath fluid per day during the study, and each change of fluid had a volume at least 300 times that of the tissue. Even with a partition coefficient strongly favoring the lipid phase, unbound drug would have been

dissipated after relatively few washes. FURCHGOTT (8) has pointed out that the rates of accumulation and dissipation are comparable for many drugs whose effects are limited by diffusion into and out of tissues.

Under these conditions, one must conclude that the prolonged blockade was due to some action on the tissue which occurred during the short period of exposure to Dibenzyline, and which was not dissipated completely over the observation period of 5 days. Data on the reactions of Dibenzyline with various chemical groupings found in tissues (14), and evidence that blockade by this agent is inhibited when specific tissue receptors are occupied by prior exposure to a high concentration of a specific agonist (7, 18, 19), combine to make it highly probably that this "action" is the formation of relatively stable chemical bonds between the blocking agent and the specific tissue receptors.

SUMMARY

Administration of thiosulfate to rats before and/or after intramuscular injection of Dibenzyline revealed that doses which almost completely prevented the development of adrenergic blockade when present during the entire period of Dibenzyline action did not significantly affect the blockade when administered two or more hours after the blocking agent. Some blockade developed when thiosulfate blood levels were allowed to decline several hours after injection of the Dibenzyline.

Exposure of rabbit aorta strips to Dibenzyline for 5 minutes *in vitro* produced a blockade which persisted for at least 5 days.

Radioactivity from Dibenzyline-C^{14} was detected in mice for as long as 7 days after intravenous injection. Measurement of radioactivity in various tissues during 24 hours after intravenous injection of 5.0 mg kg of Dibenzyline-C^{14} failed to reveal any considerable selective accumulation in fat. Comparison of these data with published values for unaltered extractable Dibenzyline indicates that much of the persistent radioactivity in parenchymatous organs was due to drug which had reacted with the tissues.

It is concluded that available data do not rule out a contribution of active haloalkylamine released from fat depots to the prolonged blockade, particularly after administration of very high dose. However, correlation of the present observations with previously published information on the pharmacology of Dibenamine, Dibenzyline and other haloalkylamines strongly supports the view that these agents produce a persistent adrenergic blockade primarily because they form ethyleneiminium

derivatives which react with specific adrenergic α-receptors. The relative stability of this drug-receptor bond prevents mass-action equilibrium between the agonist, antagonist and receptor, a "nonequilibrium" blockade.

Acknowledgements

The author wishes to acknowledge his indebtedness to Dr. Robert M. Horowitz, who was responsible for a major part of the investigation with Dibenzyline-C^{14}. This material, labeled at the methylene of the benzyl group, was synthesized by Dr. W. Gump and associates (24), and supplied through the courtesy of Smith, Kline & French Laboratories, who also provided the untagged Dibenzyline. Synthetic angiotensin was kindly supplied by Dr. C. W. Murphy, Ciba Co. Ltd.

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Thiosulfate

Über das Schicksal von Natriumthiosulfat im Organismus.

Von
Wilhelm Nyiri.

(Aus der II. medizinischen Abteilung des Krankenhauses Wieden in Wien.)

(Eingegangen am 10. Juli 1923.)

Unter den elementaren chemischen Stoffen fällt dem Schwefel im tierischen Organismus keine unbedeutende Rolle zu; dies erkennen wir schon daraus, daß im Körper sowohl organische, wie anorganische schwefelhaltige Produkte mehrfach vorkommen. Vor allem der Umstand, daß das Körpereiweiß und gewisse Abbauprodukte desselben Schwefel enthalten, spricht für die Bedeutung dieses Elementes im Organismus. Dementsprechend befassen sich auch zahlreiche Arbeiten in der Literatur mit dem Schwefelstoffwechsel. Erst in letzter Zeit hat z. B. *Hottinger*[1]) nachgewiesen, daß der Schwefel im Organismus sowohl als Reduktionsmittel, wie auch als Oxydationsmittel wirken kann. Trotzdem aber sind wir noch weit davon entfernt, ein klares Urteil über das Schicksal vieler schwefelhaltiger Produkte im Körper zu bekommen. Es hat demnach jede Beobachtung, die zur Klärung dieser komplizierten biologischen Verhältnisse, wenn auch nur in bescheidenem Ausmaße, beiträgt, eine Berechtigung.

Seitdem wir zur Feststellung der Nierenfunktion Natriumthiosulfat dem Organismus[2]) einverleiben, haben wir dem Schwefelstoffwechsel neuerdings eine größere Aufmerksamkeit zugewendet. Wenn wir nämlich dem Körper intravenös 1 g $Na_2S_2O_3$ zuführen, so treten bei erhaltener Nierenfunktion etwa 30 bis 40 Proz. dieses Salzes in den Harn über, wogegen bei gestörter Leistungsfähigkeit der Nieren dem

[1]) *Hottinger*, Schweiz. med. Wochenschr. 1923, Nr. 17.
[2]) *W. Nyiri*, Wien. klin. Wochenschr. 1922, Nr. 26; Klin. Wochenschr. 1923, Nr. 5; Die Thiosulfatprobe (Monographie). Wien-Leipzig, Deuticke, 1923.

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Grade der Schädigung entsprechend nur geringere Mengen im Harn zu finden sind. Es erscheinen demnach in unzersetztem Zustande im besten Falle 40 Proz. der einverleibten Menge Thiosulfat im Urin. Unwillkürlich hat sich uns daher die Frage aufgedrängt, was mit den restlichen 60 und mehr Prozenten im Körper geschieht.

Das Natriumthiosulfat ist bekanntlich oxydativen Vorgängen leicht zugänglich. Durch viele chemische Stoffe wird eine Zersetzung dieses Salzes in SO_2 und S hervorgerufen. Durch Oxydation dieser Körper wieder entsteht in letzter Linie Schwefelsäure, bzw. ihr Salz. Die Annahme lag daher von vornherein nahe, daß aus Natriumthiosulfat im Körper wenigstens zum Teil Natriumsulfat würde.

Mit dieser Frage befaßte sich denn auch *Trachtenberg*[1]) im Jahre 1861. In seiner Arbeit „Zur Frage über die Neutralisation überschüssiger Alkalien im Blute" verwendet er zwecks Absättigung von basischen Stoffen im Blute unter anderem das $Na_2S_2O_3$ und prüft bei dieser Gelegenheit den Harn, ob nach peroraler Verabreichung von $Na_2S_2O_3$ die ausgeschiedene Schwefelsäuremenge zunehme. Zur quantitativen Bestimmung der H_2SO_4 im Harn benutzt er die übliche gravimetrische Methode der Barytfällung. Tatsächlich findet er nach Einnahme von wenigstens einigen Grammen Natriumthiosulfat regelmäßig eine Vermehrung des Schwefelsäuregehalts im Harn. Doch begnügt sich *Trachtenberg* mit der Feststellung, daß der Überschuß an Schwefelsäure bei Einnahme von kleineren Dosen des Salzes relativ größer ist, als bei Einnahme von größeren Dosen, daß aber absolut die Vermehrung der Schwefelsäure bei großen Dosen des Mittels steigt, ohne auf nähere quantitative Beziehungen zwischen der Menge des dem Körper einverleibten Schwefels und der des im Harn erscheinenden Schwefels einzugehen.

Durch Ausarbeitung der Thiosulfatprobe war es uns interessant, einerseits die Angaben *Trachtenbergs* nachzuprüfen, andererseits nachzusehen, ob nach Ausscheidung von unzersetztem Thiosulfat die ganze restliche Menge dieses Salzes oder nur ein Teil desselben im Körper zu schwefelsaurem Natrium verbrannt wird. Es erschien uns wertvoll, als Ergänzung unserer Mitteilungen über die Thiosulfatprobe auch über das Schicksal des im Körper eine Veränderung erfahrenden Anteils von $Na_2S_2O_3$ etwas Bestimmtes aussagen zu können und für unsere Behauptungen zahlenmäßige Belege zu erbringen.

Um eine eventuelle Vermehrung der Schwefelsäureausscheidung im Harn nach Thiosulfatverabreichung feststellen zu können, mußten wir zunächst eine annähernd gleichbleibende tägliche SO_3-Ausscheidung im Harn erreichen. Wir verabreichten daher täglich eine annähernd gleiche Kost und bestimmten im 24stündigen Harn die Schwefelsäureausscheidung gravimetrisch. Als wir bei einem Patienten ein solches tägliches Gleichgewicht erzielt hatten, bestimmten wir weiterhin, ob sich in zweistündlichen Harnmengen (von 6 Uhr früh bis 12 Uhr mittags) annähernd gleiche SO_3-

[1]) *Trachtenberg*, Inauguraldissertation, Dorpat 1861.

Werte vorfanden. Da bei einer Belastung des Organismus mit 1 g $Na_2S_2O_3$ im besten Falle eine Vermehrung von auch etwa nur 1 g Schwefelsäure zu gewärtigen war, so konnte man, wenn überhaupt, diesen relativ geringen Überschuß am ehesten in einer Zweistundenportion des Harns erwarten. Eine annähernd quantitative Übereinstimmung der zweistündigen SO_3-Ausscheidungen ließ sich leicht feststellen. Darauf erhielt der Patient einmal 1 g $Na_2S_2O_3$ per os und an einem anderen Tage dieselbe Menge intravenös. Es zeigte sich jedoch kein merklicher Unterschied in den ausgeschiedenen Mengen SO_3, trotzdem beim peroralen Versuch keine Spur von unzersetztem Thiosulfat im Harn zu finden war, während nach intravenöser Verabreichung 34,9 Proz. unveränderten Salzes mit Hilfe der Jodometrie im Urin nachgewiesen werden konnten. Die nebenstehenden zwei kleinen Tabellen geben die Versuche wieder und enthalten die näheren Details (Tabelle I und II):

Tabelle I.

8h früh 1,0 $Na_2S_2O_3$ *per os* mit 200,0 H_2O.

Stunde	Harnmenge	SO_3 g
6— 8h	225	0,5653
8—10	230	0,5954
10—12	194	0,6503
12— 2	150	0,6750

Tabelle II.

8h früh 1,0 $Na_2S_2O_3$, 10proz. *intravenös.*

Stunde	Harnmenge	SO_3 g
6— 8h	86	0,6815
8—10	162	0,6706
10—12	152	0,7007
11— 2	194	0,7025

Aus den Tabellen geht deutlich hervor, daß die zweistündlichen SO_3-Werte im Harn vor der Verabreichung von $Na_2S_2O_3$ und in den folgenden Stunden etwa gleich hoch gefunden wurden. Dieser Umstand darf uns jedoch nicht verwundern, da 1 g SO_3 und weniger doch vielleicht eine zu geringe Menge vorstellt, um als Überschuß über einen schon normalerweise ziemlich beträchtlichen Schwefelsäurestoffwechsel bemerkbar zu werden.

Dementsprechend habe ich den nächsten Versuch mit einer größeren Menge $Na_2S_2O_3$ wiederholen müssen.

Freilich konnte dies nur bei peroraler Applikationsweise geschehen, da wir dem Körper intravenös nicht allzu viel Salz zuführen wollten. *Trachtenberg* gab in seiner vorhin erwähnten Arbeit an, daß nach Einnahme von 18 g und mehr Natriumthiosulfat mehrere flüssige Stühle auftraten, in denen offenbar ein nicht bestimmbarer Teil des dem Körper einverleibten $Na_2S_2O_3$ dem Stoffwechsel entging. Um dies zu vermeiden, nahm ich daher 10 g des Salzes ein. Vorher stellte ich die Gesamtausscheidungen von Schwefelsäure in den 24stündigen Harnmengen fest und bestimmte getrennt die Sulfatschwefelsäure und die Ätherschwefelsäure:

Wie aus Tabelle III ersichtlich, fingen wir am Tage der $Na_2S_2O_3$-Belastung den Harn in drei getrennten Portionen auf und am folgenden Tage in zwei Portionen; sonst wurde stets die gesamte 24stündige Menge als einzige Portion untersucht. Trotzdem ich nur 10 g $Na_2S_2O_3$

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um 8 Uhr früh eingenommen hatte, traten am Nachmittage gegen 4 Uhr zweimal leichte Diarrhöen auf, die, wie auch *Trachtenberg* schon seinerzeit an sich feststellen konnte, vollkommen ohne Tenesmen und ohne Kolikschmerzen verliefen. Ein wenn auch kleiner Teil des $Na_2S_2O_3$

Tabelle III.

Schwefelsäurebestimmungen im Harn.

Datum	Stunde	Harnmenge	Sulfatschwefelsäure SO_3 g	Ätherschwefelsäure SO_3 g	Bemerkungen
13. III.		1100	1,9658	0,0475	
14. III.		960	2,1699	0,1099	
15. III.		960	2,7382	0,1159	
16. III.	8—2h	214	2,0702	0,0238	8h früh 10,0 g $Na_2S_2O_3$ *per os* mit 200,0 g H_2O 4h nachm. zwei dünnbreiige Stuhlentleerungen
	2—8	592	3,5609	0,0609	
	8—8 früh	360	3,0194	0,0927	
17. III.	8—2h	204	0,9679	0.0133	
	2—8 früh	530	2,7670	0.0326	
18. III.		670	2,6192	0.0158	
19. III.		755	1,9100	0.0439	
20. III.		1030	2,1352	0.0274	
21. III.		680	1,9981	0,0127	

war demnach vermutlich in unzersetztem Zustande dem Organismus entwichen. Nichtsdestoweniger ist das Versuchsresultat ein durchaus zufriedenstellendes.

Die Tabelle zeigt, daß in den ersten 24 Stunden nach der Einnahme von $Na_2S_2O_3$ 8,6 g Sulfatschwefelsäure im Harn gefunden wurden, während in den folgenden 24 Stunden nur mehr 3,7 g auftraten. Sämtliche diesbezüglichen Werte der übrigen Tage sowohl vor, wie nach dem eigentlichen Versuche haben annähernd korrespondierende Zahlengrößen, deren Mittelwert etwa 2,1 g ausmacht. Rechnen wir für die beiden Tage, an denen die Schwefelsäure im Harn vermehrt war, die durchschnittliche Menge der SO_3-Ausscheidung ohne Salzbelastung auch mit etwa 2,1 g, so bekämen wir am ersten Tage ein Plus von etwa 6,5 g SO_3, und am zweiten Tage ein solches von etwa 1,6 g SO_3. Die gesamte Mehrausscheidung an Sulfatschwefelsäure beläuft sich demnach auf etwa 8,1 g.

Berechnen wir theoretisch, wieviel Gramm SO_3 bei vollständiger Oxydation aus 10 g $Na_2S_2O_3$ (reiner Substanz) entstehen können, so resultiert der Wert von 10.125 g SO_3. Praktisch können wir daher aus 10 g $Na_2S_2O_3$ auch wieder 10 g SO_3 erhalten. Aus dieser Überlegung geht hervor, daß wir in unserem Versuche 80 Proz. des dem Körper einverleibten Salzes *als Sulfatschwefelsäure wiedergefunden* haben.

Dazu kommt noch, daß, wie folgende kleine Tabelle (IV) zeigt, ein wenn auch kleiner Teil von $Na_2S_2O_3$ in unzersetztem Zustande in den Harn übertrat und in diesem jodometrisch bestimmt werden konnte. Die ausgeschiedene Menge Thiosulfat beträgt 548 mg, d. h. 5,48 Proz. Addieren wir diese 5 Proz. $Na_2S_2O_3$ zu dem früher gefundenen Werte von Sulfatschwefelsäure — ein Vorgang, den wir nach dem früher

Tabelle IV.

16. März, 8h früh, 10,0 $Na_2S_2O_3$ *per os* mit 200,0 H_2O.

Stunde	Harnmenge	Auf 10 ccm Harnmenge verbrauchte Jodlösung ccm	Ausscheidung von $Na_2S_2O_3$ mg	Proz.
8h	80	0,1	—	—
8 bis 2h	214	1,0	304	3,04
2 bis 9h abends	592	0,3	187	1,87
9h abends bis 8h früh	360	0,2	57	0,57
8h früh bis 2h nachm.	204	0,07	—	—
		Gesamtausscheidung . .		5,48 Proz.

Gesagten ohne groben Fehler tun können —, so haben wir insgesamt etwa 86 Proz. des peroral einverleibten Schwefels im Harn wiedergefunden. Wir gehen wohl kaum fehl, wenn wir annehmen, daß die übrigen 14 Proz., das ist 140 mg $Na_2S_2O_3$, mit den diarrhoischen Stühlen den Körper wieder verlassen haben, und glauben uns doch vielleicht mit aller Reserve zu dem Schluß berechtigt, daß die Oxydation des Thiosulfats im Körper mit Ausnahme des durch die Nieren hindurchtretenden Anteils eine vollständige ist und bis zur höchsten Oxydationsstufe, das ist dem Natriumsulfat, geht.

Betrachten wir im Gegensatz zu den Werten der Sulfatschwefelsäure die Ätherschwefelsäuren, so sehen wir, daß hier kaum eine nennenswerte Vermehrung im Harn nachzuweisen ist.

Ein zweiter Versuch, den wir zwecks vollständiger Verhütung der Diarrhöen mit nur 5 g $Na_2S_2O_3$ (peroral) anstellten, ergab leider kein so exaktes Resultat, indem einerseits trotz der geringeren Menge leichtes Abführen auftrat, andererseits das Plus an Schwefelsäure nicht so schlagend zutage trat, wie bei Verwendung der größeren Menge $Na_2S_2O_3$. Dieser Versuch förderte 287 mg unzersetztes $Na_2S_2O_3$ zutage, demnach 5,7 Proz. der eingeführten Menge. Aus dem Vergleich der beiden Thiosulfatbestimmungen erkennen wir, daß bei peroraler Verabreichung von 5 g und 10 g $Na_2S_2O_3$ in beiden Fällen etwa 5,5 Proz. des Salzes in unveränderter Form in den Harn übertritt. Dagegen finden wir nach Einnahme von nur 1 g dieses Salzes bei wiederholten Versuchen niemals meßbare Mengen Thiosulfat im Harn, so daß die Oxydation im Körper bei peroraler Applikation derart geringer Mengen eine vollkommene ist.

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Ausscheidung von $Na_2S_2O_3$

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Darüber, wo im Körper diese Oxydation des $Na_2S_2O_3$ vor sich geht, läßt sich zurzeit leider nichts Bestimmtes aussagen. Wie wir bereits in unserer Monographie (Die Thiosulfatprobe, Wien-Leipzig, Deuticke, 1923) erwähnt haben, ergaben Versuche, die wir zur Klärung dieser Frage unternommen hatten, ein negatives Resultat. Am wahrscheinlichsten erscheint noch die Annahme, daß die oxydierende Einwirkung auf das $Na_2S_2O_3$ mehr oder weniger allen Zellen und Gewebssäften des lebenden Körpers zukommt und wohl auch vom zirkulierenden Blute aus erfolgt.

Zusammenfassung.

Die vorliegenden Versuche befassen sich mit der Frage, wieweit die Oxydation des Natriumthiosulfats im Körper vor sich geht. Das Resultat gestattet den Schluß, daß die gesamte Menge des $Na_2S_2O_3$, die nicht die Nieren unverändert passiert, im Körper verbrannt wird. Als Endprodukt dieser Oxydation tritt eine Vermehrung der Sulfatschwefelsäure im Harn auf.

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Thiosulfate

THE FATE OF SODIUM THIOSULFATE IN THE BODY

by

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(Submitted July 10, 1923)

Of the chemical elements in the animal body, an important role is attributed to sulfur. We recognize this from the fact that many sulfur-containing products, both organic and inorganic, occur in the body. In particular, the fact that protein and some of its products of decomposition in the body contain sulfur is evidence of the importance of this element in the body. Accordingly, many articles in the literature deal with sulfur metabolism. Only recently, Hottinger[1] has shown that sulfur can act both as an oxidizing agent and a reducing agent in the body. However, despite this, we are far from being able to obtain a clear idea about the fate of many sulfur-containing compounds in the body. Therefore, every observation which contributes to the clarification of this complex, biological relationship, no matter how little, is warranted.

Since we have incorporated sodium thiosulfate into the body[2] to determine renal function, we have given greater attention to sulfur metabolism recently. If we administer 1 g $Na_2S_2O_3$ intravenously, about 30-40% of this salt passes into the urine when

[1] Hottinger, Schweiz. med. Wochenschr. 1923, Nr. 17.

[2] W. Nyiri, Wien. klin. Wochenschr. 1922, Nr. 26; Klin. Wochenschr. 1923, Nr. 5; Die Thiosulfatprobe (Monographie). Wien-Leipzig, Deuticke, 1923.

renal function is normal. However, when renal function is impaired, smaller quantities are found in the urine which correspond to the extent of the damage. Therefore, under the best conditions, 40% of the dose of thiosulfate administered appears in the urine in an undecomposed form. The problem automatically arose of what happened to the remaining 60% or more in the body.

It is known that sodium thiosulfate readily undergoes oxidative processes. This salt is decomposed into SO_2 and S by many chemicals. Sulfuric acid or its salts are then formed by oxidation of these compounds. This suggests that at least some of the sodium thiosulfate is converted intc sodium sulfate in the bcdy.

Trachtenberg[1] dealt with this problem in 1861. In his article "The Neutralization of Excess Alkalies in the Blood", he used $Na_2S_2O_3$, among other things, to saturate bases in the blood and took advantage of this opportunity to test the urine to determine whether oral administration of $Na_2S_2O_3$ would increase the quantity of sulfuric acid excreted. He employed the conventional gravimetric barium sulfate precipitation method for quantitative analysis of H_2SO_4 in the urine. He did find that an increase consistently occurred in the sulfuric acid content of the urine after ingestion of at least a few grams of sodium thiosulfate. However, Trachtenberg was satisfied with the finding that the excess of sulfuric acid is relatively larger when smaller doses of the salt are ingested than when larger doses are ingested but that, on the average, the absolute increase in sulfuric acid

[1] Trachtenberg, Dissertation, Dorpat 1861.

increases with large doses. He did not pursue in more detail the quantitative relationships between the quantity of sulfur incorporated into the body and the sulfur appearing in the urine.

We were interested in confirming Trachtenberg's findings with the thiosulfate test and also in determining whether all or only a part of the thiosulfate remaining in the body after excretion of undecomposed salt is oxidized to sodium sulfate. We thought it would be valuable to supplement our findings made by the thiosulfate test with definite information on the fate of that part of the $Na_2S_2O_3$ undergoing a change in the body and to present quantitative data for our hypotheses.

So that we could determine a possible increase in sulfuric acid excretion in the urine after thiosulfate administration, we first had to obtain a 24-hr SO_3 excretion which remained almost the same in the urine. Consequently, we gave approximately the same food every day and determined sulfuric acid excretion gravimetrically in 24-hr urine specimens. When we had reached such a daily equilibrium with a patient, we then determined whether approximately the same quantity of SO_3 was present in two-hr urine samples (collected from 6 a.m. until 12 noon). Since when the body is loaded with 1 g $Na_2S_2O_3$, only an increase of 1 g sulfuric acid at most can be anticipated, this relatively small excess would most likely be present in a two-hr urine sample. It was easy to establish an almost quantitative agreement between the two-hr SO_3 excretions. Then the patient received one dose of 1 g $Na_2S_2O_3$ orally and, on

another day, this same quantity intravenously. However, there was no appreciable difference in the quantities of SO_3 excreted although when thiosulfate was administered orally not a trace of the undecomposed salt could be found in the urine, while after intravenous administration 34.9% of the unchanged salt could be detected in the urine by iodimetry. The two small tables which are appended show the results of the experiment and contain more details (Tables I and II):

TABLE I

8 a.m., 1.0 g $Na_2S_2O_3$ orally
with 200.0 ml water

Time	Urine Volume	SO_3 in grams
6-8 a.m.	225 ml	0.5653
8-10	230	0.5954
10-12	194	0.6503
12-2 p.m.	150	0.6750

TABLE II

8 a.m.,1.0 g $Na_2S_2O_3$, 10%
intravenously

Time	Urine Volume	SO_3 in grams
6-8 a.m.	86 ml	0.6815
8-10	162	0.6706
10-12	152	0.7007
11-2 p.m.	194	0.7025

From the tables it can be seen clearly that SO_3 values for two-hr urine specimens were found to be about the same before administration of $Na_2S_2O_3$ and in the following hours. However, this fact should not surprise us since 1 g SO_3 or less is

probably too small a quantity to be observed as an excess over the sulfuric acid metabolism which is rather substantial under normal conditions.

Therefore, I had to repeat the next experiment with a larger quantity of $Na_2S_2O_3$.

This could only be done with the oral route of administration since we do not want to introduce too much salt into the body intravenously. In his previously cited work, Trachtenberg reported that several liquid stools were passed after the ingestion of 18 g or more of sodium thiosulfate. Apparently they contained an undetermined percentage of the $Na_2S_2O_3$ which was not metabolized by the body. Therefore, I used 10 g of the salt to avoid this. First I determined the total excretion of sulfuric acid in 24-hr urine samples and determined sulfate sulfuric acid and ether sulfuric acid separately.

As can be seen from Table III, we collected three separate samples on the day of $Na_2S_2O_3$ loading and two samples on the next day. Otherwise, determinations were always run on the entire 24-hr samples. Although I only ingested 10 g $Na_2S_2O_3$ at 8:00 a.m., two watery bowel movements occurred around 4 p.m. without any tenesmus or colic pains, as Trachtenberg had already established. Therefore, it was suspected that $Na_2S_2O_3$, even though it was just a small part of it, was excreted unchanged from the body. However, the results of the test were quite satisfactory.

TABLE III

Urinary Sulfuric Acid Determinations

Date	Times	Urine Volume	Sulfate Sulfuric Acid, g SO_3	Ether Sulfuric Acid, g SO_3	Comments
Mar. 13		1100	1.9658	0.0475	
Mar. 14		960	2.1699	0.1099	
Mar. 15		960	2.7382	0.1159	
Mar. 16	8-2	214	2.0702	0.238	10.0 g $Na_2S_2O_3$ orally at 8 a.m. with 200.0 g water at 4 p.m. 2 watery bowel movements
	2-8	592	3.5609	0.0609	
	8-8 a.m.	360	3.0194	0.0927	
Mar. 17	8-2	204	0.9679	0.0133	
	2-8 a.m.	530	2.7670	0.0326	
Mar. 18		670	2.6192	0.0158	
Mar. 19		755	1.9100	0.0439	
Mar. 20		1030	2.1352	0.0274	
Mar. 21		680	1.9981	0.0127	

The table shows that 8.6 g of sulfate sulfuric acid were found in the urine in the first 24 hours after ingestion of $Na_2S_2O_3$, while only another 3.7 g appeared during the next 24 hours. All of the values for the other days, both before and after the test, have about the same numerical values with an average of about 2.1 g. If we assume that the average quantity of SO_3 excreted for the two days in which urinary sulfuric acid was elevated would also have been about 2.1 g without salt loading, we would obtain an excess of about 6.5 g SO_3 on the first day and an excess of 1.6 g SO_3 on the second day. Thus,

the entire excess excretion of sulfate sulfuric acid would then be about 8.1 g.

If we calculate theoretically how many grams of SO_3 would be formed by complete oxidation of 10 g $Na_2S_2O_3$ (pure compound), we get a value of 10.125 g SO_3. In practice, therefore, we could obtain 10 g SO_3 from 10 g $Na_2S_2O_3$. From this it follows that in our experiment we recovered 80% of the ingested salt as sulfate sulfuric acid.

As the following small table (IV) shows, it also follows that a small percentage of $Na_2S_2O_3$ went unchanged into the urine and could be determined iodometrically in the urine. The quantity of thiosulfate excreted was 548 mg, i.e. 5.48%.

TABLE IV

March 16, 8 a.m., 10 g $Na_2S_2O_3$ orally with 200.0 [ml] H_2O

Times	Urine Volume	Iodine Solution used for 10 ml Urine (ml)	$Na_2S_2O_3$ Excretion mg	%
8	80	0.1	-	-
8-2	214	1.0	304	3.04
2-9 p.m.	592	0.3	187	1.87
9 p.m.-8 a.m.	360	0.2	57	0.57
8 a.m.-2 p.m.	204	0.07	-	-
		Total excretion		5.48%

If we add this 5% $Na_2S_2O_3$ to the previously established value for sulfate sulfuric acid, a procedure which we can do without great error as was stated above, we have recovered a total of about 86% of the ingested sulfur in the urine. We would scarcely

be wrong to assume that the remaining 14%, i.e. 140 mg $Na_2S_2O_3$, was excreted from the body with the loose bowel movements, and perhaps we would be justified in concluding that with the exception of the portion excreted by the kidneys, the oxidation of thiosulfate in the body is complete and that it proceeds to the highest state of oxidation, i.e. sodium sulfate.

If, on the other hand, we compare the values for sulfate sulfuric acid with those for ether sulfuric acid, it can be seen that the increase in the urine is hardly significant.

Unfortunately, a second experiment in which we used only 5 g $Na_2S_2O_3$ (orally) to prevent diarrhea completely did not give such accurate results. Despite the small quantity of loose stools which formed, the excess of sulfuric acid was not as pronounced as when the larger quantity of $Na_2S_2O_3$ was used. This experiment showed that there were 287 mg of unchanged $Na_2S_2O_3$ or 5.7% of the quantity ingested. By comparing the two thiosulfate determinations, we found that administration orally of both 5 g and 10 g $Na_2S_2O_3$ showed that about 5.5% of the salt passed unchanged into the urine. However, after ingestion of only 1 g of this salt, repeated experiments showed immeasureably small quantities of thiosulfate in the urine so that oxidation in the body is complete when small doses of this kind are administered orally.

In the meantime, it is unfortunate that nothing definite can be said about where in the body this oxidation of $Na_2S_2O_3$

occurs. As we have already mentioned in our monograph (Die Thiosulfatprobe, Wien-Leipzig, Deuticke, 1923), experiments which we carried out to solve this problem gave negative results. The most likely hypothesis appears to be that $Na_2S_2O_3$ is oxidized more or less by all cells and tissue fluids in the living body and probably also by the circulating blood.

SUMMARY

The present experiment deals with the problem of the extent to which sodium thiosulfate is oxidized in the body. The results confirm the conclusion that all $Na_2S_2O_3$ which is not passed unchanged by the kidneys is oxidized in the body. An increase in urinary sulfate sulfuric acid appears as the end product of this oxidation.

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Discussion : Vote de clôture.

Concernant les remarques de BOCK à propos de l'éosinophilie de la panmyélopathie d'uréthane je voudrais seulement rappeler qu'on trouve souvent la même éosinophilie non seulement dans l'agranulocytose mais aussi dans les anémies aplastiques de benzol. Je crois que nous pouvons dire qu'il y a deux formes de cellules desquelles nous ne savons encore rien ou très peu de leur fonction, c'est les éosinophiles et les lymphocytes.

Je suis très heureux que LEIBETSEDER a pu confirmer nos constatations expérimentales avec uréthane. Je ne crois personnellement pas qu'il s'agit ici d'un phénomène central parce qu'on peut très bien démontrer le même effet dans les cultures de la moelle osseuse. La constatation d'une anémie dans les expériences avec l'uréthane dépend surtout du temps d'observation, vu la longue durée de vie des érythrocytes (120 jours).

Concernant le rapport de GOLDECK, je voudrais seulement ajouter qu'il y a [illegible] t individuel très différent qui peut-être dépend du contenu individuel en ferments et enzymes qui sont bloqués par ces substances.

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Thiosulfate

LE MÉTABOLISME DE L'HYPOSULFITE DE SODIUM CHEZ LES LEUCÉMIQUES SON ACTION HYPERPLASIQUE ET ANTICYTOSTATIQUE

Par W. PAOLINO et F. P. GAVOSTO.

Nous nous sommes occupés du problème des différences de rapidité et extension du développement des tissus hyperplasiques et néoplasiques : différences qu'on remarque de cas à cas, et, dans chaque cas en différentes phases du cours de la maladie. Comme première hypothèse, ce qui est l'objet de cette étude, nous avons avancé la possibilité que cette différence soit d'origine périphérique, c'est-à-dire dans les tissus mêmes. Nous avons par conséquent supposé que le différent potentiel de développement des tissus hyperplasiques et néoplasiques, et l'avidité de ces tissus au égard aux tissus normaux, dépend de la disponibilité et d'une particulière et primitive capacité de fixer et d'utiliser, d'une façon générique et sélective, les facteurs essentiels de développement, soit les enzymes, nécessaires à l'utilisation des matériaux constitutifs plutôt que l'utilisation directe de ces derniers. Il est désormais reconnu qu'en vue du développement cellulaire une suffisante et spécifique dotation d'enzymes soit nécessaire. Parmi les groupes d'enzymes nous avons fixé notre attention sur ceux qui agissent au moyen du radical sulfhydrile. Leur importance dans les processus prolifératifs des tissus normaux et pathologiques est soutenue par de nombreuses recherches. Parmi les plus anciennes nous rappelons celles de HAMMET qui conclut

Le Sang, 21, 1950

pour une action stimulante sur le développement du tissu animal et végétal par les groupes sulfhydriliques en contraste avec l'action inhibante des groupes sulfoxyliques (SO_2, SO_3) dérivés des premiers par oxydation. BACKER en a démontré l'influence sur la prolifération des fibroblastes et des cellules épithéliales *in vitro;* SHEARER, RAPKINE, EPHRUSSI, BRACHET l'ont démontrée dans les processus de division cellulaire. La métamorphose des gyrins est accélérée par l'hyposulfite de Na (BINET et MAGROU). Dans les tumeurs végétales il y a une augmentation du glutathion (BINET et MAGROU); dans les hépatomes expérimentaux le développement de la tumeur est précédé par une augmentation du glutathion hépatique (BOYLAND, MAWSON; IKI) ainsi que dans la peau traitée par du goudron (SPASOKOUKOTZKY). Des recherches plus récentes ont confirmé ces données. PARCKER et KRACKE (1946) rapportent au glutathion une action accélérante des mitoses chez les leucémiques. FRIEDMANN et MARRIAN (1948) ont démontré que l'inhibition des mitoses par l'acide maléique et les quinones est parallèle à la perte des groupes sulfhydriques des cellules, en liant ces substances aux groupes SH cellulaires. L'administration intra-musculaire de thioglycolate et de thiomalate porte, chez les rats, avec une tumeur transplantable, à une accélération de l'accroissement tandis que la méthionine, la cistéine, le thiolactate sont inactifs; les substances qui, comme l'iodoacétate et le maléate, lient les groupes SH, en retardent l'accroissement (BRUNSCHWIG et coll.). Parmi plusieurs acides aminés expérimentés, seule la cystéine démontra sa capacité d'inhiber, en cas de carence, l'apparition des leucémies expérimentales de méthylcolanthrène (WHITE, WHITE et MIDER) (1947). Aussi, dans l'inhibition du développement des tumeurs est évidente l'importance des groupes SH. D'excellents résultats furent obtenus dans les tumeurs transplantables avec miosalvarsan (action sur les groupes SH par l'oxyde arsénieux) (TANAKA et coll.); disparition de l'action inhibante des hydrocarbures par l'administration des cystéine, méthionine, glutathion (WHITE); on observa encore la diminution des groupes SH dans les organes d'animaux après l'administration de benzopyrène (RONDONI et BORETTI). On ne peut donc douter, bien qu'on ne la connaisse pas définitivement, de l'importance des groupes sulfhydriliques; leur action fait part des processus d'oxydo-réduction, qui sont à la base des phénomènes du métabolisme cellulaire.

Pour nos recherches cliniques nous avons employé le thiosulfate de soude, ne pouvant pas disposer de glutathion ni d'acides aminés sulfurés en quantité suffisante. Au thiosulfate de soude on reconnaît la capacité de participer à la formation de composés organiques sulfurés et du glutathion même (ARNOVLJEVITCH, BINET et coll.) et de former par conséquent des composés avec action sulfhydrilique, et, enfin, par son énergique et rapide activité réduisante, d'influer d'une façon directe

sur l'équilibre oxydo-réductif de l'organisme. On a encore un avantage dans la possibilité d'en suivre le métabolisme par le dosage dans le sérum et dans l'urine. Nous avons donc fait nos recherches par l'administration intra-veineuse de 2 gr. de thiosulfate de soude. Nous avons étudié : 1° les conditions cliniques des malades; 2° le dosage du thiosulfate dans le sérum et dans les urines; 3° la consommation d'oxygène par les malades. Nous avons adopté ce dernier critérium parce que, bien qu'on ne connaisse pas encore exactement les causes de l'augmentation du métabolisme basal chez les leucémiques et beaucoup d'hémopathiques, néanmoins, sur la base d'une expérience personnelle, et des données de la plupart des observateurs les plus récents, il semble que cette augmentation peut être rapportée aux dimensions du développement du tissu hyperplasique. C'est en considérant cette augmentation du métabolisme basal chez les leucémiques, que nous avons fait nos recherches de contrôle, non seulement sur des sujets normaux, mais aussi sur des hyperthyroïdiens.

Nous avons injecté 2 gr. de thiosulfate de soude et déterminé son taux dans le sérum après 15', 30', 45'. Nous avons trouvé les valeurs les plus hautes après 15'; 30' après le taux diminuait déjà sensiblement, devenant presque toujours zéro après 45'. Dans l'évaluation nous avons tenu compte de la valeur obtenue après 15'. Les différences des moyennes obtenues dans les différents groupes n'ont pas de signification au point de vue statistique, cependant on peut dire que les valeurs les étant nettement plus basses les valeurs des sujets normaux (mgr. 5,4 p. 100), et plus basses encore celles des leucémiques (mgr. 4,5 p. 100). Les autres cas ont démontré des valeurs à peu près normales.

Nous avons examiné l'élimination urinaire dans les trois heures suivant l'injection. L'examen fractionné nous a permis de constater qu'après deux heures, pratiquement, il n'était plus éliminé de thiosulfate. Nous rapportons par chaque groupe les valeurs moyennes d'élimination en pourcentage de la quantité de thiosulfate injectée. Le pourcentage d'élimination le plus élevé a été observé chez les hyperthyroïdiens (4 cas avec une moyenne de 21,7 p. 100 $\pm$ 2,2), quelque peu plus bas a été le pourcentage des sujets normaux (21 cas avec une moyenne de 18,2 p. 100 $\pm$ 2,9); nettement plus bas a été le pourcentage d'élimination des leucémiques (11 cas de différentes formes de leucémie avec une moyenne de 12,7 p. 100 $\pm$ 2,6); les cas de maladie de Hodgkin et de lymphosarcome ont donné un pourcentage d'élimination intermédiaire entre les normaux et les leucémiques, tandis que les cas d'anémie de Biermer et de polycythémie donnèrent des valeurs égales à celles de leucémiques.

Des épreuves exécutées sur le même sujet, quelques jours après, démontrèrent que les valeurs, soit d'augmentation dans le sérum que

d'élimination urinaire, étaient constantes, chez les sujets normaux ainsi que chez les groupes de malades.

En répétant l'épreuve chez le même sujet après 8-10 jours d'administration continuelle journalière de 2 gr. de thiosulfate, les valeurs, tant dans le sérum que dans les urines, ne présentèrent pas de modifications chez les sujets normaux, chez les hyperthyroïdiens et chez les leucémiques (myéloses chroniques leucémiques), tandis que dans les deux cas de polycythémie on remarqua une nette augmentation de l'élimination urinaire (de 286 mgr. soit 14 p. 100, à 488 mgr. soit 24 p. 100 et de 252 mgr. soit 13 p. 100 à 350 mgr. soit 17 p. 100), les valeurs n'ayant nullement varié dans le sérum après 15'.

Pour ce qui concerne la consommation d'oxygène, on a étudié ses éventuelles modifications dans les trois heures immédiatement successives à la première injection et le métabolisme basal après 8-10 jours d'administration quotidienne. Les modifications immédiates de la consommation d'oxygène furent de médiocre importance. Chez les hyperthyroïdiens, nous avons vu une augmentation moyenne de 4 p. 100; chez les normaux de 5 p. 100; chez les leucémiques de 8,6 p. 100. Après l'administration prolongée le métabolisme basal n'a pas absolument varié chez les normaux; il a augmenté constamment, et, sauf un cas, d'une façon significative chez les leucémiques. Une autre détermination exécutée 4-5 jours après l'achèvement des injections du thiosulfate, nous a permis de constater que chez les leucémiques les valeurs du métabolisme basal revenaient presque toujours aux valeurs de départ.

Après l'administration prolongée de thiosulfate on remarqua une tendance à l'augmentation du métabolisme, moindre cependant que chez les leucémiques, ainsi que dans l'anémie de Biermer, et le peu de cas de maladie d'Hodgkin et de lymphosarcome chez lesquels, au commencement de ces recherches, nous avions prolongé l'administration de thiosulfate.

En résumé, pour ce qui concerne la consommation d'oxygène, nous pouvons dire que le thiosulfate, injecté intra-veineusement, détermine de petites variations immédiatement après l'injection, particulièrement accentuées, dans le sens de l'augmentation, chez les sujets leucémiques; l'administration du thiosulfate pendant plusieurs jours de suite ne donne pas lieu à des modifications du métabolisme basal chez les sujets normaux et hyperthyroïdiens, tandis que chez les leucémiques il détermine une augmentation presque toujours nette et quelquefois considérable, qui toutefois tend à disparaître quelques jours après. Les effets du thiosulfate sur la consommation d'oxygène et son élimination mettent en évidence le comportement nettement opposé des hyperthyroïdiens et des leucémiques, et démontrent que le comportement particulier de ces derniers n'est pas à rapporter à l'existence d'un état d'hyperconsommation d'oxygène.

Pour ce qui regarde les rapports entre le comportement de la consommation d'oxygène et le métabolisme du thiosulfate injecté, notre casuistique, observée dans son ensemble, ne permet aucun rapprochement; par l'examen séparé des cas de leucémie (bien qu'il s'agisse de formes cliniquement différentes) on remarque l'existence d'un rapport : exactement, l'élimination urinaire de thiosulfate est d'autant plus faible qu'est plus élevée la valeur du métabolisme basal de départ et plus forte est l'augmentation de la consommation d'oxygène suivant immédiatement l'injection.

Au point de vue clinique nous avons observé, à la suite d'une seule injection, l'apparition de fièvre dans les cas de lymphadénose leucémique, de maladie d'Hodgkin, de lymphosarcome. A la suite de plusieurs injections, augmentation de température, des adénopathies et de la splénomégalie dans les lymphadénoses leucémiques et dans l'Hodgkin. Après une série d'injections, les cas de lymphadénose leucémique, d'Hodgkin et de lymphosarcome ont démontré une résistance sensible, et parfois totale, aux irradiations avec les rayons X. Les leucocytes n'ont pas présenté de variations sensibles, les cas de myélose leucémique, au contraire, ont été améliorés comme d'habitude par la roentgenthérapie. Dans un cas de myélose subleucémique aiguë (leucocytes 10,000 avec 29 p. 100 d'hémocytoblastes), sans fièvre et en conditions d'équilibre depuis trois mois, après seulement 6 injections de thiosulfate, on eut une aggravation imprévue des symptômes (fièvre élevée, angine nécrotique, leucocytes de 10,000 à 52,000 et puis à 150,000 avec 90 p. 100 d'hémocytoblastes) et la mort dans l'espace de 10 jours. A la suite de ce résultat et de la radio-résistance observée dans les cas de lymphadénose leucémique, d'Hodgkin et de lymphosarcome, il nous a paru nécessaire de limiter nos recherches aux cas de myélose leucémique, pour lesquelles nous n'avions pas remarqué de conséquences dangereuses évidentes.

Sur la base de notre hypothèse, que le comportement particulier du thiosulfate injecté et les suites cliniques observées dans les cas de leucémie, d'Hodgkin, de lymphosarcome fussent à rapporter à l'action réduisante et de formation de sulfhydriles par le thiosulfate, nous avons fait une contre-épreuve, essayant de traiter ces malades avec des substances antisulfhydriliques ou oxydantes. Nous avons choisi parmi les premières le cyanure de mercure. Nous avons traité un cas de lymphadénose leucémique avec 2 cgr. de cyanure tous les deux jours, c'est-à-dire comme pour un traitement antisyphilitique. Nous n'avions pas l'intention d'obtenir un effet thérapeutique, parce que nous pensions que l'action antisulfhydrylique du cyanure devait se dérouler d'une façon générique sur les sulfhydriles de tout l'organisme, et amenant par conséquent un dommage pareil, et peut-être majeur, pour les tissus normaux; on ne pouvait pas en effet présumer une action sélective sur

les sulfhydriles du tissu leucémique, quoiqu'il semble qu'il se vérifie à l'occasion de processus donnant lieu à une hyperconsommation génetique (grossesse, maladie fébrile). Par ce traitement nous avons observé : une augmentation initiale des leucocytes et une nette réduction successive des adénopathies et de la splénomégalie, avec une médiocre diminution du métabolisme basal. Nous avons cependant interrompu le traitement après 8 injections, parce que parallèlement aussi, les conditions générales du malade s'aggravaient. Il s'est donc vérifié ce que nous avions supposé. Jusqu'à présent, nous n'avons pas répété d'autres épreuves avec le cyanure. Comme substance oxydante nous avons employé la vitamine K, en l'administrant par des injections biquotidiennes intra-veineuses de 50 mgr. chacune. Ce traitement, nous l'avons pratiqué en 3 cas de myélose leucémique. En aucun cas nous n'avons obtenu une diminution de la splénomégalie ni des leucocytes. Le métabolisme basal est diminué nettement dans un cas, peu dans l'autre, il n'a pas varié dans le troisième, après 10 jours de traitement. Nous n'avons pas encore eu l'occasion de traiter des cas de lymphadénose leucémique : nous ne pouvons donc faire une comparaison concluante, étant donnée aussi la différence du comportement clinique des myéloses et des lymphadénoses eu égard au thiosulfate. En général, sur la base de ces dernières observations, on peut penser que la prolifération du tissu est à rapporter à l'action des groupes sulfhydriliques plutôt qu'à l'équilibre oxydo-réductif en sens général et que, par conséquent, les résultats obtenus par l'administration du thiosulfate sont plutôt à rapporter à sa possibilité d'augmenter la dotation de sulfhydrile, que de modifier l'équilibre oxydo-réductif.

De plus, grâce à nos observations susdites, on remarque que l'emploi du thiosulfate peut produire des réactions nuisibles à des sujets leucémiques, ou, génériquement, affectés par des processus hyperplasiques, et aussi on peut reconnaître dans une substance déterminée une des causes possibles de ces aggravations imprévues qui très facilement sont appelées spontanées.

Discussion : L. WEISSBECKER.

Theorien über den Wirkungsmechanismus betreffen biochemisch gesehen nur SH Gruppen (Thioprive Substanzen, PETERS, BACQ) oder den Nucleinsäurestoffwechsel. As und Nitrogen mustard erfassen ausserdem lebenswichtige Redoxsysteme, blockieren die Keto-Enoltautomerie (Abfall der red. Ascorbinsäure bei Anstieg der Dehydroascorbinsäure des Menschen). Kobaltsalze sind ebenfalls wirksame Zytostatika. Wirkung bei chron. myeloischer Leukämie. As, CO und Nitrogen mustard blockieren ebenfalls die Wasserstoffübertragung in Fermenten, deren prosthetische Gruppe einen koordinativ 4-wertigen Stickstoff (Thiazoliniumstickstoff im Aneurin, Pyridiniumstickstoff im Vitamin B_6 und Nikotinsäu-

reamid u. a.). Unter Behandlung mit Zytostatikas wird das Redoxpotential des strömenden Blutes positiver. Erweiterung der Theorie von den thiolopriven Substanzen auch auf Blockade von Redoxsystemen mit Keto-Enoltautomerie und koordinativ 4-wertigen Stickstoff. Zytostatika verursachen eine Redoxblockade, ein « Aredoxie ».

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DAS VERHALTEN DER BLUTPHAGOCYTEN UNTER CHEMOTHERAPEUTISCHEN KUREN

Von St. SANDKÜHLER (Heidelberg).

Bei der klinischen Erprobung neuer Arzneistoffe und bei der Überwachung als toxisch bekannter Substanzen ist die Kontrolle von Nebenwirkungen auf eine relativ geringe Zahl von Methoden beschränkt. Die routinemässige Blutuntersuchung ist seit langem selbstverständlich. Trotzdem ist man eigentlich wenig davon überzeugt, damit genug zu tun und den grösseren Teil der möglichen Veränderungen zu erfassen, selbst wenn man nur das Blut berücksichtigt.

Während Einzeluntersuchungen der Phagocytosefähigkeit schon von verschiedenen Untersuchern durchgeführt wurden (v. PHILIPPSBORN, KESTERMANN und VOGT und neuerdings JERSILD) haben wir bei der Erprobung von Urethan und Pentamidin erstmals laufende Phagocytosekontrollen unternommen. Wir nahmen hierzu eine Aufschwemmung von Staphylokokken (besonders gut ist Staph. aureus haemolyticus) und anfangs ausserdem Tusche. 0,2 cm³ der Aufschwemmung gaben wir zu 1 cm³ defibrinierten Blutes, strichen aus der im Brutschrank

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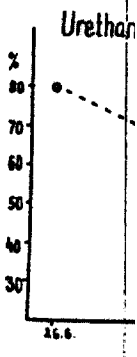

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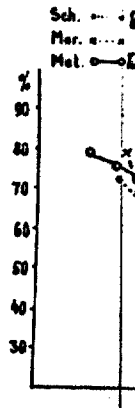

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Le Sang 21: 344 (1950)

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THE METABOLISM OF SODIUM HYPOSULFITE IN LEUKEMIAS. ITS HYPERPLASTIC AND ANTICYTOSTATIC ACTION

by W. Paolino and F. P. Cavosto

We have become involved in the problem dealing with differences in the growth rate and extension of hyperplastic tissue and in that of neoplastic development. Differences are observed from case to case, and in each case, during different phases of the disease. The first hypothesis and the one which is the object of the present study, is based on the possibility that this difference is of a peripheral origin, that is, the difference originates in the tissues themselves. As a consequence, we have presumed that hyperplastic and neoplastic tissue possess different potentials for development, and an avidity for normal tissues, depending on their availability and on a particular and primitive capacity to fix and utilize these cells in a generic and selective manner. The factors essential for the development are either enzymes, necessary for the utilization of growth materials, or else, the direct utilization of these growth materials. It is already well known that in view of cellular development, a sufficient and specific number of enzymes are required. Among these groups of enzymes we should emphasize those which act by means of the sulhydryl radical. The importance of sulhydryl enzymes in the proliferation of normal and pathological tissues is upheld by a great number of studies. Among the oldest known studies are those by HAMMET who concluded that the sulhydryl group enzymes have a stimulatory effect on the development of animal and vegetable tissue as opposed to the inhibitory action exhibited by the sulfonylic

groups (SO_2, SO_3) which are derived from the sulfhydryl groups by oxidation. BACKER showed that sulhydryl enzymes have an effect on the proliferation of fibroblasts and epithelial cells in vitro. SHEARER, RAPKINE, EPHRUSSI, BRACHET showed that these enzymes have an effect on cell division. The metamorphosis of gyrins is accelerated by sodium hyposulfite (BINET AND MAGROU). In vegetal tumors there is an increase in glutathione content (BINET and MAGROU). In experimental hepatomas the development of the tumor is preceded by an increase in hepatic glutathione content (BOYLAND, MAWSON: IKI); skin treated with tar also shows an increase in glutathione (SPASOKOUKOTZKY). More recent research has confirmed these findings. PARCKER and KRACKE (1946) reported that glutathione has an accelerating effect on mitoses in leukemia. FRIEDMANN and MARRIAN (1948) showed that inhibition of mitoses by maleic acid and by quinones parallels the loss of sulfhydryl groups within the cells, by binding these substances to the SH groups in the cell. The intramuscular administration of thioglycolate and thiomalate produces an increase in the growth rate of tumors transplanted in rats, while on the other hand, methionine, cysteine, and thiolactate are inactive. Substances such as iodoacetate and maleate bind with SH groups and retard their growth (BRUNSCHWIG et al.). Among the several amino acids tested only cysteine showed the capacity to inhibit, in cases of deficiency, the appearance of experimental leukemias induced with methylcholanthrene (WHITE, WHITE and MIDER) (1947). Thus, the SH groups evidently appear to be important in the development of tumors. Excellent results have been obtained in transplantable tumors treated with miosalvarsan (arsenic

oxide has an effect on the SH groups) (TANAKA et al.). The inhibitory activity of hydrocarbons disappeared following the administration of cysteine, methionine, and glutathione (WHITE); following administration of benzpyrene, several animal organs show a decrease in the content of SH groups (RONDONI and BORETTI). Although we cannot know for certain, there is a great deal of evidence to indicate that the sulhydryl groups are important; they take part in the oxidation-reduction processes, that are the basis for the phenomena involved in cellular metabolism.

In our clinical studies we have used sodium thiosulfate, since we did not have glutathione or any of the sulfur containing amino acids available in sufficient quantities. Sodium thiosulfate takes part in the formation of organic sulfur compounds and even in the formation of glutathione itself, (ARNOVLJEVITCH, BINET et al.) and as a consequence it can form compounds with sulfhydryl action; finally by its energetic and rapid reducing activity, sodium thiosulfate can affect directly the oxidative-reduction balance within the system. Furthermore, one additional advantage is that the metabolism of sodium thiosulfate can be monitored both in the serum and in the urine. Therefore, in our studies, we administered sodium thiosulfate intravenously in a dose of 2 grams. We studied the following parameters: 1. the clinical conditions of the patients; 2. the determination of thiosulfate in the serum and in the urine; 3. the consumption of oxygen by the patients. We adopted the latter criterion, even though we did not know, at this point, the exact causes for the increase in the basal metabolism observed in leukemia as well as in other hemopathies; nevertheless, based on our personal

experience, and on the data obtained by the majority of investigators most recently, it appears that this increase may be related to the degree of development of the hyperplastic tissue. Since we were wondering about the elevated metabolic rate in patients with leukemia, we carried out controls not only on normal subjects but also on patients with hyperthyroidism.

We injected two grams of sodium thiosulfate and then determined its serum level after 15, 30 and 45 minutes. We found that the highest values occurred after 15 minutes; 30 minutes after administration the levels decreased appreciably and at 45 minutes, the values were nearly always zero. In our evaluation we took into consideration the value obtained after 15 minutes. The differences among the average values obtained within the different groups were not significant from a statistical point of view; however, we can definitely state that the values were clearly lower than the values obtained in normal subjects (5.4 mg%) and even lower than those observed in leukemic patients (4.5 mg%). The other cases showed nearly normal levels of thiosulfate.

We determined the urinary excretion of thiosulfate three hours after injection. The fraction examined showed that two hours after injection practically no thiosulfate was excreted. We shall report the average values for each group, expressing the excretion of thiosulfate as a percentage of the original quantity injected. The highest amount of thiosulfate excreted was observed in the hyperthyroid patients (four cases with an average value of 21.7 per cent $\pm$ 2.2); the normal subjects had a somewhat lower value (21 cases with an average of 18.2

per cent ± 2.9); definitely the lowest percentage of thiosulfate excretion was observed in the patients with leukemia (11 cases with different forms of leukemia had an average value of 12.7 per cent ± 2.6). Patients with Hodgkin's disease and with lymphosarcoma had a percentage of thiosulfate excretion intermediate between that of the normal subjects and those with leukemia; patients with Biermer's anemia and with polycythemia had values equal to those observed in patients with leukemia.

Tests carried out on the subjects several days later showed that in both normal subjects and in patients with pathological conditions, the serum levels may have increased while the urinary excretion remained constant.

After eight to ten days of continued daily administration of 2 grams of sodium thiosulfate, the tests performed repeatedly in the same subject showed that the values observed in the serum and in the urine did not show any modifications in normal subjects, in hyperthyroid patients and in patients with leukemia (chronic myeloid leukemia); in the two patients with polycythemia there was a definite increase in the urinary excretion of thiosulfate (286 mg or 14 per cent to 488 mg or 24 per cent and 252 mg or 13 per cent to 350 mg or 17 per cent). The serum values did not show any variation after 15 minutes.

Any eventual oxygen consumption was studied three hours after the first injection was administered; basal metabolism was studied after eight to ten days following daily administration of sodium thiosulfate. The immediate changes in oxygen consumption were of moderate importance only. In the hyperthyroid patients, we saw an average increase of 4 per cent; in normal patients the

average increase was 5 per cent; in the patient with leukemia the increase was 8.6 per cent. Following prolonged administration of thiosulfate the absolute basal metabolism did not show absolute variation among the normal subjects. The basal metabolism increased constantly and except for one case, the increase was significant among the leukemia patients. Another determination performed four to five days after the injections of thiosulfate were given, proved that the basal metabolism rate returned nearly to the initial values among the leukemia patients.

Following prolonged administration of thiosulfate, there was a tendency for the basal metabolism to increase; however, this increase was lower among the leukemic patients and among patients with Biermer's anemia; and only slight among the patients with Hodgkin's disease and lymphosarcoma; at the beginning of our research, we continued the administration of thiosulfate in these groups.

In summary, thiosulfate injected intravenously produced small variations in oxygen consumption especially immediately after its injection; this increase was particularly marked among the leukemic patients; administration of thiosulfate for several successive days did not produce any significant variations in the basal metabolism of normal subjects and in hyperthyroid patients; in leukemic patients, thiosulfate produced nearly always a definite and sometimes considerable increase, which, nevertheless, tended to disappear several days later. The effects of thiosulfate on oxygen consumption and its elimination demonstrated diametrically opposed results in hyperthyroid

patients and in those with leukemia; it also showed that patients with leukemia do not manifest a state of oxygen hyperconsumption.

However, any relationship between the results observed with oxygen consumption and the metabolism of the injected thiosulfate could not be drawn based on the overall results obtained. Nevertheless, separation of the cases, as for example, those with leukemia (although these patients did have different clinical forms of the disease), showed that there was a relationship; the urinary excretion of thiosulfate was lowest when the initial basal metabolic rate was high and the increase in oxygen consumption was highest immediately after the injection was made.

From the clinical point of view, we observed fever after only one injection of thiosulfate was administered to patients with lymphadenous leukemia, with Hodgkin's disease and with lymphosarcoma. Following several injections, there was a rise in temperature, adenopathy and splenomegaly in patients with lymphadenous leukemia and in patients with Hodgkin's disease. Following a series of injections patients with lymphadenous leukemia, with Hodgkin's disease and with lymphosarcoma showed an appreciable resistance, which in some cases, was total when exposed to X-ray irradiation. The leukocytes did not show any appreciable variations; however, patients with myeloid leukemia did improve, as is to be expected normally, after exposure to radiation therapy. In one patient with acute myeloid subleukemia (10,000 leukocytes with 29 per cent hemocytoblasts) without fever and in a stabilized state during

the past three months, there was an abrupt aggravation of the symptoms after only six injections of thiosulfate (elevated fever, necrotic angina, 10,000 leukocytes increased to 52,000 and then to 150,000 leukocytes with 90 per cent hemocytoblasts) and death occurred within ten days. Following this unexpected result and the resistance to X-ray therapy observed in patients with lymphadenous leukemia, Hodgkin's disease and lymphosarcoma, it appeared that it was necessary for us to limit our research to cases of myeloid leukemia, for whom we did not observe any fatal consequences.

Based on our initial hypothesis as well as on the results of clinical observations of patients with leukemia, Hodgkin's disease, lymphosarcoma, the behavior of injected thiosulfate appears to be related to the reducing action of this compound and the formation of sulfhydryls by thiosulfate. We then performed a counter-experiment in an attempt to treat these patients with anti-sulhydryl or oxidizing substances. Among the first compounds to be tested was mercury cyanide. We treated one patient with lymphadenous leukemia with two grams of cyanide every other day, that is, the same schedule as that used in the treatment of syphilis. We had no intention of obtaining a therapeutic effect, because we believed that the anti-sulfhydryl action of cyanide extends generically and involves all the sulhydryls in the entire organism and thus may result in similar or even perhaps major damage to normal tissues. We could not presume that the sulhydryls would have a selective action on leukemic tissue, despite the fact that occasionally there are processes of genetic hyperconsumption involved

(obesity, feverish states). The results of mercury cyanide treatment showed that there was an initial increase of leukocytes and then a definite successive decrease of adenopathies and of splenomegaly with a moderate decrease in basal metabolism. However, we did interrupt the treatment with mercury after eight injections, because the patients' general condition seemed to worsen. Therefore, what we had believed to be true was verified. Until the present time, we did not perform any other tests with cyanide. As oxidizing substances we have used vitamin K, by administering the vitamin in intravenous injections of 50 mg twice daily. Vitamin K injections were administered to three patients with myeloid leukemia. None of the patients showed any decrease in splenomegaly or in the number of leukocytes. Basal metabolic rate decreased definitely in one case, and perhaps in another patient; it did not vary at all in the third patient even after ten days of vitamin K treatment. We did not have the opportunity to treat the patients with lymphadenous leukemia; perhaps we would have been able to make a concluding comparison, by including the differences of clinical behavior to thiosulfate observed among the patients with myeloid and lymphadenous leukemia. In general, based on these latter observations, it may be stated that the proliferation of tissue is related to the action of the sulhydryl groups rather than to oxidation-reduction balance in a general sense, and as a consequence, the results obtained by the administration of thiosulfate are due more so to its ability to increase the supply of sulfhydryl rather than its ability to modify the oxidation-reduction balance.

Furthermore, based on our previously described observation, we can conclude that the use of thiosulfate may produce harmful reactions in patients with leukemias, or generically, thiosulfate may affect the hyperplastic processes and it may prove possible to discover that one of these definite substances is the possible cause of these unexpected aggravations of leukemia that are often too readily dismissed as spontaneous.

Discussion: L. WEISSBEEKER

There are theories on the mechanism of action pertaining to the biochemical aspects of only the SH groups (thiol substances, PETER, BACQ or nucleic acid metabolism). For example, nitrogen mustard affects the vitally important redox system, blocking the keto-enol tautomers, releasing the reduced ascorbic acid while increasing the dehydroascorbic acid in man. Cobalt salts are also significant cytostatics and their effect in chronic myeloid leukemia is known. As for carbon monoxide and nitrogen mustard, they also block the water transport system, which has coordinate four valence prosthetic groups of nitrogen (Thiazolinium-nitrogen in Aneurin, Pyridinium nitrogen in Vitamin B_6 and nicotinic acid, etc.). Treatment with cytostatics produces a positive redox potential for flowing blood. There is an increasing number of theories on thiol substances, on the blockade of the redox system with Keto-enol tautomers and coordinate four valence nitrogens. Cytostatics cause a redox blockade known as "Aredoxia".

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VENOUS THROMBOSIS AFTER INFUSION WITH GELATIN SOLUTIONS CONTAINING MERCURIAL PRESERVATIVES*

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In a clinical study[7] designed to test the suitability of certain gelatin solutions as plasma substitutes, the only untoward effect encountered was the frequent occurrence of thrombosis of the injected veins. This reaction both was undesirable and restricted seriously the number of veins available for repeated infusions. Because of the high incidence and considerable extent of venous thrombosis it seemed likely that the solutions contained a thrombosing substance.

The present study indicates that mercurial preservatives were the probable cause of this reaction.

A. *Observations on Patients. Methods and Case Material.* Eighty injections of 5 different gelatin solutions were made in 39 patients. With the exception of 4 injections of 500 to 700 cc. each infusion was 1000 cc. Before administration the fluid was warmed to room temperature in a water bath. Fluid was administered by gravity in from 80 to 120 minutes through a standard venoclysis set† employing a 19 gauge needle. No technical difficulties were encountered.

The 39 patients ranged in age from 20 to 84 years, the majority being over 50 years. They had various chronic diseases such as rheumatoid arthritis, thrombo-angiitis obliterans, Laennec's cirrhosis, chronic glomerulonephritis and cerebral arteriosclerosis. Of these patients, 34 were injected wtth 2 or more preparations at suitable intervals to make certain that differences in response were not attributable to differences in patient material.

Five minor febrile reactions, 3 with chill, occurred during the first 18 injections. It was believed that these may have been due to insufficient preparation of the equipment. Therefore, more elaborate care was taken in cleansing glassware and tubing. Following this precaution‡ no further febrile reaction occurred.

Results. In general the infusion of gelatin solutions was well tolerated. No significant changes occurred in body temperature, pulse, respiration or blood pressure during or after the infusions, other than the 5 febrile reactions previously noted.

Thrombosis was observed in 27 of 80 veins injected with gelatin solutions. The lesion was apparent within 24 hours of injection, the veins becoming hard and cordlike. The hardened vein extended for distances of 3 to 30 cm. from the site of injection. Occasionally the vessel wall seemed thickened near the site of injection although it remained patent. In such

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Research Service, First (Columbia) Division, Goldwater Memorial Hospital.

† Upjohn Infusion Set, supplied by Upjohn Company, Kalamazoo, Mich.

‡ The procedure adopted was as follows: Dismantling of separate parts of equipment, washing with green soap, soaking in green soap for 20 minutes, rinsing in running tap water for 1 hour, rinsing in triply distilled water, packaging, and autoclaving at 15 pounds for 20 minutes.

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instances the veins were not classified as thrombosed. Veins were classified as thrombosed when venepuncture showed them to be bloodless. Repeated venepuncture on an appreciable number of these vessels after several months showed the veins to be occluded. However, in some cases the veins were blood-containing after a few months. With 3 exceptions the thrombosed veins were painless and non-tender. In these 3 instances the patients had complained of pain along the course of the vessel during the infusion. No clinical evidence of embolism from the thrombosed sites was observed.

Table 1 shows the incidence of thrombosis after intravenous injections with 5 different gelatin solutions, 4 of which contained mercurial preservatives.

B. Observations on Rabbits. Production of Thrombosis. The thrombosing action of phenyl mercuric borate in 3 gelatin preparations and in 10% glucose solution was tested in rabbits. Control groups were injected with 10% glucose and with 1 gelatin preparation free of the mercurial preservative. A single dose of each preparation, equivalent to 20 cc. per kilo body weight, was injected into the marginal ear vein of the rabbit. The site of injection was then examined daily for the appearance of thrombosis. On the 4th day after injection biopsy of the site of injection was obtained by punching out a circular section of the ear, 1 cm. in diameter. Tissues were fixed and examined histologically.

The results (Table 2) correspond to the observations on patients. Thromboses occurred frequently with preparations containing mercurial preservatives, whereas only 1 of 12 injections with non-preserved gelatin was followed by thrombosis. However, unlike the experience with patients, the intravenous injection of 10% glucose produced thrombosis in 4 of 6 instances. The most severe and extensive thromboses followed the injection of 10% glucose containing phenyl mercuric borate in concentration of 4 mg./100 cc. In 4 instances gangrene of the surrounding tissues appeared 2 weeks after the injections.

Thrombosis was apparent within 24 to 72 hours of the time of injection. The microscopic appearance of sections taken 4 days after injection is described as follows: Thrombi consisted of fibrin, platelets, coagulated red blood cells and widely

Table 1.—Incidence of Thromboses After Intravenous Injections of Gelatin and Glucose Solutions

Preparation*	Preservative	(mg./100 cc.)	No. injections	No. thromboses
Upjohn B20610-27	Phenyl mercuric borate	4.0	16	12
Upjohn B20610-45	Phenyl mercuric borate	1.0	10	5
Knox P7-20	Phenyl mercuric borate	2.5	12	5
Knox PM10-20	Merthiolate†	3.4	15	5
Knox PX10-20	None		27	0
10% glucose in water	Phenyl mercuric borate	4.0	7	5
10% glucose in water	None		7	0

* The Knox preparation was characterized by high viscosity and the Upjohn by low viscosity.
† Mercury content equivalent to phenyl mercuric borate 2.5 mg. per 100 cc.

The 2 Upjohn preparations were essentially alike except for differences in concentration of the preservatives. The 3 Knox preparations were also alike but for differences in the preservatives. Table 1 shows that thromboses occurred frequently after injection with gelatin solutions containing either phenyl mercuric borate or merthiolate, whereas no thromboses followed injection with a gelatin solution (Knox PX10-20) containing no preservative. This effect of the mercurial preservatives was likewise demonstrated in control studies employing 1000 cc. of 10% glucose. Five of 7 patients had extensive thromboses after injection with 10% glucose containing phenyl mercuric borate. By contrast none of 7 patients receiving 10% glucose alone had subsequent thromboses.

scattered leukocytes with faded nuclei. The thrombotic elements formed masses which projected into the lumen or lined the endothelium like a membrane. Veins containing thrombi were widely dilated, and many revealed interrupted elastic lamellæ. About the walls of several veins were dense edema, fibrin precipitates and slight infiltration of lymphoid, mononuclear and rare polymorphonuclear leukocytes. A few instances of perivascular hemorrhage were also found. No necrosis or leukocytic infiltration of the vessel wall was seen.

different concentrations was added to the gelatin solution in 2 groups of rabbits, and in another group sodium thiosulfate was added.* The results are shown in Table 3.

Thrombosis occurred in 8 of 10 veins injected with Upjohn gelatin solution B20610–27 containing phenyl mercuric borate, 4 mg./100 cc. The same incidence was obtained with this preparation in a previous series (Table 2). The addition of either sodium thiosulfate or of methionine to the gelatin solution appears to give some degree of protection against

TABLE 2.—INCIDENCE OF THROMBOSIS IN RABBIT EARS AFTER INTRAVENOUS INJECTION OF DIFFERENT GELATIN AND GLUCOSE SOLUTIONS

Solution	Phen. merc. bor. (mg./100 cc.)	No. injections	No. thromboses	Av. length of thrombus (cm.)	Sequelæ
Knox PX10-20	..	12	1	0.60	
Upjohn B20610-27	4.0	10	8	1.85	
Knox P7-20	2.5	10	4	0.80	
Knox P7-180	2.5	10	8	1.25	
10% glucose	4.0	6	5	4.70	4 had skin gangrene
10% glucose	..	6	4	1.20	

TABLE 3.—THROMBOSIS IN THE RABBIT EAR AFTER A SINGLE INTRAVENOUS INJECTION OF A GELATIN SOLUTION CONTAINING PHENYL MERCURIC BORATE: PARTIAL PROTECTION WITH SULFUR-CONTAINING COMPOUNDS

Preparations	Adjuvant (mg./100) cc.		No. injections	No. thromboses	Av. length of thrombus
Upjohn B20610-27*	None		10	8	2.2
Upjohn B20610-27*	Methionine	8	10	4	1.1
Upjohn B20610-27*	Methionine	16	10	4	1.2
Upjohn B20610-27*	$Na_2S_2O_3$	15	10	1	0.5

* This gelatin preparation contains phenyl mercuric borate 4 mg. per 100 cc.

PROTECTION AGAINST THROMBOSIS. An attempt was made to prevent the thrombosing action of phenyl mercuric borate by adding various sulfur-containing compounds to the gelatin solution. The selections of these substances was based upon earlier reports indicating a protective effect by sulfur-containing compounds in chloroform[8] and by heavy metal poisoning.[3,4,10] The following experiments were performed: Four series of 10 rabbits each were employed. One gelatin preparation containing phenyl mercuric borate was used throughout. One group received this gelatin preparation alone. Methionine in

the thrombosing action of phenyl mercuric borate. Differences between the control series and that receiving sodium thiosulfate are highly significant. However, in the case of methionine these differences are not of statistical importance.

Discussion. The clinical observations show that intravenous injections of gelatin solutions containing phenyl mercuric borate or merthiolate frequently lead to thrombosis of the injected vein. Other workers[5] employing the same preparations and similar technique have observed thromboses much less often. The reason for this difference in incidence is not

* An attempt to test the effect of cystine was unsuccessful because of the insolubility of this substance in gelatin solutions.

clear. The experimental production of venous thrombosis in the rabbit ear vein tends to support our clinical findings.

The theory has been advanced that the toxicity of mercury and of arsenic may be due largely to inhibition of —SH enzymes. Recently Cook[1,2] and his coworkers have shown that phenyl mercuric nitrate depresses the activity of certain enzyme systems important in tissue respiration, and that this depressive action can be prevented by compounds containing sulfhydril groups. The detoxifying capacity of certain dithiol compounds (BAL)[6,9,11] for arsenic and mercury are attributed to the presence of —SH groups. Such a reaction could explain the low incidence of thrombosis with the use of plasma containing this mercurial preservative and the relatively high incidence of thrombosis obtained with the use of gelatin or glucose solutions containing the preservative. Plasma, which is made up of complete proteins containing reactive sulfur groups, might be expected to protect against mercuric ions more readily than either gelatin or glucose.

However, in the present study another explanation seems indicated, since methionine and sodium thiosulfate appeared to protect against thrombosis caused by phenyl mercuric borate. The protection, therefore, cannot be ascribed to the specific presence of —SH groups. It seems more likely that these substances converted the mercuric ions to the mercurous form, which would render the compound less irritative. A similar reaction has been observed between mercuric chloride and sodium formaldehyde sulfoxalate.[10]

Summary and Conclusions. 1. Local venous thrombosis occurred frequently in patients injected with gelatin solutions containing phenyl mercuric borate or merthiolate.

2. Venous thrombosis did not occur in patients injected with gelatin solutions free of these mercurial preservatives.

3. Similar findings were obtained in experimental studies on ear veins of rabbits.

4. The addition of sodium thiosulfate or methionine to gelatin solutions containing phenyl mercuric borate appeared to give partial protection against the thrombosing action of this mercurial preservative.

The authors wish to express their thanks to the Knox Gelatine Company and to the Upjohn Company for their helpful coöperation in furnishing materials for this study.

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THE FATE OF CERTAIN SULFUR COMPOUNDS WHEN FED TO THE DOG.*

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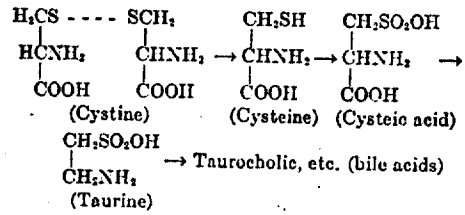
It is a generally accepted fact that the synthesis of the sulfur-containing bile acids proceeds according to the following equations:



The cystine used by the body for the synthesis of bile acids is derived from the common protein catabolism and includes both endogenous and exogenous factors (1). Taurine appears to be available for combination with cholic acid at all times and hence is not the limiting factor which determines the amount of bile acids which the body can synthesize. Our experimental work with the above as well as with certain closely related compounds has been restricted to the determination of the ability of the body to oxidize the sulfur to sulfates and to utilize the nitrogen. We have also been interested in the relative rates of excretion of the nitrogen and of the sulfur of cystine and in the possible conjugation of taurine with urea to form taurocarbamic acid. The substances studied afford an excellent opportunity for the correlation of chemical structure with fate in the body. The demands of the

* Aided by a grant from the Research Board of the University of California.

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body for sulfur are probably entirely supplied by cystine and not by the ingestion of other substances which we have administered to the dog. However, certain of the compounds, particularly taurine (2), occur as normal constituents of food products and are ingested in small quantities.

The experiments of Salkowski (3), Smith (4), and Heffter (5) with respect to the ability of the dog to oxidize the sulfur which is contained in sulfonic acids to sulfuric acid are at variance, and similarly, the statement that thiosulfuric acid appears in the urine when cystine is fed (6) is not confirmed by the experiments of all who have worked on this subject (7). Experiments which were carried out in this laboratory by Schmidt (8) and his co-workers show clearly that when taurine is ingested by man there is no marked increase in urinary sulfates and that contrary to the reports of Salkowski taurine is not excreted as taurocarbamic acid but as free taurine (9).

EXPERIMENTAL.

The experimental work was carried out on dogs which were kept on constant diets containing a low but not a minimal amount of protein. The dosage of the particular substance given was added to a part of the food which was fed first. Certain of the animals, particularly Dog 1, consumed the food rapidly, the others more slowly. Urine collections were made in a metabolism cage in the usual manner and the dogs were catheterized at the end of the 24 hour period. Estimations of the sulfur fractions were carried out with the aid of the methods of Folin (10) and of Denis (11) and the amino nitrogen estimations were performed with the aid of Van Slyke's method (12). Cysteic acid was prepared from cystine according to the method described by Friedmann (13). Taurine was obtained from the abalone (*Haliotis*) by the method described by Schmidt and Watson (2). For the preparation of isethionic acid, taurine was treated with an excess of nitrous acid as suggested by W. Gibbs (14), and after acidifying and evaporating the solution, the residue was extracted with absolute alcohol from which, on cooling, the product crystallizes.

The results are given in Tables I to VI and a résumé, showing the significant figures of these experiments, is contained in Table VII. In the interpretation of the data, due consideration must be given

ur Compounds

y supplied by cystine and not
which we have administered
the compounds, particularly
uents of food products and are

), Smith (4), and Heffter (5)
og to oxidize the sulfur which
furic acid are at variance, and
sulfuric acid appears in the
onfirmed by the experiments of
ject (7). Experiments which
y by Schmidt (8) and his co-
urine is ingested by man there
sulfates and that contrary to
not excreted as taurocarbamic

ENTAL.

rried out on dogs which were
a low but not a minimal amount
particular substance given was
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ed from the abalone (*Haliotis*) by
and Watson (2). For the prep-
ne was treated with an excess of
Gibbs (14), and after acidifying
the residue was extracted with
cooling, the product crystallizes.
I to VI and a résumé, showing the
iments, is contained in Table VII.
, due consideration must be given

TABLE I.

Experiment 1. Dog 1.

Day of experiment.	Total sulfur.	Inorganic sulfur.	Ethereal sulfur.	Neutral sulfur.	Total nitrogen.	α-amino nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*	
1	0.291	0.159	0.030	0.103	4.23	170	Weight 13.9 kilos.
2	0.254	0.129	0.012	0.123	3.70	118	
3	0.206	0.121	0.010	0.075	3.36	90	
4	1.277	0.145	0.015	1.117	4.20	493	5.85 gm. taurine = {1.5 gm. S; 0.66 gm. N}
5	0.328	0.152	0.022	0.154	3.46	134	
6	0.827	0.212	0.014	0.602	3.81	166	6.7 gm. cysteic acid as Na salt = {1.123 gm. S; 0.49 gm. N}
7	0.298	0.085	0.006	0.207	3.33	144	
8	0.915	0.178	0.013	0.724	3.78	178	7.0 gm. cysteic acid as Na salt = {1.171 gm. S; 0.510 gm. N}
9	0.342	0.075	0.010	0.256	3.28	105	
10	1.120	0.185	0.010	0.925	4.10	184	3.7 gm. isethionic acid* as Na salt = 0.795 gm. S. Slight diarrhea, also diuresis.
11	0.746	0.111	0.013	0.622	3.76	157	Soft stools.
12	0.637	0.134	0.014	0.489	3.44	141	
13	0.680	0.120	0.015	0.545	3.15	100	2.8 gm. isethionic acid* as Na salt = 0.613 gm. S.
14	0.359	0.131	0.012	0.215	3.33	94	
15	1.432	0.150	0.012	1.291	3.28	103	8.2 gm. isethionic acid† as Na salt = 1.766 gm. S. Inorganic S = 0.012 gm.
16	0.244	0.121	0.016	0.107	3.55	108	
17	1.371	0.698	0.016	0.656	4.06	301	7.5 gm. cystine = {2.00 gm. S; 0.878 gm. N}
18	0.675	0.213	0.020	0.443	3.80	253	Soft stools.
19	0.298	0.185	0.013	0.100	3.36	90	
20	0.275	0.142	0.007	0.126	3.56	122	7 gm. taurocholic acid = {0.441 gm. S; 0.195 gm. N}
21	0.244	0.142	0.009	0.094	3.74	106	Soft stools. Weight 15.9 kilos.

* Prepared from taurine by treatment with HNO_2.

† Kahlbaum preparation—50 per cent aqueous solution.

Daily Diet.

Cracker meal	200 gm.
Crisco	40 "
Sucrose	60 "
Fullers' earth	50 "
Condensed milk	100 cc.

Distilled water sufficient to make a "stiff dough."

TABLE II.

Experiment 2. Dog 2.

Day of experiment.	Total sulfur.	Inorganic sulfur.	Ethereal sulfur.	Neutral sulfur.	Total nitrogen.	α-amino nitrogen.	Remarks.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*	
1	0.103	0.042	0.009	0.052	3.28	49	Weight 12.7 kilos.
2	0.103	0.053	0.009	0.041	3.11	60	
3	0.097	0.047	0.008	0.042	2.86	50	
4	0.919	0.752	0.005	0.163	3.30	109	7.5 gm. cystine = {2.0 gm. S; 0.878 gm. N}
5	0.267	0.164	0.005	0.098	2.90	79	
6	0.147	0.077	0.010	0.061	2.55	77	
7	0.712	0.073	0.007	0.632	2.76	63	11 cc. of Kahlbaum's 50 per cent isethionic acid as Na salt = 1.035 gm. S.*
8	0.118	0.039	0.007	0.073	2.87	66	
9	0.079	0.023	0.009	0.048	2.54	50	
10	0.526	0.080	0.006	0.440	2.76	55	5.8 gm. cysteic acid as Na salt = {0.989 gm. S; 0.425 gm. N}
11	0.189	0.133	0.010	0.046	2.64	67	
12	0.125	0.015	0.006	0.104	2.67	56	
13	1.203	0.051	0.005	1.147	3.03	515	6 gm. taurine = {1.54 gm. S; 0.67 gm. N}
14	0.262	0.021	0.008	0.233	2.87	84	
15	0.146	0.036	0.007	0.102	2.52	76	
16	0.125	0.030	0.012	0.083	3.05	65	
17	0.111	0.028	0.006	0.077	2.50	68	
18	0.888	0.708	0.006	0.174	3.10	155	7.5 gm. cystine = {2.0 gm. S; 0.878 gm. N}
19	0.317	0.131	0.017	0.166	2.60	95	
20	0.224	0.101	0.009	0.111	2.80	46	
21	0.152	0.048	0.034	0.070	2.80	68	

* Contains 0.013 gm. S as sulfate.

Diet.

Cracker meal	175 gm.
Crisco	25 "
Sucrose	80 "
Fullers' earth	40 "
Condensed milk	90 cc.
20 per cent beef extract	10 "

TE II.

. 2. Dog 2.

	Remarks.
	Weight 12.7 kilos.
	7.5 gm. cystine = {2.0 gm. S; 0.878 gm. N}
3	11 cc. of Kahlbaum's 50 per cent isethionic acid as Na salt = 1.035 gm. S.*
55	5.8 gm. cysteic acid as Na salt = {0.989 gm. S; 0.425 gm. N}
15	6 gm. taurine = {1.54 gm. S; 0.67 gm. N}
55	7.5 gm. cystine = {2.0 gm. S; 0.878 gm. N}

e.

Diet.

	175 gm.
	25 "
	80 "
	40 "
	90 cc.
	10 "

to variability for it is difficult in experiments of several weeks duration to eliminate daily fluctuations in the output of urinary constituents and the selection of normal values is difficult. In the résumé the normal values were obtained either by taking the average of several days of the fore period or by using the figures preceding the experimental day.

TABLE III.

Experiment 3. Dog 2.*

Day of experiment.	Urinary sulfur.			Urinary nitrogen.		Remarks.
	Total.	Inorganic.	Neutral plus ethereal.	Total.	α-amino nitrogen.	
	gm.	*gm.*	*gm.*	*gm.*	*mg.*	
1	0.398	0.113	0.185			Weight 14.5 kilos.
2	0.247	0.106	0.141			
3	0.671	0.473	0.198			2 gm. $Na_2S_2O_3 \cdot 5H_2O$ = 0.516 gm. S†
4	0.179	0.082	0.097			
5	0.537	0.426†	0.111			1.5 gm. Na_2SO_3 = 0.381 gm. S‡§
6	0.149	0.071	0.078			
7	0.404	0.300	0.104			1 gm. $Na_2S_2O_3 \cdot 5H_2O$ = 0.258 gm. S‖
8	0.155	0.075	0.080	2.49	52	
9	0.946	0.765	0.181	3.21	75	7.5 gm. cystine = {2.0 gm. S; 0.878 gm. N}
10	0.361	0.196	0.165	2.48	59	

* About a month elapsed between Experiments 2 and 3, during which time the dog had been returned to the animal house and received a meat and bread diet.

† When the urine was acidified with H_3PO_4 and distilled, S and SO_2 were obtained in large amounts.

‡ 0.046 gm. S was present as sulfate.

§ Distillation shows only traces of sulfites in the urine.

‖ Urine contains only a small amount of thiosulfate.

Diet.

Cracker meal	150 gm.
Crisco	20 "
Sucrose	65 "
Fullers' earth	40 "
Condensed milk	75 cc.
20 per cent beef extract	10 "

TABLE IV.

Experiment 4. Dog 3.*

Day of experiment.	Urinary sulfur.			Total nitrogen.	Remarks.
	Total.	Inorganic plus ethereal.	Neutral.		
	gm.	gm.	gm.	gm.	
1	0.110	0.074	0.036	2.50	Weight 11.4 kilos.
2	0.126	0.083	0.043	2.68	
3	0.112	0.072	0.040	2.38	
4	1.084	0.799	0.285	2.77	7 gm. cystine = {0.819 gm. N, 1.87 gm. S}
5	0.241	0.186	0.055	2.02	
6	0.136	0.095	0.041	1.96	

* Young growing dog.

Diet.

Cracker meal	75 gm.
Crisco	15 "
Sucrose	30 "
Fullers' earth	25 "
Condensed milk	50 cc.

TABLE V.

Experiment 5.* Dog 4 (13.5 kilos).

Day of experiment.	Total sulfur.	Inorganic sulfur.	Ethereal sulfur.	Neutral sulfur.	Total nitrogen.	Remarks.
	gm.	gm.	gm.	gm.	gm.	
1	0.135	0.091	0.005	0.039	2.50	
2	0.164	0.104	0.009	0.051	2.51	
3	0.180	0.118	0.007	0.055	2.58	0.210 gm. S as purified taurocholic acid given by mouth.
4	0.168	0.101	0.004	0.063	2.42	
5	0.166	0.097	0.007	0.061	2.40	

* Experiments 5 and 6 were carried out in this laboratory by Mr. Thomas Watson.

Daily Diet.

Lard	30 gm.
Butter	10 "
Cracker meal	200 "
Sugar	60 "
Milk	100 cc.
Kaolin	50 "

-ur Compounds

Dog 3.*

Remarks.

nt 11.4 kilos.

.. cystine = {0.819 gm. N, 1.87 gm. S}

..... 75 gm.
..... 15 "
..... 30 "
..... 25 "
..... 50 cc.

E V.
og 4 (13.5 kilos).

Remarks.

210 gm. S as purified taurocholic acid given by mouth.

d out in this laboratory by Mr. Thomas

y Diet.
..... 30 gm.
..... 10 "
..... 200 "
..... 60 "
..... 100 cc.
..... 50 "

With cysteic acid there is an increase neither in urinary sulfates nor in amino nitrogen[1] which indicates that deamination took place but that the remainder of the molecule was apparently excreted unchanged. Neither sulfurous nor thiosulfuric acid was found in the urine. In contrast to these findings are the results which were obtained on feeding taurine. The amino nitrogen figures indicate that taurine does not combine with urea to form taurocarbamic acid and the sulfur values indicate that there is no appreciable oxidation of the sulfur. These facts agree with the previous findings of Schmidt and Allen on man (9). Cysteic acid, which contains an amino group in a position alpha

TABLE VI.

Experiment 6.* Dog 4 (13.5 kilos).

Day of experiment.	Total sulfur.	Inorganic sulfur.	Ethereal sulfur.	Neutral sulfur.	Total nitrogen.	Remarks.
gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
1	0.175	0.095	0.012	0.068	2.70	
2	0.177	0.098	0.012	0.067	2.66	
3	0.171	0.096	0.009	0.066	2.62	
4	0.945	0.852	0.020	0.073	3.76	8.7 gm. cystine by mouth = {2.32 gm. S, 1.01 gm. N}
5	0.456	0.430	0.013	0.013	2.56	
6	0.187	0.098	0.011	0.078	2.58	
7	0.175	0.096	0.011	0.068	2.68	

* The daily diet was the same as in Experiment 5.

to the carboxyl group, is deaminized on passage through the body despite the fact that it also contains a sulfonic group, while taurine, which reacts *in vitro* in all respects like an α-aminocarboxylic acid, is not so deaminized. The replacement of the amino group of taurine by a hydroxyl group to give isethionic acid does not lead to an increase in urinary sulfates. A marked increase in the output of both sulfur and nitrogen coincident with the administration of the first dose of isethionic acid to Dog 1 was found. Apparently a factor other than the substance administered was responsible for the increase in endogenous metab-

[1] Cysteic acid yields its nitrogen quantitatively when treated with HNO_2.

TABLE VII.

*Résumé Showing the Urinary Recovery of the Substances Ingested.**

Substance ingested.	Inorganic sulfur.			Neutral sulfur.			Total sulfur.			Total nitrogen.			α-amino nitrogen.		
	Experimental value.	Normal value.	Recovery.	Experimental value.	Normal value.	Recovery.	Experimental value.	Normal value.	Recovery.	Experimental value.	Normal value.	Recovery.	Experimental value.	Normal value.	Recovery.
Experiment 1.															
	gm.	*gm.*	*per cent*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*per cent*	*mg.*	*mg.*	*per cent*
Taurine	No increase.			1.117	0.117	67	1.277	0.263	68	4.20	3.50	106	493	114	58
Cysteic acid 1.															
Output 1st day	0.212	0.141	6	0.602	0.117	43	0.827	0.263	50	3.81	3.50	63	No increase.		
" 2nd "				0.207	0.117	8	0.298	0.263	3						
						51			53						
Cysteic acid 2.															
Output 1st day	0.178	0.141	3	0.724	0.117	52	0.915	0.263	56	3.78	3.50	74	No increase.		
" 2nd "				0.256	0.117	12	0.342	0.263	6						
						64			62						
Isethionic acid 1.															
Output 1st day				0.925	0.489	55	1.120	0.637	61†						
" 2nd "				0.622	0.489	17	0.746	0.637	14						
						72			75						

Cysteic acid 1:															
Output 1st day	0.212	0.141	6	0.602	0.117	43	0.827	0.263	50	3.81	3.50	63	No increase.		
" 2nd "				0.207	0.117	8	0.298	0.263	3						
						51			53						
Cysteic acid 2.															
Output 1st day	0.178	0.141	3	0.724	0.117	52	0.915	0.263	56	3.78	3.50	74	No increase.		
" 2nd "				0.256	0.117	12	0.342	0.263	6						
						64			62						
Isethionic acid 1.															
Output 1st day				0.925	0.489	55	1.120	0.637	61†						
" 2nd "				0.622	0.489	17	0.746	0.637	14						
						72			75						
Isethionic acid 2				0.545	0.215	54	0.680	0.359	52						
" " 3				1.291	0.107	61	1.452	0.244	68						
Cystine.															
Output 1st day	0.698	0.156	27	0.656	0.107	28	1.371	0.272	55	4.06	3.55	58	301	110	22
" 2nd "	0.213	0.156	3	0.443	0.107	17	0.675	0.272	20	3.80	3.55	28	253	110	16
			30			45			75			86			38
Taurocholic acid.§															
Output 1st day.‡	0.698	0.156	27				0.822	0.272	28	3.87	3.55	36			
" 2nd " .‡	0.213	0.156	3				0.339	0.272	3	3.66	3.55	12			
			30						31			48			
Experiment 2.															
Cystine.															
Output 1st day	0.752	0.047	35	0.163	0.045	6	0.919	0.101	41	3.30	2.90	46	109	53	6
" 2nd "	0.164	0.047	6	0.098	0.045	3	0.267	0.101	8	No increase.					
" 3rd "	0.077	0.047	2	0.061	0.045	1	0.147	0.101	2	" "					
			43			10			51						

* Blank spaces indicate that the output values are within the limits of the normal daily variation.

† The abnormally high values for the output of nitrogen and sulfur were apparently due to factors other than the ingestion of isethionic acid. The sulfur values of the 2nd day following the ingestion of isethionic acid are taken as normal values for the purpose of calculating sulfur output.

‡ The calculations are on the basis of catabolized cystine.

§ The figures in Table I indicate that taurocholic acid is not eliminated in the urine.

TABLE VII—*Concluded.*

Substance ingested.	Inorganic sulfur.			Neutral sulfur.			Total sulfur.			Total nitrogen.			α-amino nitrogen.		
	Experimental value.	Normal value.	Recovery.	Experimental value.	Normal value.	Recovery.	Experimental value.	Normal value.	Recovery.	Experimental value.	Normal value.	Recovery.	Experimental value.	Normal value.	Recovery.
Experiment 2—*Concluded.*															
	gm.	*gm.*	*per cent*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*per cent*	*mg.*	*mg.*	*per cent*
Isethionic acid.															
Output 1st day				0.632	0.045	57	0.712	0.101	59						
" 2nd "				0.073	0.015	3	0.118	0.101	1						
						60			60						
Cysteic acid.															
Output 1st day				0.440	0.046	40	0.526	0.125	41	2.76	2.59	40	No	increase.	
" 2nd "	0.133	0.050	10				0.189	0.125	6						
									47						
Taurine.															
Output 1st day				1.147	0.102	68	1.203	0.146	69	3.03	2.70	49	515	76	66
" 2nd "				0.223	0.102	9	0.263	0.146	8	2.87	2.70	25			
						77			77			74			
Cystine.															
Output 1st day	0.708	0.048	33	0.174	0.070	5	0.888	0.152	34	3.10	2.60	57	155	68	10
" 2nd "	0.134	0.048	4	0.166	0.070	5	0.317	0.152	8				95	68	3
" 3rd "	0.104	0.048	3	0.111	0.070	2	0.224	0.152	4						13
			40			12			46						

Experiment 3.]

Compounds																
Cysteic acid.																
Output 1st day	0.133	0.050	10	0.440	0.046	40	0.526	0.125	41	2.76	2.50	[illegible]				
" 2nd "							0.189	0.125	6							
									47							
Taurine.																
Output 1st day				1.147	0.102	68	1.203	0.146	69	3.03	2.70	49	515	76	66	
" 2nd "				0.233	0.102	9	0.263	0.146	8	2.87	2.70	25				
						77			77			74				
Cystine.																
Output 1st day	0.708	0.018	33	0.171	0.070	5	0.888	0.152	34	3.10	2.60	57	155	68	10	
" 2nd "	0.134	0.018	4	0.166	0.070	5	0.317	0.152	8				95	68	3	
" 3rd "	0.101	0.018	3	0.111	0.070	2	0.224	0.152	4						13	
			40			12			46							
Experiment 3.‖																
Sodium thiosulfate	0.473	0.076	77	0.198	0.085	22	0.671	0.168	98							
" sulfite	0.426	0.076	92	0.117	0.085	8	0.537	0.168	97							
" thiosulfate	0.300	0.076	87	0.101	0.085	7	0.404	0.168	92							
Cystine.																
Output 1st day	0.765	0.076	35	0.181	0.085	5	0.946	0.168	39	3.21	2.48	83				
" 2nd "	0.196	0.076	6	0.165	0.085	4	0.361	0.168	10							
			41			9			49							
Experiment 4.¶																
Cystine.																
Output 1st day	0.799	0.076	39	0.285	0.011	13	1.081	0.116	52	2.77	2.20	70				
" 2nd "	0.186	0.076	6				0.241	0.116	7							
			45						59							
Experiment 6.																
Cystine.																
Output 1st day	0.852	0.096	33				0.945	0.175	33	3.76	2.62	113				
" 2nd "	0.430	0.096	14				0.456	0.175	12							
			47						45							

‖ In this experiment the figures given in the neutral sulfur column are neutral plus ethereal sulfur.
¶ In this experiment the figures given in the inorganic sulfur column are for total sulfates.

olism since subsequent doses of the same substance to this animal and to another dog did not show the effect.

No appreciable amount of thiosulfuric acid was found in the urine following the ingestion of either cysteic acid, taurine, or isethionic acid. This raises the question why other experimenters found thiosulfates on feeding compounds containing sulfonic acid radicals. It is possible that bacterial action was a factor concerned in their experiments. The presence of small quantities of sulfurous or thiosulfuric acid in urine is easily determined by acidifying the urine with H_3PO_4 and distilling in an atmosphere of CO_2. Our experiments with sodium sulfite and sodium thiosulfate indicate that if such substances as isethionic acid and taurine are reduced to sulfurous acid and this compound is absorbed, oxidation is both complete and rapid.[2] If large quantities of thiosulfuric acid are formed from the administered substance by bacterial action and absorption subsequently takes place the urine of the animal will contain thiosulfuric acid, the amount depending on the dosage of thiosulfate which is absorbed. Our experiments with sulfites and thiosulfates are in agreement with the results of Salkowski (15), Lasch (16), and Rost and Franz (17), and show that moderate doses are readily oxidized to sulfates.

Administration of a 7.5 gm. dose of cystine to Dog 1 not only led to a considerable oxidation (30 per cent) of the amino-acid as seen from the increase in urinary sulfates, but curiously, about one-half of the amount administered was excreted in the urine unchanged, the elimination of cystine extending over a period of 2 days. The figures for both the neutral sulfur and the amino nitrogen support this statement and the presence of cystine in the urine was established by subsequent isolation with the aid of Gaskell's method (18). The elimination of unaltered cystine in the urine of Dog 1 was probably due to unusually rapid absorption but it is by no means clear why the elimination of free amino-acid extended over the 2nd day. Similar doses of cystine administered to Dogs 2 (Experiments 2 and 3), 3 (Experiment 4), and 4 (Experiment 6) were followed by minimal increases in neutral sulfur. The major portions of the absorbed cystine were oxidized as evidenced by a very marked increase in the elimination of urinary

[2] Unpublished investigations with sulfurous acid carried out by one of us (C. L. A. S.) on man gave similar results.

he same substance to this
how the effect.
lfuric acid was found in the
ther cysteic acid, taurine, or
stion why other experimenters
unds containing sulfonic acid
ial action was a factor con-
presence of small quantities of
rine is easily determined by
distilling in an atmosphere of
m sulfite and sodium thiosul-
as isethionic acid and taurine
this compound is absorbed,
rapid.[2] If large quantities of
the administered substance by
sequently takes place the urine
ric acid, the amount depending
is absorbed. Our experiments
in agreement with the results
Rost and Franz (17), and show
dized to sulfates.
e of cystine to Dog 1 not only
0 per cent) of the amino-acid
y sulfates, but curiously, about
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sulfates. Ingestion of 7.5 gm. of cystine did not lead to the appearance of either sulfurous or thiosulfuric acid in the urine of the experimental animals.

From the results of Experiments 1, 2 (second dose), 3, 4, and 6 (see also résumé table) it will be noted that following the ingestion of cystine the magnitude of the figures for nitrogen elimination is larger than the magnitude of the corresponding sulfur figures. From this list we may possibly eliminate the comparison of the nitrogen and sulfur figures of Experiments 1, 2 (second dose), and 4 as being not far outside of the limits of the normal variability but the difference in the percentages of cystine nitrogen and sulfur recovered in the urine as found in Experiments 3 and 4 cannot be so eliminated. If the difference is due to a specific demand on the part of the animal for anabolic sulfur a retention of both nitrogen and sulfur would be expected since sulfur is built into the protein molecule in the form of cystine. It is possible that we are dealing with a phenomenon analogous to that studied by Folin and Denis (19). They found that as a result of bacterial action on the nitrogenous material which has passed into the large intestines the ammonia content of the portal blood is increased. If cystine is decomposed by bacterial action to NH_3 and H_2S the former can by absorption enter the blood stream while the latter may be eliminated through the intestines.

Our results also show that the elimination of nitrogen after ingestion of cystine precedes that of sulfur. This fact is shown in Experiments 2, 3, 4, and 6. Certain of the figures for the output of sulfur on the 2nd and 3rd day after ingestion of cystine probably lie within the variability of the normal value; all, however, point in the same direction. The lag in the excretion of sulfur is apparently not due to a lag in the elimination of sulfates since the experiment with Na_2SO_3 indicates very prompt elimination. It appears to us more reasonable to assume that deamination of the cystine precedes the oxidation of sulfur, with the result that the elimination of sulfur lags behind the nitrogen. This explanation is in accord with the idea of Lewis (20) that the breaking up of the cystine molecule is dependent upon and takes place subsequent to deamination.

A negative Pettenkofer test for bile acids and normal values for urinary sulfur and nitrogen were obtained following the in-

gestion of taurocholic acid and indicate that the urine is not a channel for the elimination of bile acids. Even in icterus the amount of bile acids eliminated in the urine per 24 hours does not exceed several hundred milligrams.[3]

SUMMARY.

Experiments were carried out on dogs to determine the fate of certain sulfur compounds with especial reference to those concerned in bile metabolsim.

It was found that taurine is excreted in the urine unchanged and not as taurocarbamic acid as stated by Salkowski. Cysteic acid is deaminized, but the remainder of the molecule is excreted in the urine unchanged. Administration of isethionic acid is not followed by an increase in urinary sulfates as stated by several investigators. The urine is not a channel for the elimination of bile acids when the latter are fed. When given in large doses the major portion of the absorbed cystine is oxidized to sulfates which are eliminated in the urine. With the exception of large doses of sodium thiosulfate the ingestion of the various sulfur compounds under consideration did not lead to the appearance of appreciable amounts of sulfurous or thiosulfuric acid in the urine.

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[3] Unpublished investigations of Schmidt and Merrill.

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The reaction may prove useful in the management of mycobacterial infections other than tuberculosis — for example, leprosy (Levine, 1951). In an investigation on the use of the test for the detection of mycobacterial infections in cattle, Fisher and Gregory (1951) concluded that the reaction might have an important application in the diagnosis of Johne's disease, a condition in which sensitivity to mammalian tuberculin is usually either weak or absent.

Conclusions.

Reactions between human sera and sheep erythrocytes coated with fractions of tubercle bacilli were not sufficiently specific or sensitive to make the test a helpful one in the diagnosis of tuberculosis, irrespective of whether the hæmagglutination or the hæmolytic method was used. Correlation between clinical state and serological titre in tuberculous subjects, although present, was not close; thus little reliance could be placed on serological titre as a means of the clinical assessment.

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THE TREATMENT OF LEAD POISONING BY THE INTRAVENOUS ADMINISTRATION OF SODIUM THIOSULPHATE.

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AMONG the various agents which have been used or recommended in the treatment of lead poisoning, sodium thiosulphate appears to have suffered from undeserved neglect.

Among the earliest investigators to use sodium thiosulphate intravenously in metallic poisonings were Dennie and McBride (1923, 1924), who in 1920 began the treatment of poisoning by various metallic compounds by this means. Among poisonings successfully treated were those due to arsenic, mercury, bismuth and lead. Their use of sodium thiosulphate was suggested by the work of Ravaut (1920), who treated arsphenamine dermatitis with this agent. Ockerblad (1923) successfully treated with sodium thiosulphate given intravenously a patient suffering from acute lead poisoning, who was passing red cells, albumin and casts in the urine. Dennie and McBride (1924) quote the treatment of a similar patient by R. Mayer, three patients by L. Milne, and three patients by C. Nelson, with excellent results.

In the subsequent period between the appearance of the work referred to above and the work of Pincus (1936), several papers appeared on the use of sodium thiosulphate in the treatment of metallic poisoning due to arsenic, bismuth and mercury compounds.

Semon (1924) reported its successful use in two cases of mercury stomatitis, in one of bismuth stomatitis, in one of bismuth salivation, in one of arsenical jaundice, and in one of acute dermatitis arising after two intravenous injections of sulpharsenol. In the last-mentioned case the pruritus had subsided the day after one dose of 0·5 gramme. In the former cases the dose was 0·45 to 0·6 gramme in 5·0 millilitres of water on alternate days. Three to four doses were sufficient. Semon stated that it was of value also when taken orally in a case of mercurial stomatitis and eruptions, and also as a local antidote to the effects of arsenical injections outside a vein.

Halliday and Sutherland (1925) describe a case of very severe arsenical poisoning in which the patient at one stage was moribund. The patient was treated at first with 30 grains of the stock salt given orally every four hours while the preparation of the pure salt was being awaited. There were no toxic effects from the thiosulphate, but no improvement in the patient's condition, which was deteriorating; after three days the intravenous injections were commenced, the dose being 0·45 gramme daily for ten days and then 0·45 gramme on alternate days for seven days. After the first injection the downward trend was arrested and the patient eventually made a complete recovery.

Aub, Fairhall, Minot and Reznikoff (1926) referred to the earlier work on the treatment of lead poisoning with this agent and stated that the method was worthy of further study.

In spite of this early success the method apparently failed to secure general acceptance.

As late as 1944, Cantarow and Trumper (1944) stated that whereas some beneficial effect was observed in clinical lead poisoning by a few investigators (McBride and Dennie, 1923, 1924; Hegler, 1933), the majority of reports in clinical and experimental plumbism indicated that this agent was of little or no value in this regard (Curtis and Young, 1928; Linguerri, 1933).

Curtis and Young (1928), however, treated their lead-poisoned animals with thiosulphate given orally. They found that sodium thiosulphate given by mouth in doses of 0·05 gramme per kilogram of body weight to guinea-pigs, rabbits and rats previously poisoned by lead did not appreciably affect the excretion of lead. Since their treatment was oral, their results cannot be adduced as evidence against the value of this agent when used intravenously.

Early in 1933 Pincus (1936) commenced to use the method at Mount Isa, where in the period from 1931 to 1936 there were about 300 cases of compensable lead poisoning and many others of lesser degree of severity. Although he was not the only medical practitioner treating these cases, his experience firstly of the then recognized methods of treatment and subsequently of the thiosulphate method was very extensive. He soon came to prefer this method to any other. At this time nothing was definitely known as to the mode of action of the thiosulphate. His experience showed that after thorough treatment with thiosulphate recurrence of symptoms without further exposure was very rare, whereas it was common after calcium therapy.

Further, at Mount Isa it was frequently observed that the blue line (Burton's line) rapidly disappeared under thiosulphate treatment, which indicated that lead sulphide was probably being dissolved, and either eliminated or deposited elsewhere.

Dennie (1924) stated that Meyers had proved beyond doubt that sodium thiosulphate increased the elimination of metals from the body in cases of metallic poisoning, but no figures were given by him.

Subsequent to the appearance of the note by Pincus (1936) several papers on the effect of sodium thiosulphate in experimental lead poisoning in animals and in clinical lead poisoning in humans appeared.

Schmitt and Lossie (1938) found that the intravenous administration of "S-hydril" (that is, stabilized sodium thiosulphate) in one case of clinical lead poisoning resulted in a sudden clinical improvement. The blood lead content was significantly decreased after eight days and returned to normal after fourteen days.

Schmitt and Lossie (1939) stated that sodium thiosulphate administration relieved clinical symptoms, lowered blood lead content and eliminated lead from depots.

In a series of papers on experimental work with rabbits, guinea-pigs and fish, Binet, Pérel and Glotz (1939), Binet and Perel (1939), Binet, Chauchard and Pérel (1940) showed that the intravenous injection of 0·2 gramme of lead acetate into adult rabbits caused coproporphyrinuria within forty-eight hours, which persisted for weeks if not treated, but disappeared in two days if one gramme of sodium thiosulphate was injected daily; that sodium thiosulphate counteracted the toxic action of lead salts in experimental lead poisoning paralysis and prevented paralysis from occurring; and that this salt, suitably administered, protected fish and guinea-pigs from lethal doses of lead subacetate.

Sabatini, Molfino and Mazzatini (1937) showed that intramuscular injections of various preparations of sulphur were followed by diuresis and increased urinary lead output. Patients showed a rapid improvement in general condition and a progressive diminution in the clinical symptoms of lead poisoning. Their paper is not available in Australia, and the abstract does not specify the method of administration.

The Present Investigation.

In view of the fact that the value of this agent is apparently still not widely recognized and that the search for other satisfactory agents still goes on, as witness the somewhat unfavourable results of the use of BAL (British anti-Lewisite—2,3-dimercaptopropanol) (Vigliani and Zurlo, 1951), it has seemed worth while to describe in more detail than appears in the note of Pincus (1936) some of the results obtained with this agent at Mount Isa and subsequently in Melbourne.

Most of the work discussed in this paper was done in 1934, 1935 and 1936. Experience in the subsequent years has confirmed the value of the method. It is proposed to deal in a separate paper with the effects of the intravenous use of sodium thiosulphate on urinary lead concentration at Mount Isa, and with recent work in Melbourne on the effects on blood lead concentration and on lead excretion in urine and fæces.

In about the year 1935 Mount Isa was the largest single lead mine in the world, employing over 1200 men and producing 100 tons of pig lead per day. There were during the years 1931 to 1936 the following number of cases of compensable lead poisoning: 1931, 69 cases; 1932, 95 cases; 1933, 77 cases; 1934, 1935 and 1936, 71 cases.

There were two hospitals (that of Mount Isa Mines, Limited, and the Mount Isa District Hospital) at which the patients were treated, probably about half the numbers in each. Pincus, who had been at Mount Isa from 1931 and was superintendent of the district hospital, commenced the use of sodium thiosulphate early in 1933, so his experience with this agent and previously with the then recognized methods of treatment was extensive.

There were in earlier years some very severe cases of permanent disability due to lead poisoning. In the later period from September, 1934, to the end of 1936, there were among the 68 cases four cases of very severe abdominal colic and vomiting, and two of extensor paralysis in addition to other disabilities. In the majority of cases the patients complained of less severe symptoms than those mentioned, such as abdominal pain (hypogastric and epigastric), constipation, muscular weakness, fatigue, loss of weight, headache, sleeplessness, metallic taste in the mouth, pains in the region of the joints (especially knees and elbows), pain in the back in the lumbar region, and cramping pain in the muscles, especially of the calves.

From September, 1934, onwards the cases were assessed by a medical board of three members. The present writer was not responsible for the treatment given, but as chairman of the board examined all patients and carried out investigations on the stippled cell counts, the ratio of monocytes *plus* large lymphocytes to small lymphocytes, the urinary lead concentrations, and any other appropriate tests on all of them.

In addition to the above-mentioned compensation cases there were numerous other employees who were suffering from milder symptoms of similar type, and who were (i) treated but remained at their previous work, which had caused their condition, or (ii) treated but transferred to other work of less hazardous nature, or (iii) not treated but merely transferred to work of a less hazardous nature.

In order to give some idea of the condition of the subjects, Table I gives a list of 16 cases at Mount Isa for which definite data are still available to the writer, and of two recent cases in Melbourne (Cases XII and XVIII).

The patients listed in Table I were all treated with sodium thiosulphate given intravenously (30 grains in a few millilitres of water on alternate days). In some of the cases it was the only treatment given. In some the colic was controlled at first with calcium given intravenously as the chloride or gluconate for one or more doses.

In one case (Case IX) the severe colic required morphine for a couple of days.

In a few of the cases a week or ten days had to elapse before the patient began to feel much improvement; but in the majority the improvement in the patient's clinical condition was rapid.

TABLE I.

Case Number.	Stippled Cells per 1,000,000 Red Cells.[2]			Ratio of Monocytes *plus* Large Lymphocytes to Small Lymphocytes	Urinary Lead Excretion. (Milligrammes per Litre.)
	Coarsely Stippled.	Finely Stippled.	Total.		
I	3050	1527	4580	0·965	0·19
II	1280	640	1920	1·07	0·48
III	5100	0	5100	0·94	
IV	720	1100	1820	1·20	0·134
V	2530	1370	3700	0·905	
VI	1250	0	1250	0·50	0·17
VII	480	1600	2080	1·66	
VIII	6400		6400	0·48	0·24
IX	—	100	100	1·45	0·18
					Several days after treatment commenced.
X	2530	0	2530	1·2	1·215
XI	1700	900	2600	1·48	0·23
XII[1]	4200	16,000	20,200	0·84	0·83
XIII	3940	2060	6000	1·5	0·32
XIV	—	—	—	1·9	
XV	210	0	210	0·75	0·19
XVI	1180	2880	4060	1·08	0·10
XVII	0	1450	1450	2·0	0·315
XVIII[1]	0	5400	5400	1·9	0·10

[1] For Cases XII and XVIII, stippled cell counts were made with a magnification of 900, all others with a magnification of 600. Cases XII and XVIII were Melbourne cases, and occurred in 1941 and 1941. Cases listed in this table do not necessarily represent the most severely poisoned subjects, but in Cases VI, VIII, IX and XII the patients were severely affected and in hospital. Case IX was one of severe abdominal colic and vomiting. In this case there were never more than 360 finely stippled cells per 1,000,000 red cells, although the blood was examined on several occasions; the patient was not very anæmic.

[2] Light field illumination, Seller's stain.

It was no part of the function of the medical board simply to remove from the lead hazard persons who were suffering from lead poisoning of a compensable degree and leave them without treatment, just as controls.

The assessment of the value of the method depends, therefore, to a considerable extent on the clinical judgement of the members of the board, and that opinion was that improvement under this method of treatment was definitely more rapid than could be accounted for by mere removal of the subject from the lead hazard.

Effect of Sodium Thiosulphate Therapy on Ratio.[1]

However, in order to give a numerical estimate of the improvement in condition a comparison has been made of the ratio of monocytes *plus* large lymphocytes to small lymphocytes before treatment began, and at a short interval afterwards. Ferguson and Ferguson (1934) and Shiels (1937, 1938, 1950) have already shown the value of this ratio as an indication of the clinical condition of persons who are suffering from lead poisoning, of those who are in the state of recovery or have just recovered from lead poisoning, and of those who have absorbed significant amounts of lead but are without symptoms of lead poisoning.

The tests were not carried out every day for any particular subject; in some instances a few days, in others a week or two, elapsed between tests, so that the intervals shown in column 7 are really greater than the actual intervals required to bring about improvement.

The *t* test of Fisher (1950)—that is, the ratio of the mean to the standard error of the mean—has been applied to the mean increase in the ratio. Its value was 6·919. When $n = 17$ (one less than the number of observations of the increase), this corresponds to a *P* value of less than 0·001. Thus there was a highly significant increase in the ratio shortly after commencement of the thiosulphate treatment.

In the cases considered in this paper the number of injections required to bring about a really satisfactory condition of well-being varied from two or three to a dozen or so.

The average ratio for these 18 subjects before treatment—namely, 1·23—was very close to that found for 65 cases of lead poisoning at Mount Isa—namely, 1·21.

[1] "The ratio" is that of monocytes *plus* large lymphocytes to small lymphocytes.

TABLE II.

Showing the Ratio for Each Subject Just Before Treatment Began and Again a Short Time Later.

1 Case Number.	1 Date.	3 Ratio on Date in Column 2.	4 Date.	5 Ratio on Date in Column 4.[2]	6 Increase in the Ratio.	7 Approximate Number of Days Since Treatment Began at which Ratio in Column 5 was Obtained.
I	3/ 4/36	0·97	14/ 4/36	5·30	4·33	11
II	13/ 5/36	1·07	18/ 5/36	3·20	2·13	3
III	23/ 2/36	0·94	5/ 3/36	3·82	2·80	11
IV	12/ 3/36	1·20	30/ 3/36	2·04	0·84	18
V	13/ 3/36	0·97	21/ 4/36	4·40	3·43	8
VI	21/11/34	0·70	27/11/34	3·88	3·18	6
VII	22/ 4/36	1·66	27/ 4/36	2·46	0·80	3
VIII	20/12/34	0·48	14/ 1/35	1·40	0·92	25
IX	23/ 1/35	1·45	28/ 2/35	2·20	0·75	5
X	21/ 6/35	1·20	19/ 6/35	4·90	3·70	25
XI	1/ 5/35	1·48	10/ 5/36	2·80	1·32	6
XII	8/ 6/41	0·84	19/ 6/41	4·20	3·36	10
XIII	12/ 5/36	1·50	26/ 5/36	2·35	0·85	14
XIV	3/ 1/36	1·90	10/ 1/36	3·80	1·90	13
XV[3]	12/ 2/36	0·75	9/ 4/36	2·90	2·15	56
XVI	12/ 2/36	1·03	18/ 5/36	2·05	0·97	6
XVII	26/ 5/36	2·00	15/ 6/36	2·40	0·40	20
XVIII[3]	8/ 2/41	1·00	10/ 4/41	3·81	1·91	21
Mean		1·227		3·219	1·992	
Standard error					0·2879	—
					$t = 6·919$ P (for $n = 17$) $< 0·001$	

[1] This subject had already had several injections when the test was performed on February 12, 1936. Only one test was performed between February 12 and April 10.

[2] In this case injections did not commence until March 20, 1941.

[3] At the time when the results shown in Column 5 were obtained, the subjects all showed very great improvement in their clinical condition, in many cases feeling very well indeed.

The average ratio after some days' treatment—namely, 3·22—approached the average value—namely, 3·72—for 109 persons at Mount Isa exposed to generally similar lead hazards, but without any symptoms or with very mild symptoms.

Comment.

Thus removal from work and a short course of thiosulphate injections changed the condition of subjects with lead poisoning almost into that of persons who had absorbed some lead but had no symptoms.

These facts are summarized in Table III.

Table IV shows the change in the distribution of the values of the ratio among the 18 subjects as a result of removal from work and treatment. Before treatment none of the values of the ratio exceeded 2, whereas the values of the ratio determined in some cases during treatment and in others at the end of the treatment exceeded 2 in all cases except one.

The improvement in clinical condition and in the ratio is not due merely to removal from work. This is shown by the facts that similar rapid clinical improvement occurred in the case of a person who had been away from work for over five months before the treatment started (Case XVIII, Tables I and II), and in the case of a number of persons with similar symptoms of a milder nature

TABLE III.
The Ratio.

Subjects.	Ratio.	Ratio.	Subjects.
18 persons with lead poisoning; before treatment.	1·23	1·21	65 persons with lead poisoning at Mount Isa.
The same 18 persons after some days' treatment.	3·22	3·72	109 persons at Mount Isa exposed to lead hazard but without lead poisoning.

than those shown by the subjects referred to in Tables I and II, who were treated while still remaining at work under the lead hazards which produced their symptoms.

Shortly after leaving work, the subject in Case XVIII had been treated with some improvement by the intravenous administration of calcium gluconate, vitamin *D* and a diet rich in calcium. When examined by the writer he still complained of weariness and lack of energy, the earlier improvement not having been maintained. After four injections he was feeling much better, and after several further injections (up to April 10, 1941) he was feeling very well indeed, and had no relapse.

TABLE IV.
Distribution of Values of the Ratio, Showing Number of Individuals Having Ratio within the Range of Values Set Out.

Range of Values of the Ratio.	Before Treatment.	During and After Treatment.
0 to 1·0 ..	7	0
1·1 to 2·0 ..	11	1
2·1 to 3·0 ..	0	8
3·1 to 4·0 ..	0	5
4·1 to 5·0 ..	0	3
5·1 to 6·0 ..	0	1

Summary.

1. The value of the intravenous administration of sodium thiosulphate in the treatment of acute and chronic lead poisoning has been discussed.

2. It has been shown that the treatment causes rapid improvement in clinical condition without recurrence of symptoms.

3. The treatment causes an increase in the ratio of monocytes *plus* large lymphocytes to small lymphocytes, which is highly significant statistically.

Acknowledgements.

Grateful acknowledgements are made to Dr. S. Barnett and Dr. E. Ryan, formerly of Mount Isa Mines, Limited, to Dr. F. F. Pincus, to Dr. Rae Dungan and to the Medical Superintendent of the Royal Melbourne Hospital, for access to patients and clinical notes on some of them. The help of the late Ian Robertson, B.Sc., in supplying a number of references and abstracts, is also gratefully acknowledged.

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The Tohoku Journal of Experimental Medicine, Vol. 63, No. 4, 1956

The Influence of Internally Used Sodium Thiosulphate upon the Oxygen Equilibrium of Hemoglobin

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By

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(Received for publication, May 20, 1955)

The work of most of investigators[1-5] into the blood has proved that the oxygen equilibrium of hemoglobin is affected by the hydrogen ion concentration, by the carbon dioxide pressure, by the salts concentration and by the temperature of blood. From the time, when L. Pauling[6] theoretically clarified the relationship between the molecular structure and oxygen equilibrium of hemoglobin, its biological function was more substantially connected to its chemical structure. In this connection we have experienced a new phenomenon which has not yet been reported. We observed a moderate cyanosis in a diabetic patient who had taken orally 3 g. of sodium thiosulphate a day for 2 months. At first we thought that this cyanosis was due to the formation of methemoglobin or other altered hemoglobin which could not transport oxygen. But this was not correct. The hemoglobin of this patient could completely be combined with oxygen by saturating the blood with pure oxygen but could not be fully oxygenated by saturating with air. Therefore, the affinity of the hemoglobin of this patient to oxygen should be reduced. On studying the blood of this patient we discovered that the effect of the dosed sodium thiosulphate upon the hemoglobin and oxygen equilibrium was greater than any other physiological factors ever known. Thus, we can shift the oxygen hemoglobin dissociation curve to the right by oral administration of sodium thiosulphate. The effect of sodium thiosulphate is not direct action upon hemoglobin. It is very interesting that we can affect artificially in vivo the dissociation curve of hemoglobin and oxygen without any side effect. To confirm the above fact we have made the following experiments.

Experimental

Methods and Materials

As subjects, we used our inpatients, and we administered 3 g. of

sodium thiosulphate a day. After a week or two, various degrees of cyanosis came to be discernible on these patients ranging from the slightest to the medium degree. At this stage we measured the oxygen saturation of arterial blood by van Slyke's method.[7] The most important matter in the circumstances, we must remember, is that the carbon monoxide capacity method has always to be adopted to decide the 100 per cent of oxygen capacity, because saturation with air produces a quite different result in this case. On having measured oxygen saturation of arterial blood, the dosage of sodium thiosulphate was stopped for a whole week, and then the same measurements were made again, of which the result is shown in Table I. In case cyanosis was remarkable, we measured the carbon monoxide capacity of the blood by saturating it with pure carbon monoxide gas and then we measured the oxygen capacity of the same blood by saturating with air, and of these we drew a comparison between the each. Furthermore, we measured the oxygen capacity by Drabkin's cyanmethemoglobin method[1] and drew a comparison of the above. (Table II) Next, after having measured the oxygen capacity saturating the blood with pure oxygen on one hand and with carbon monoxide on the other, we also drew a comparison of capacities between them. (Table III)

Further, to the normal blood was added the same quantity of distilled water on one hand and the same quantity of 1/100 N sodium thiosulphate on the other, and after several hours a comparison between carbon monoxide capacity of above two was drawn.

TABLE I

Oxygen Saturation of Hemoglobin of Arterial Blood before and during Cyanosis Produced by Sodium Thiosulphate Administration and after its Disappearance

Name of patient	Before	During		After
Kanno	96	91	87	96
Chiba	97	95	89	95
Nishida	95	89		
Kumada	94	88	88	94
Takeuchi		92		98
Minowa	94	89		93
Namba	97	93	87	
Wakatsuki		75	85	97
Matutani	95	91		96
Mean	95.4	88.5		95.6

TABLE II

O_2-Capacity Determined by Saturation with Air, CO-Capacity and O_2-Capacity Determined Spectrophotometrically by the Method of Drabkin, of the Same Blood of Patients who Showed Cyanosis by Thiosulphate Administration

Case No.	O_2-Capacity	CO-Capacity	O_2-Capacity determined photometrically
1	13.3		14.8
2	16.1		19.9
3	19.2	21.8	
4	13.9	15.4	15.8
5	13.5	15.8	
6	14.6	16.4	16.7
7	16.7	17.7	18.0

TABLE III

CO-Capacity and O_2-Capacity Determined by Saturation with Pure Oxygen of the Blood of Patients who Showed Cyanosis by Taking Sodium Thiosulphate

Case No.	CO-Capacity	O_2-Capacity
8	15.8	61.0
9	22.8	22.9
10	21.3	21.1
11	20.3	20.5
12	17.8	17.9

Results

As the Table I clearly shows, the oxygen saturation of arterial blood of the patients are lower while the sodium thiosulphate being dosed than before they begin their dosage. Moreover, the lowering is far below the physiological range. And the values measured after a week of free interval have regained the same normal value as measured before the dosage. By the experiments shown in Table I, it can be clearly seen that the hemoglobin of a patient while taking sodium thiosulphate does not fully saturate with oxygen under the normal alveolar oxygen tension. Therefore, we made experiments in saturating the blood with air. In spite of the fact that the hemoglobin of normal blood can be saturated almost completely under the oxygen pressure (about 150 mm. Hg) of the air, the hemoglobin of the patients does not combine with oxygen under the same oxygen pressure to the full extent, as shown in Table II. By this alone, however, it can not

be denied that a portion of hemoglobin may have become the derivative combining with carbon monoxide though it does not combine with oxygen. In fact the formation of sulfhemoglobin can not be excluded. But there is shown in Table III a fact that denies the above question. Though the blood of a patient with cyanosis can not be saturated under the atmospheric oxygen partial pressure (ca. 150 mm. Hg), it can perfectly be saturated with pure oxygen under the atmospheric pressure (760 mm. Hg). Table II proves that the hemoglobin content of the blood measured by carbon monoxide corresponds to the value yielded by measuring spectrophotometrically. On the above fact we assert that the cause of cyanosis is not the derivative which does not combine with carbon monoxide or pure oxygen.

Having gone through the above experiments, we realized that the hemoglobin of a patient under the administration of sodium thiosulphate shows diminished degree of affinity to oxygen. To study whether it is really due to the direct action of the sodium thiosulphate or not, we observed the comparison between the two oxygen saturations of hemoglobin: one is of the blood left alone after having been added the same quantity of distilled water and the other is of the same blood added same quantity of 1/100 *N* sodium thiosulphate solution. No difference between them, however, could be found at all.

Again, to testify if the oxygen dissociation curve of hemoglobin is affected by sodium thiosulphate immediately after its entering into blood in vivo or not, we compared the two arterial bloods: one taken 10 minutes after the intravenous injection of 20 m*l*. 10% solution of sodium thiosulphate and the other taken before the injection. The result is shown in Table IV. No difference can be seen in the oxygen saturations of hemoglobin of arterial blood before and after the injection.

TABLE IV

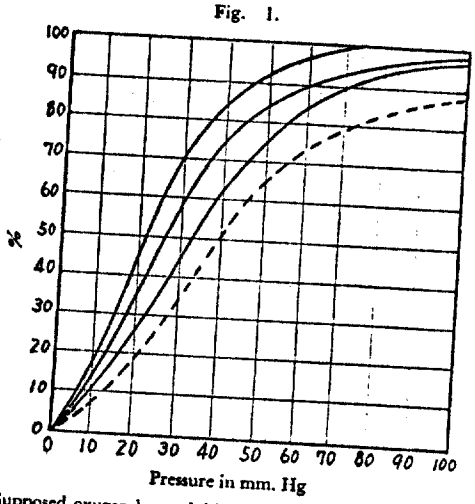
Oxygen Saturation of Arterial Hemoglobin before and after Intravenuous Injection of 10% Thiosulphate Solution

Case	Before	After
M.K.	94	94
S.G.	93	92
T.B.	95	94
O.S.	97	97
A.K.	98	98
H.T.	95	95
N.T.	97	97

Discussion

We proved that cyanosis gradually appears while the patients are being given sodium thiosulphate. By the above experiment, we confirmed that the cyanosis is not caused by the deterioration of the hemoglobin to another derivative which does not combine with oxygen, but by the decrease in affinity of hemoglobin to oxygen. The partial pressure of oxygen in the arterial blood is not considered to have been diminished in such a degree, because neither dyspnea nor symptom of oxygen lack can be seen. Accordingly this denatured hemoglobin shows the oxygen saturation about 89% at about 100 mm. Hg pressure of oxygen. We could not decide the oxygen dissociation curve of hemoglobin on this blood, but the dissociation curve as rough approximation should be supposed to be as shown in Fig. 1. The curve on the left side has been quoted from that decided by Henderson.

Fig. 1.



--- Supposed oxygen hemoglobin dissociation curve of a patient showing cyanosis by administration of sodium thiosulphate. —— Dissociation curve taken from Henderson's data.

The figure shows that the dissociation curve shifts further to the right by sodium thiosulphate administration than the shift caused by the other physiological factors. The shift can not be considered as the direct action of sodium thiosulphate on the dissociation curve, from the point that the sodium thiosulphate added to blood has no effect at all. Moreover, no such change can be observed immediately after the intravenous injection of sodium thiosulphate. Through the above experiments, we are com-

pelled to decide that either the nature of hemoglobin or factors influencing its oxygen equilibrium may have gradually been changed after a definite duration by sodium thiosulphate administration. Anyhow, we can shift the oxygen hemoglobin dissociation curve to the right by artificial means. This fact may contain some significance not only in the theoretical view point but also in the practical application.

Summary and Conclusion

Patients orally given sodium thiosulphate 3 g. daily showed moderate cyanosis. The cause of the cyanosis was proved as oxygen unsaturation of hemoglobin of arterial blood. This unsaturation was due to the shift of oxygen hemoglobin dissociation curve to the right. Thus, one can decrease artificially the affinity of hemoglobin to oxygen in vivo without any discernible side effect.

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ENZYMIC TRANSFER OF SULFUR FROM MERCAPTOPYRUVATE TO SULFITE OR SULFINATES

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Thiosulfate is known to be a normal constituent of urine in man[1] and higher animals[2], but its mode of formation has up to now been unknown. It has been suggested that thiosulfate is formed by oxidation of hydrogen sulfide, the latter being liberated by intestinal microorganisms or formed through the cysteine desulfhydrase reaction. We have briefly reported[3] that the ability of tissues to oxidize hydrogen sulfide to thiosulfate is a general heme-catalyzed reaction which probably bears no physiological importance. However an enzymic reaction was discovered, by which thiosulfate is formed from β-mercaptopyruvate and sulfite. When sulfite is replaced in this reaction by the structurally related sulfinates, thiosulfonates are formed instead of thiosulfates. The present paper deals with the transsulfuration reactions of mercaptopyruvate.

MATERIALS

Ammonium mercaptopyruvate, commercially available from Bios Inc., was used in some experiments. It was twice recrystallized from an acetone-water mixture, as the original product was found to be decomposed to some extent. In later experiments sodium mercaptopyruvate prepared according to PARRON[4] was used. L-Cysteinesulfinic acid was prepared according to LAVINE[5] and hypotaurine[6] was a gift from Dr. F. KIEFFER of this laboratory. Water homogenates of rat tissues were prepared with a Bühler or Turmix blender.

METHODS

Thiosulfate was determined by a recently developed colorimetric procedure[7] based on the conversion of thiosulfate to thiocyanate by cyanide and cupric ions, and a subsequent colorimetric determination of the thiocyanate with ferric ions. Since mercaptopyruvate interferes with this determination, it was removed by precipitation with cadmium ions. This step was carried out under slightly acid conditions, as considerable amounts of thiosulfate were carried down with the precipitate under more alkaline conditions. Since the assay systems also contained phosphate buffer, the main component of the precipitate was cadmium phosphate. Cadmium ions predominantly precipitate the secondary and tertiary phosphate ions; thus the desired slightly acid medium was directly produced in this step by the primary phosphate remaining in the supernatant. The determination was carried out as follows. To 3.0 ml reaction mixture, containing 0.1 *M* phosphate buffer at pH 7.4, 0.5 ml 1 *M* cadmium acetate was added at the end of reaction. The precipitate was centrifuged off and to 0.50 ml of the supernatant 0.40 ml 1 *M* ammonia, 3.30 ml water, 0.5 ml 0.1 *M* KCN, 0.30 ml 0.1 *M* cupric chloride and 0.50 ml ferric nitrate reagent (100 g $Fe(NO_3)_3 \cdot 9H_2O$ and 200 ml HNO_3, sp.gr. 1.40 and water to 1000 ml) were added. The additions were made in the order indicated and the samples were thoroughly mixed before each new addition. The optical density in a 1 cm cuvette was then determined at 460 mμ. Recovery

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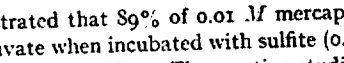
experiments demonstrated that 94% of thiosulfate added to the complete system was recovered in the final test and the values were correspondingly corrected. Thiosulfonates do not interfere with this determination of thiosulfate if they are not present in too large an excess (demonstrated with ethanethiosulfonate and *p*-toluenethiosulfonate). Pyruvate was determined in the cadmium supernatants by the salicylaldehyde method[8]. Cyanide labile sulfur (from thiosulfonates) was determined in the sulfinate systems by the following method. The remaining mercaptopyruvate was removed by cadmium precipitation as in the thiosulfate determination. To 0.50 ml supernatant was added 0.20 ml 1 *M* ammonia and 0.70 ml 0.1 *M* KCN and the mixture was heated for 30 min in boiling water. To the cooled sample 0.50 ml ferric nitrate reagent was then added, the sample was made up to 5 ml and the optical density was determined at 460 mμ. As sulfinic acids give a red colour with ferric ions, a blank determination was carried out, in which the ferric nitrate was added to the sample before the cyanide, and the heating step omitted.

Paper chromatography was carried out directly on the sulfinate systems after removal of proteins by heat denaturation (1 min at 100° C) and centrifugation. 10 μl was put on Whatman paper No. 1 and the chromatograms were obtained by descending development with water-saturated phenol and the spots were revealed with ninhydrin.

RESULTS

Preliminary experiments demonstrated that 89% of 0.01 *M* mercaptopyruvate was converted to thiosulfate and pyruvate when incubated with sulfite (0.02 *M*) and liver homogenate (2%) for 30 min at 37° C and pH 7.4. The reaction studied was thus the following one:



$$SCH_2COCOO^- + SO_3^{2-} = CH_3COCOO^- + S_2O_3^{2-}.$$

The factor in the liver homogenate was heat-sensitive (complete inactivation occurred in 5 min at 55° C, whereas 20% of the activity remained after 5 min at 50° C) and nondialyzable. Experiments were then undertaken in order to develop a suitable assay system. As the enzyme was highly active it was decided to lower the temperature to 20° C and to shorten the reaction time to 10 min. The effect of pH on the system under these conditions is shown in Fig. 1. It is evident that the enzyme has an optimum around pH 7.4–8, and the following experiments were consequently carried out at this pH. In Fig. 2 is demonstrated the effect of sulfite concentration. Maximum activity was obtained with 0.02 *M* sulfite; higher concentrations were slightly inhibitory. Fig. 3 demonstrates the effect of mercaptopyruvate concentration. Saturation of the enzyme with mercaptopyruvate was not obtained in this experiment. In order to save the valuable mercaptopyruvate it was decided to use this compound at 0.01 *M* concentration in the standard test. Fig. 4 finally shows the relation between thiosulfate formation and enzyme concentration in this standard test. The activities of different rat tissues were studied by means of this test. The results are shown in Table I. The most active tissue is liver, followed by kidney and blood. The activity of blood is confined to the cell fraction, whereas blood serum is practically inactive. Brain and muscle showed some activity but this was attributed to the blood content of these tissues.

Sulfinates as sulfur acceptors

When mercaptopyruvate and L-cysteinesulfinate were incubated together with rat liver homogenate, only insignificant amounts of thiosulfate were formed. A certain degree of formation of thiosulfate was in fact expected, as cysteinesulfinate yields sulfite when incubated with liver[9]. Paper chromatography of the reaction mixture demonstrated not only the expected cysteinesulfinate spot with an R_F of 0.14 but

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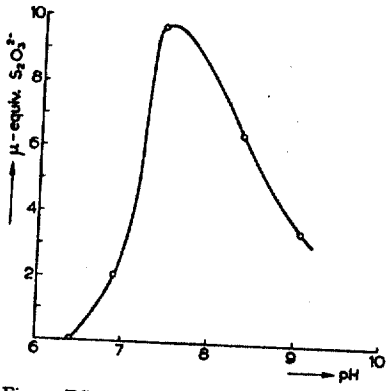

Fig. 1. Effect of pH on thiosulfate formation. Test conditions: Mercaptopyruvate 0.01 *M*, sulfite 0.02 *M*, phosphate 0.1 *M*, pH in the whole system as indicated. Each sample contained 0.5 ml 2% rat liver homogenate, final volume 3.0 ml. After 10 min at 20° C the reaction was stopped by the addition of 0.5 ml 1 *M* cadmium acetate and the necessary amount of NaOH or HCl to bring the pH back to 7.4 (as measured before the cadmium acetate addition). All samples were then brought to a final volume of 4.1 ml before centrifugation. Thiosulfate is determined in the supernatant as described in METHODS.

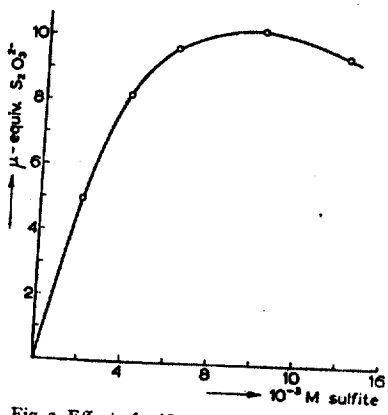

Fig. 2. Effect of sulfite concentration. Mercaptopyruvate 0.01 *M*, phosphate 0.1 *M*, pH 7.4, reaction time 10 min and temperature 20° C. Each sample, with a final volume of 3.0 ml contained 0.5 ml 2% liver homogenate.

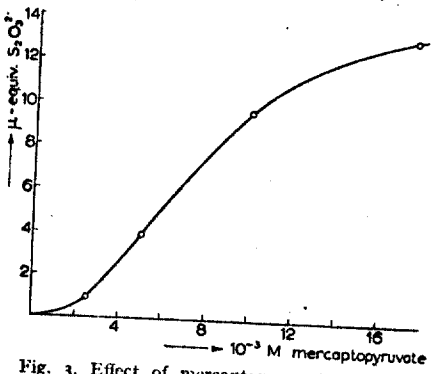

Fig. 3. Effect of mercaptopyruvate concentration. Sulfite 0.02 *M* and mercaptopyruvate as indicated, other conditions as in Fig. 2.

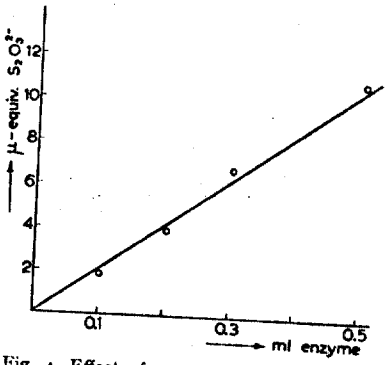

Fig. 4. Effect of enzyme concentration. Mercaptopyruvate 0.01 *M*, sulfite 0.02 *M* and indicated amount of 2% liver homogenate. Other conditions as in Fig. 2.

also a ninhydrin-positive unknown spot with an R_F of 0.06. When heat-denatured liver homogenate was used, only the cysteinesulfinate spot was detected. Sulfinates can add on sulfur, yielding thiosulfonates, and it was therefore expected that the unknown compound was a thiosulfonate. When thiosulfonates are heated with cyanide, they are converted to the corresponding sulfinate and thiocyanate[10] and this reaction was used for identifying the unknown compound. When 0.5 ml of the deproteinized reaction mixture was heated with 1.0 ml 0.1 *M* KCN for 30 min at

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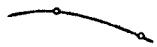

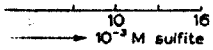

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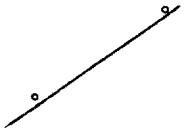

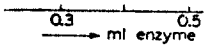

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TABLE I

ENZYME ACTIVITY OF RAT TISSUES

Tissue	Activity μequiv. $S_2O_3^{2-}$ formed per mg fresh weight
Liver	0.87 (0.62–1.12)
Kidney	0.46 (0.29–0.70)
Blood	0.33 (0.26–0.45)
Serum	0.01
Blood cells	0.40
Muscle	0.06 (0.03–0.08)
Brain	0.05 (0.04–0.06)

The standard assay system contained 0.5 ml of a 2% homogenate in the case of liver, kidney and blood, and 0.5 ml of a 10% homogenate in the case of brain and muscle. All values, except those for serum and blood cells are the averages of 3 determinations on tissues from different animals. Ranges indicated in brackets.

100° C considerable amounts of thiocyanate were formed. Paper chromatography showed that the unknown spot had by that time disappeared and that the cysteinesulfinate spot was more intense. Consequently, the unknown compound was the thiosulfonate analogue of cysteinesulfinate. The name of the corresponding acid, $HS_2O_2CH_2CH(NH_2)COOH$, would be alaninethiosulfonic acid* according to existing rules. As the thiosulfonate group is about as polar as the sulfonate group, alaninethiosulfonate should have on a paper chromatogram an R_F value similar to the R_F value of cysteate, which was actually observed. When the sodium salt of hypotaurine (aminoethanesulfinic acid) was used instead of cysteinesulfinate, a similar reaction occurred. Paper chromatography demonstrated two ninhydrin-positive spots, one from hypotaurine with an R_F of 0.65 and one from an unknown more polar compound with an R_F of 0.36. After heating with cyanide, only a more intense hypotaurine spot remained and concomitantly the formation of thiocyanate was demonstrated. The unknown compound was thus aminoethanethiosulfonate $NH_2C_2H_4S_2O_2^-$. The amount of thiosulfonate formed in these reactions could be obtained be determining cyanide-labile sulfur. When 0.5 ml of a 10% liver homogenate was incubated with 30 μ-moles each of mercaptopyruvate and cysteinesulfinate (in the presence of 0.1 M phosphate buffer pH 7.4 and in a final volume of 3.0 ml), 7.4 μmoles of thiosulfonate had been formed after 30 min at 30° C. When cysteinesulfinate was replaced by aminoethanesulfinate under otherwise equal conditions, 16.8 μmoles of thiosulfonate were formed. Aminoethanesulfinate is thus more active as sulfur acceptor than cysteinesulfinate.

DISCUSSION

GAST *et al.*[1] have found that the sulfur-containing amino acids in the diet are the source of excreted thiosulfate. As the enzymic formation of thiosulfate from mercaptopyruvate and sulfite has now been demonstrated, it is possible to formulate a sequence of established reactions, through which one molecule of thiosulfate can be produced from 2 molecules of cysteine. One cysteine molecule is transaminated, yielding mercaptopyruvate[11]. Another cysteine molecule is first oxidized to cysteinesulfinate and sulfite is then formed through the desulfinase system[9,12]. Sulfur is finally transferred from mercaptopyruvate to sulfite. It is of interest, that FROMAGEOT AND ROYANÉ[13] observed an increased excretion of thiosulfonate after the administration of cysteinesulfinate to rabbits.

* Cysteinesulfinic acid is more appropriately called alaninesulfinic acid; however, the established name cysteinesulfinic acid is used in this paper.

References p. 329.

An enzymic desulfuration of mercaptopyruvate was first demonstrated by MEISTER *et al.*[11], who observed that pyruvate and free sulfur were formed when mercaptopyruvate was incubated with a liver preparation. This reaction was slow and incomplete even in the presence of large amounts of liver preparation, but in the presence of certain thiol compounds the reaction became rapid and complete. Under these conditions hydrogen sulfide is formed instead of free sulfur. It is probable that in this case sulfur is first transferred from mercaptopyrucate to the thiol compound, resulting in a persulfide formation. Persulfides are however very unstable and will react with the excess thiol, giving the corresponding disulfide and hydrogen sulfide. Another desulfuration reaction of mercaptopyruvate was discovered by WOOD AND FIEDLER[14], who found that thiocyanate was formed when mercaptopyruvate was incubated with liver in the presence of cyanide. This reaction was attributed to rhodanese, an enzyme catalyzing the formation of thiocyanate from thiosulfate and cyanide. Experiments by the present author[15] demonstrated that the two reactions are catalyzed by different enzymes, and this was recently confirmed by FIEDLER AND WOOD[16]. The present work has demonstrated a third type of desulfuration reaction of mercaptopyruvate in which sulfite or sulfinates are the sulfur acceptors. It is of interest that all the sulfur acceptors that are active in these reactions (thiol, compounds, cyanide, sulfite or sulfinates) have strong nucleophilic properties. It is consequently possible that these desulfuration reactions, or better transsulfuration reactions, involve a nucleophilic attack on the sulfur-carbon bond in mercaptopyruvate. The adjacent carbonyl group in mercaptopyruvate will make this bond more susceptible to this attack, in contrast to the stabilizing effect of the amino group in cysteine. Thus an electronic interpretation is offered for the fact that cysteine is inactive in these transsulfuration reactions and must first be transaminated before the sulfur can be detached. An objection to this theory seems to be that cysteine yields hydrogen sulfide when incubated with liver[17], but evidence has recently been obtained by CHATAGNER AND SAURET-IGNAZI[18] that a transamination is the first step in this reaction.

The demonstration of an enzymic formation of thiosulfonates from cysteine-derived metabolites, which are normally formed in the body, is of interest, since it is the first indication of the biological occurrence of thiosulfonates. These compounds have not yet been directly demonstrated in the body, but an observation of SMITH AND TULLER[19] might be mentioned in this connection. In a study of sulfur-containing compounds in human blood serum by a paper-chromatographic technique, these authors observed an unknown spot with an R_F value similar to that of taurine. However, the unknown compound contained reducing sulfur, in contrast to taurine. This unknown compound might well be aminoethanethiosulfonic acid, for which we suggest the shorter name of *thiotaurine*, in analogy with the name of hypotaurine for aminoethanesulfinic acid. (Alaninethiosulfonic acid may then be called thiocysteic acid; however, this name could also be given to the compound $COSH(NH_2)CHCH_2SO_3H$.) Further studies are being made at present on the synthesis and metabolism of these thiosulfonates. The activity of other thiosulfonates as sulfur donors in the rhodanese reaction has previously been demonstrated[20].

ACKNOWLEDGEMENT

The author is very grateful to Professor C. FROMAGEOT for his kind interest in this work.

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SUMMARY

An enzyme has been found in rat tissues, which forms thiosulfate from mercaptopyruvate and sulfite. Liver, kidney and blood are the most active tissues. When sulfite is replaced by sulfinates, thiosulfonates are formed. The formation of the salts of two new compounds, aminoethanethiosulfonic acid (thiotaurine) and alaninethiosulfonic acid has been demonstrated. The significance of these reactions in sulfur metabolism is discussed.

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DIALYZABILITY OF HISTONES*

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Recent investigations by ultracentrifugation[1-4], electrophoresis[3-6], salting-out[8,9], isoelectric precipitation[8], or chromatography[5,10,11] have shown that histone is a complex which can be resolved into several fractions. The findings differ in regard to the number and ratio of histone components and in regard to their amino acid content. The inconsistencies appear to be due in part to the natural tendency of histones to form aggregates, and in part to the application of non-quantitative fractionation procedures. For example, it has been our experience[2] and also that of DAVISON *et al.*[4] and CRAMPTON[11] that dialysis has marked effects upon histone preparations and seems to

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(Aus der I. Chirurg. Abteilung des Bürgerspitals Verona.)

Natrium-thiosulfat, eine gefäßwandschädigende Substanz.

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Thiosulfate

Von C. STEGER.

Die Anwendung von Natrium-thiosulfat zur Behandlung der Hyperthyreose geht auf *Biancalana* (1934, 1935, 1936 usw.) zurück. Dieses Pharmakon ist jedoch in den letzten Jahren für diesen Zweck vielfach in Vergessenheit geraten und durch andere Substanzen (Thiouracil usw.) mit erhöhter Wirkung ersetzt worden.

Die Eignung dieser Substanz zur Behandlung der Hyperthyreose beruht auf der spezifischen Beeinflussung des Jodhaushaltes im Organismus. In der Tat zieht diese Substanz das Jod entsprechend der Formel

$$2\,Na_2S_2O_3 + 2\,I = 2\,NaI + Na_2S_4O_6$$

an sich; sie vermindert bei stets gleichbleibender Jodzufuhr dadurch den Jodgehalt des Blutes, der Schilddrüse und erhöht die Jodausscheidung im Harn und im Stuhl. Na-thiosulfat verhindert somit durch den hervorgerufenen Jodmangel die Synthese des aktiven Schilddrüsenhormons.

Der Mangel des aktiven Schilddrüsenhormons ruft im Organismus eine Mehrleistung des Hypophysenvorderlappens hervor. Dieser Mehrleistung entspricht in morphologischer Hinsicht eine Hyperplasie der thyreotropen Zellen und in funktioneller Hinsicht eine gesteigerte Bildung und Ausschüttung thyreotropen Hormons. Dieses ruft das histologische Bild einer aktiven Schilddrüse hervor. Die Schilddrüse ist makroskopisch durch die Zunahme des Organvolumens, infolge Neubildung von Follikeln und durch die dunkelrote Farbe des Organs, infolge der Mehrdurchblutung gekennzeichnet. Mikroskopisch erkennt man eine Verminderung des Kolloids, eine Änderung der Form und der Weite der Follikel. Die Zellen des Follikelepithels zeigen Volumsvermehrung und nehmen zylindrische Formen an.

Eingegangen: 28. 1. 1953.

Schweiz. Zeitsch. Allg. Pat

Ich habe diese Studien in der letzten Zeit an der Rattenschilddrüse wiederholt (1952) und die histomorphologischen Untersuchungen auf andere Organe ausgedehnt (1953 b). Gleichzeitig habe ich an ihnen histochemische Untersuchungen (Phosphatasennachweis) durchgeführt (1953 a).

Im Laufe dieser Arbeiten bin ich zu interessanten Ergebnissen gelangt, die bereits an anderer Stelle (1953 b) beschrieben sind. Es sei hier eine kurze Betrachtung der Gefäßwandveränderungen angeschlossen.

Technik.

Als Versuchstiere dienten männliche Ratten. Es wurden 2 Gruppen von je 4 Tieren untersucht. Die Vorbehandlungszeit der ersten Gruppe betrug 4 Wochen, die der zweiten Gruppe 3 Monate. Jedes Tier erhielt Na-thiosulfat 0,05 g pro die intramuskulär.

Nach der Behandlungszeit werden die Tiere mit je einem Kontrolltier durch Nackenschlag getötet und die entfernten Organe in die Fixierungslösungen gegeben.

Die histochemische Darstellung der alkalischen Glycerophosphatase erfolgte nach der von *Meier* (1950) modifizierten Originalmethode *Gomoris* (1939, 1941, 1946).

Zum histomorphologischen Studium werden die üblichen Färbemethoden nach Fixierung in Alkohol durchgeführt.

Betrachtungen der Ergebnisse.

1. *Nachweis der alkalischen Glycerophosphatase in Schilddrüse und Nebennierenrinde.*

Die Schilddrüse und die Nebennierenrinde zeigen die deutlichsten histochemisch erfaßbaren Kapillarwandveränderungen, die bereits 4 Wochen nach der Pharmakonverabreichung ersichtlich sind. Abgesehen von der durch Na-thiosulfat hervorgerufenen Gefäßerweiterung in der Schilddrüse, wie sie allen Antithyroid-Stoffen zukommt, sei, im Vergleich zum Kontrollorgan, auf die deutliche Verminderung der Reaktionsintensität der alkalischen Glycerophosphatase in den Kapillarwänden hingewiesen. Zur Erklärung dieser Reaktionsintensitätsverminderung wird die hormonale Umstellung oder die bestehende Gefäßdilatation herangezogen. Die Wand würde somit ausgedehnt und verdünnt und die Reaktion auf größeren Raum verteilt. Nach Thiouracilbehandlung sind die hormonale Umstellung und die Dehnung und Verdünnung der Gefäßwände viel ausgesprochener als nach Na-thiosulfat-Einwirkung; die Intensität der alkalischen Phosphatasereaktion je-

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doch ist erhöht (*Kroon*, 1949; *Steger*, 1950). Man muß somit mit Recht an eine spezifische Wirkung des Pharmakons (Na-thiosulfat) auf die Gefäßwand denken.

In der Nebennierenrinde beobachtet man nach Verabreichung von Na-thiosulfat eine Erweiterung der Kapillaren. *Li Voti* (1949) hat nach Thiouracilgaben dasselbe Bild erhalten und die Gefäßerweiterung als Ausdruck einer verminderten Aktivität des Organs angesehen. Das histochemische Bild der gedehnten Gefäßwände entspricht dem der Schilddrüse; es besteht eine Verminderung der Reaktionsintensität der alkalischen Glycerophosphatase. Dieser Befund steht im Gegensatz zu dem nach Thiouracilbehandlung erhaltenen, bei dem die kapillaren Gefäße stärker erweitert und die Wände stärker verdünnt sind und die Reaktionsintensität der alkalischen Phosphatase leicht vermehrt ist. Auch dieser Befund läßt den Schluß einer spezifischen Wandschädigung durch Na-thiosulfat zu.

2. *Nachweis der Gefäßwandveränderungen mit den üblichen Färbemethoden.*

Nach länger dauernder Behandlung mit Na-thiosulfat (3 Monate) sind deutlich Gefäßveränderungen in der Niere erkennbar. Die Glomerula sind atrophisch, hyalin, ausgesprochen zellarm. Die Kapillarschlingen nehmen nur mehr teilweise den Raum der Bowmanschen Kapsel ein. Die einzelnen Schlingen sind nicht mehr zu differenzieren, da sie von einer homogenen, eosinophilen Substanz durchtränkt sind. Diese Substanz färbt sich nach der Malloryschen Methode bläulich an.

Die proximalen Nierentubuli sind zum Teil stark erweitert und von einem Epithel ausgekleidet, das sehr stark abgeflacht ist und einem Endothel ähnlich sieht. Diese erweiterten Tubuli enthalten ebenfalls die bereits genannte homogene eosinophile Substanz. Die peripheren Nierentubuli hingegen zeigen keinerlei morphologisch erfaßbaren Veränderungen. Daraus folgt, daß das angewandte

Fig. 1. Niere nach 3 Monate dauernder Behandlung mit Natrium-thiosulfat. Durch die Gefäßwandveränderungen sind die Glomerulumschlingen von einer homogenen, eosinophilen Substanz durchtränkt und die proximalen teilweise erweiterten Tubuli von derselben erfüllt.

Fig. 2. Leber nach 3 Monate dauernder Behandlung mit Natrium-thiosulfat. Verbreiterung der interstitiellen Räume. Vermehrung der Kupfferschen Sternzellen im Leberparenchym.

Fig

Fig

Pharmakon die Kapillarschlingen des Glomerulum lädiert, so daß diese für Plasmasubstanzen durchlässig werden. Zudem hat es eine schädigende Wirkung in jenem Gebiet des Kanälchensystems, welches für die Sekretion und Resorption von Stoffen besondere Bedeutung hat (Fig. 1).

Die Nierenarteriolen sind zum Teil erweitert, zum Teil weisen sie eine Endothelzellwucherung auf. Dadurch wird das Lumen eingeengt oder vollständig obliteriert. Die gewucherten Endothelzellen sind von der bereits erwähnten plasmatischen Substanz durchtränkt.

Im Leberparenchym besteht eine Vermehrung der interstitiellen Flüssigkeit. Diese ist am deutlichsten längs der Leberzellbalken, von denen die Kapillaren an einigen Stellen abgehoben sind, erkennbar. Es besteht auch in diesem Organ das Bild eines vermehrten Flüssigkeitsdurchtrittes durch die Kapillarwände auf Grund der Pharmakonwirkung. Kurz möchte ich noch an die bereits beschriebenen, durch Na-thiosulfat hervorgerufenen Befunde (1953 b) einer Vermehrung der Kupfferschen Sternzellen im Leberparenchym und des Bindegewebes um die Zentralvene und in den Kiernanschen Scheiden hinweisen (Fig. 2).

Diskussion.

Die durch Na-thiosulfat verursachten Läsionen beziehen sich auf die Gefäßwände, besonders der Kapillaren, im Sinne einer erhöhten Wanddurchlässigkeit. Es wird der Blutflüssigkeit vermehrter Übertritt in die Gewebe gestattet. In der Niere jedoch werden durch elektive Störung des Filters plasmatische Substanzen in das Tubulussystem ausgeschieden; dies wird am besten mit dem Ausdruck Plasmurie gekennzeichnet.

Natriumthiosulfat bewirkt zudem eine deutliche Verminderung der Reaktionsintensität der alkalischen Glycerophosphatase in den Kapillarwänden verschiedener Organe. Dies wurde auf Grund vergleichender Beobachtungen mit Organen normaler Ratten und solcher von mit Thiouracil behandelten Tieren festgestellt. Das Pharmakon hat in den Kapillarwänden eine elektive Wirkung.

Die Gefäßerweiterung der endokrinen Organe ist nach *Li Voti* (1949) Ausdruck einer verminderten Aktivität derselben. Es fragt sich nun, ob mit der verminderten Aktivität der Organe auch eine solche der Gefäßwände parallel geht, wodurch die verminderte

Reaktionsintensität der alkalischen Glycerophosphatase, abgesehen von der Gefäßdilatation und der Wandverdünnung, erklärt werden könnte. Die Befunde nach Thiouracilbehandlung sprechen dieser Annahme entgegen. Die Organaktivität ist nach Thiouracileinfluß stärker vermindert als nach Na-thiosulfat, und die Kapillaren sind dilatierter, ihre Wände verdünnter; trotzdem besteht eine Zunahme der histochemischen Reaktion.

Erhöhte Wanddurchlässigkeit und verminderte Reaktionsintensität der alkalischen Phosphatase gehen als Ausdruck der Pharmakonwirkung parallel. Der Angriffspunkt liegt wahrscheinlich in den Endothelzellen oder in der Kittsubstanz zwischen denselben, denn nur auf diese Weise ist die Permeabilitätssteigerung erklärlich. Das histologische Bild erinnert an die seröse Entzündung *Roeßles*. Ich möchte in einer späteren Arbeit nach Berücksichtigung weiterer Studien auf dieses Problem näher eingehen.

Was den Aktionsmechanismus des Natrium-thiosulfats anbetrifft, so können verschiedene Möglichkeiten ins Auge gefaßt werden:

a) Sind die durch das Pharmakon hervorgerufenen Veränderungen Ausdruck einer hormonalen Gleichgewichtsstörung, im speziellen zwischen Hypophyse und Schilddrüse?

b) Verursacht das verabreichte Pharmakon eine Änderung des Schilddrüsenhormons in seiner chemischen Zusammensetzung, so daß als Ausdruck einer Dysthyreose die Gefäßwandschädigung zu erklären ist?

c) Hat das Pharmakon eine spezifische toxische Wirkung auf die Gefäßwände, speziell der Kapillaren?

Auf Grund meiner bisherigen Untersuchungen möchte ich am ehesten letzteren Punkt ins Auge fassen. Es liegen für die beiden ersten Möglichkeiten zu wenig Beweise und Gegenbeweise vor. Ich hoffe jedoch, in nächster Zeit einige Beiträge für meine bisherigen Annahmen liefern zu können.

Zusammenfassung.

Nach Verabreichung von Natrium-thiosulfat konnten typische Gefäßwandläsionen, speziell der Kapillaren, festgestellt werden. Die Läsionen sind erkenntlich einerseits an einer Durchlässigkeitssteigerung der Gefäßwände für Blutflüssigkeit und plasmatische Substanzen, andererseits an der Verminderung der Intensität der alkalischen Phosphatasereaktion der Gefäßwände.

Résumé.

Des lésions des parois vasculaires, en particulier des capillaires, ont pu être mises en évidence après l'administration de thiosulfate de sodium. Ses lésions sont caractérisées par une augmentation de la perméabilité pariétale au liquide sanguin et aux substances plasmatiques d'une part, par une diminution d'intensité de la réaction alcaline de la phosphatase des parois vasculaires d'autre part.

Summary.

Administration of sodium thiosulfate elicits typical lesions of the blood vessels, especially of the capillaries, which results in increased permeability of the vessel walls for plasma and reduced intensity of their alkaline phosphatase reaction.

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30b
thiosulfate

Sodium Thiosulfate, a Substance Injurious to the Walls of the Vascular System

C. Steger

The use of sodium thiosulfate in the treatment of hyperthyroidism goes back to Biancalana (1934, 1935, 1936 and so on). This drug, however, for this purpose, has fallen into oblivion in later years and has been replaced by other substances (thiouracil, and so forth with heightened activity.

The qualification of this substance to treat hyperthyroidism is based on the specific influence on the iodine content in organisms. Actually, this substance affects iodine according to the formula $2\ Na_2S_2O_3 + 2\ I = 2\ NaI + Na_2S_4O_6$. With a constant invariable iodine supply, it diminishes the iodine content of the blood and thyroid gland in this way and elevates the excretion of iodine in urine and feces. Na-thiosulfate prevents the synthesis of the active thyroid hormone through the iodine deficiency which it brings about.

The deficiency of active thyroid hormone puts an overload on the anterior lobe of the pituitary of the organism. This overload, in regard to morphology, corresponds to a hyperplasia of the thyrotropic cells, and in regard to function corresponds to an increased formation and excretion of thyrotropic hormone. The thyroid is characterized macroscopically by an increase in organ volume as a consequence of the new follicle development and by the dark red color of the organ due to increased flow of blood. A reduction in colloidin can be detected microscopically by a change in form and diameter of the follicles. The follicular epithelial cells display an increase in volume and assume a cylindrical shape.

I have repeated these studies recently on the thyroid glands of rats (1952) and extended the histomorphological investigations to other organs. I have simultaneously carried out histochemical investigations on these (phosphatase determinations) (1953 a). Here might be added a short reflection on the changes in the walls of the vascular system.

METHOD

Male rats served as experimental animals. 2 groups of 4 animals were investigated. The pretreatment period for the first group was 4 weeks, with 3 months for the second group. Each animal received 0.05 g of Na-thiosulfate pro die intramuscularly.

After the period of treatment the animals, each with a control, were sacrificed with broken necks, and the organs removed were placed in a fixing solution.

The histochemical preparation of alkaline glycerophosphatase followed the original method of Gomoris (1939, 1941, 1946) as modified by Meier (1950).

The usual staining methods were carried out for the histomorphological studies, after alcoholic fixation.

ANALYSIS OF THE RESULTS

1. DETERMINATION OF THE GLYCEROPHOSPHATASE IN THE THYROID GLAND AND ADRENAL CORTEX

The thyroid gland and the adrenal cortex showed distinct histochemically detectable changes in the capillary walls, which are obvious 4 weeks after the drug administration. Apart from the vascular dilation in the thyroid gland brought about by Na-thiosulfate, as occurs with all antithyroid substances, there might be indicated, in comparison with the organs of the controls, a distinct reduction in the reaction intensity of alkaline glycerophosphatase in the capillary walls. To explain this decrease in reaction intensity, the hormonal reversal or the existing dilation in the blood vessel walls is called upon.

The walls may be extended and weakened and the reaction diffused over a greater area. Following treatment with thiouracil the hormonal reversal and the dilation and weakening of the vascular walls are more pronounced than after the action of Na-thiosulfate; the intensity of the alkaline phosphatase reaction is therefore elevated (Kroon, 1949; Steger, 1950). With reason, a specific action by the drug (Na-thiosulfate) on the vascular walls must be considered.

A dilation of the capillaries of the adrenal cortex was observed after administration of Na-thiosulfate. Li Voti (1949) got the same picture after thiouracil administration, and viewed the vascular dilation as an expression of diminished activity of the organ. The histochemical picture of the dilated vessel walls corresponds to that of the thyroid gland; it consists of a reduction in the reaction intensity of the alkaline glycerophospotase. This finding is contrary to that obtained after thiouracil treatment, with which the walls are further dilated and more greatly weakened and the intensity of the alkaline phosphatase reaction is slightly reduced. This finding also admits the conclusion of a specific injury to the walls by Na-thiosulfate.

2. DETERMINATION OF CHANGES IN THE WALLS OF THE VASCULAR SYSTEM WITH THE USUAL STAINING METHODS

After an extensive treatment with Na-thiosulfate (3 months), distinct changes in the vascular walls are discernible in the kidney. The glomeruli are atrophic, hyaline and expressly diminished in cells. The capillary loops only partially occupy the space in Bowman's capsule. The individual loops can no longer be differentiated, since they are infiltrated with an eosinophilic substance. This substance stains blue by Mallory's method.

The proximal tubules of the kidneys are very dilated in part and coated by an epithelium that is very flattened and appears similar to an endothelium. In all cases these dilated tubules contain the homogenous eosinophilic substance already mentioned. The peripheral tubules of the kidneys, on the contrary, show no detectable morphological changes. It follows, therefore, that the drug used damages the capillary loops of the glomerlum, so that it is permeable to plasma substances. Besides, it has an injurious effect in that area of the system which has a special significance for the secretion and resorption of substances (fig. 1).

The kidney arterioles are somewhat dilated, and exhibit in part an endothelium proliferation. From this, the lumen is narrowed or completely obliterated. The proliferated endothelial cells are infiltrated by the plasma substances already mentioned.

In the liver parenchyma there is a reduction of interstitial fluid. This is most clearly recognizible along the liver cell bands, from which the capillaries rise at several places. There is also a picture in this organ of a decreased fluid permeability through the capillary walls because of the drug effect. I would like briefly to refer to the findings of an increase in the Kupffer stellate cells in the liver parenchyma, and of the connective tissue around the central vein, and in Kiernan's space (Fig. 2), brought on by Na-thiosulfate and already described (1953 b).

DISCUSSION

The lesions caused by Na-thiosulfate are related to the walls of the vascular system, especially the capillaries, in the sense of an elevated permeability of the walls. This allows the blood flow an enlarged passage into the tissues. In the kidney, through a selective disturbance of the filters, plasma substances were excreted into the tubular system; this is best characterized by the expression plasmauria.

Sodium thiosulfate causes a distinct reduction in the reaction intensity of alkaline glycerophosphotase in the capillary walls of various organs. This was

Fig. 1.

Fig. 1. Kidney after a three month period of treatment with sodium thiosulfate. From the changes in the walls of the vascular system, the glomerular loops are infiltrated with a homogenous eosinophilic substance and the proximal partially distended tubules are impregnated with the same material.

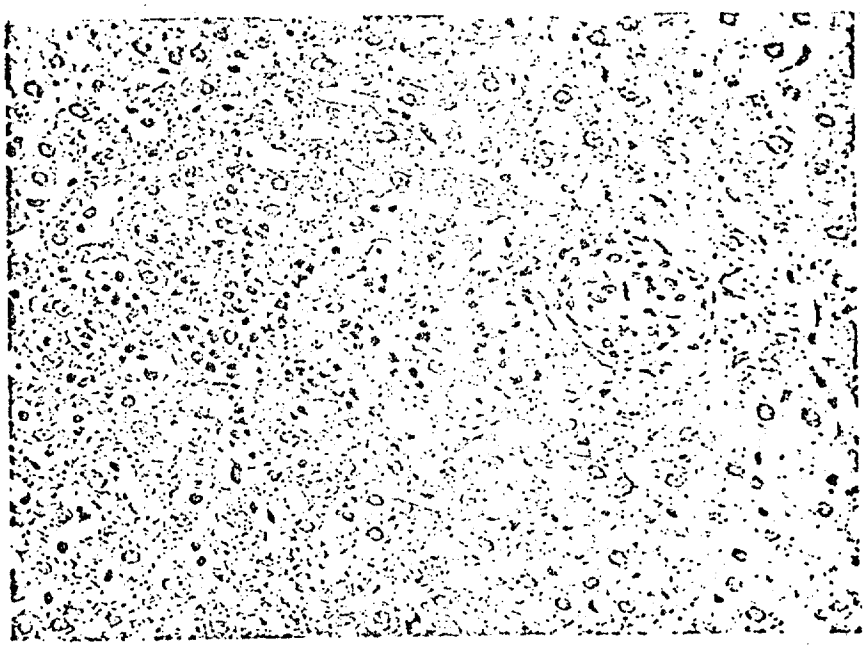

Fig. 2.

Fig. 2. Liver after a three month period of treatment with sodium thiosulfate. Spreading of the interstitial spaces. Decrease of Kupffer stellate cells in the liver parenchyma.

This was established on the basis of comparing observations on the organs of normal rats and those animals treated with thiouracil. The drug has a selective action on the capillary walls.

The dilation of the vessels of the endocrine organs is, according to Li Voti (1949), an expression of diminished activity. The question now is, whether there is a parallel between the diminished activity of the organs and the vascular walls, by which the diminished reaction intensity of alkaline glycerophosphotase can be explained, apart from dilation of the vessels and weakening of the walls. The findings following thiouracil treatment are in opposition to this assumption. The activity of the organs is decreased further than after Na-thiosulfate, the capillaries are more dilated and their walls are weaker; in spite of this there is an increase in the histochemical reaction.

Elevated permeability of the walls ans diminished reaction intensity of alkaline phosphatase are expressions of the parallel action of the drug. The point of attack probably lies in the endothelial cells or in the connective material between them, for the elevated permeability can only be explained in this manner. The histological picture is reminiscent of the serous inflammation of Roessles. After consideration of further studies on this problem, I would like to go into it in greater detail in later work.

Various possibilities can be borne in mind as to the action mechanism of sodium thiosulfate:

a) Are the changes brought about by the drug an expression of hormonal imbalance, particularly between the pituitary and thyroid glands?

b) Does administration of the drug cause a change in the chemical composition so that the damage to the vascular walls can be explained as an expression of thyroid disfunction?

c) Has the drug a specific toxic effect on the vascular walls, especially on the capillaries?

On the basis of my investigations till now, I would bear this last point in mind chiefly. There is too little evidence and counterevidence for the first two possibilities. I hope, therefore, the next time, to make some contributions to what, until now are my assumptions.

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relatively simple method for evaluating the action of *SU* 4885 where interpretation of results from determination of total corticosteroids may be ambiguous.

Recently, Butt *et al.*[7] have reported on a similar fractionation technique to investigate the effect of *SU* 4885. They utilized silica-gel chromatography to effect separation of the ketosteroids. Our results support the claim of Butt *et al.*[7] that the 'fractionation' method has greater sensitivity than the total 17-ketogenic steroid method.

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The Metabolic State of Thiosulphate

OUR earlier experimental work using labelled thiosulphate has shown that the two atoms of sulphur in this compound have different fates in the course of metabolism in the animal organism[1]. In rats, the inner sulphur atom is eliminated very quickly from the organism in the form of sulphate, while the outer atom is transformed into the sulphate much more slowly, going probably through a number of intermediate stages. The results of our investigations have shown that the thiosulphate may be the metabolic precursor of the sulphate, and that, however small its concentration in rat urine may be, the endogenous pool of thiosulphate is large (ref. 1b).

In the work reported here, rats were injected subcutaneously with 0·8 mgm. of D,L-cystine labelled with sulphur-35 dissolved in 0·5 per cent sodium carbonate, and at the same time with 200 mgm. non-radioactive sodium thiosulphate ($Na_2S_2O_3.5H_2O$). The dose of thiosulphate was repeated after 24 hr. The urine was collected quantitatively at set intervals of time. As a control, we used rats to which radioactive cystine had been given without the simultaneous administration of thiosulphate. The thiosulphate excreted in the collected urine was separated and estimated quantitatively by the method of Cast *et al.*[2], the residual sulphate was removed by adding barium chloride, and the remaining sulphur compounds which were neither thiosulphate nor inorganic sulphate were treated as residual sulphur. The total activity of a given sample of urine, the activity of the thiosulphate, and that of residual sulphur were determined. The activity of the inorganic sulphate was calculated indirectly from the difference.

Table 1. ACTIVITIES OF EXCRETED THIOSULPHATE, SULPHATE AND RESIDUAL SULPHUR

Results are expressed as a percentage of total activity of urine, after injection of 0·8 mgm. of cystine-^{35}S (4 animals)

Time (hr.)	Activity of thiosulphate		Activity of sulphate		Activity of residual sulphur	
	Range	Average	Range	Average	Range	Average
0–6	1·79–2·28	2·1	46·5–78·5	71·8	19·3–31·4	26·0
6–24	1·88–3·22	2·47	55·7–77	67·4	20·7–44·5	30·1
24–48	2·45–3·40	2·64	51·2–68·8	62·6	28·1–15·5	31·2
48–72	1·69–2·71	2·36	55–72	60·8	26·4–43·5	30·5

Table 2. ACTIVITIES OF EXCRETED THIOSULPHATE, SULPHATE AND RESIDUAL SULPHUR

Results are expressed as a percentage of total activity of urine, after injection of 0·8 mgm. cystine-^{35}S and 200 mgm. $Na_2S_2O_3.5H_2O$. The dose of $Na_2S_2O_3.5H_2O$ was repeated after 24 hr. (6 animals)

Time (hr.)	Activity of thiosulphate		Activity of sulphate		Activity of residual sulphur	
	Range	Average	Range	Average	Range	Average
0–6	38·5–53·2	43·6	31·7–51·8	44	12·0–15·0	13·5
6–24	1·9–19·5	7·6	56·7–77·0	65·5	21·2–30·0	25·4
0–24	29–41	33	47–52	49	12–19	18
24–48	10·2–30·9	18·95	30·0–60·4	51	19·7–39·2	28
48–72	2·5–7·3	5·3	42·5–57·7	52	37·0–52·5	42·2

Table 1 shows the proportion of radioactivity of the different fractions of sulphur in urine after giving rats cystine alone. Table 2 similarly illustrates the results of experiments when both radioactive cystine and non-radioactive thiosulphate were injected.

As may be seen from Tables 1 and 2 only an insignificant percentage of activity is excreted in the form of thiosulphate after the injection of labelled cystine without the addition of thiosulphate. On the other hand, if at the same time a massive dose of unlabelled thiosulphate is given, 33 per cent of the sulphur produced by the metabolism of cystine is excreted in the urine in the form of thiosulphate in the course of 24 hr. Even after the next 48 hr., considerable quantities of radioactive thiosulphate are excreted when additional thiosulphate has been injected.

Our explanation of these facts is that external introduction of a large dose of thiosulphate inhibits the oxidation of the endogenic thiosulphate. We assume that thiosulphate given from an external source mixes with the pool of endogenous thiosulphate, and in consequence both the excretion and the oxidation of the endogenous thiosulphate take place at the same rate as that of the thiosulphate administered from an external source. A scheme of the relation between the exogenous and endogenous thiosulphate is given in Fig. 1, where $*A_e$ is the sulphur of the endogenous thiosulphate; $*A$ is the sulphur of the endogenous thiosulphate excreted in the urine in the form of thiosulphate; $*B$ is the sulphur in the

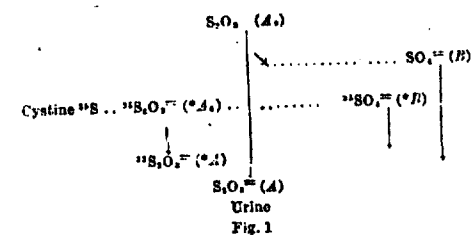

Fig. 1

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sulphates originating from the radioactive cystine excreted in urine; A_0 is the sulphur of the injected thiosulphate; A is the sulphur of the injected thiosulphate excreted in the urine in the form of thiosulphate; B is the sulphur in the sulphates originating from the injected thiosulphate excreted in urine.

Quantitatively, if the endogenous and exogenous thiosulphate behave in an identical manner, the following relation would be valid: $*A_0/*A = A_0/A$.

We had previously estimated[1] that under normal conditions the rat excretes 0·05–0·1 mgm. of sulphur in the form of the thiosulphate and about 3 mgm. of sulphate sulphur during 24 hr.; that is, the ratio of these two kinds of sulphur may be expressed numerically as 0·01–0·03. The same relation exists between activity of thiosulphate and sulphate after injection of labelled cystine alone (Table 1). After injecting rats with labelled thiosulphate, we showed that the relation of excreted thiosulphate sulphur to the sulphate sulphur (A/B) is given by the average values 0·61, 0·19 and 0·53 for periods 0–6, 6–24 and 0–24 hr. respectively.

If we assume that thiosulphate is the normal precursor of the sulphate and that the small quantity in the urine is only the result of its rapid oxidation in the animal organism, after the injection of thiosulphate the ratio of the sulphur of the endogenous thiosulphate to the sulphur of the endogenous sulphate should be approximately the same as that of the introduced thiosulphate sulphur to the sulphate originating from this: $*A/*B = A/B$. Taking the figures from Table 2, we obtain the following values of $*A/*B$: 0·99, 0·11, 0·67 for periods 0–6, 6–24 and 0–24 hr. respectively.

The figures obtained for $*A/*B$ as compared with values of A/B lie in the range of experimental deviation and these results indicate with great probability that the endogenous thiosulphate behaves like the exogenous, and hence it is the fundamental precursor of the sulphate originating in the organism of the rat.

A full description of the experiments and calculations will be given elsewhere.

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Polysaccharides of *Mycobacterium phlei*

INVESTIGATIONS of the polysaccharides of *Mycobacterium phlei* have been few and mainly concerned with those secreted into the culture medium. Thus Masucci and McAlpine[1] found that a polysaccharide which they isolated from culture filtrates contained D-mannose and D-arabinose. Tennent and Watson[2] isolated a similar product and showed that the molecular weight was 7,300. In the present investigation the intracellular polysaccharides of *M. phlei* were studied.

The organisms were grown on a culture medium (10 litres) consisting of 'Oxoid' bacteriological peptone, 1 per cent; 'Lab. Lemco', 1 per cent; sodium chloride, 0·5 per cent, and glycerol, 5 per cent at 37° for 16 days. The cells were separated from the medium, washed with 0·14 *M* sodium chloride, suspended in phosphate buffer, *p*H 8, and disintegrated in batches of 250 ml. by shaking at 0° for 5 hr. with ballotini glass balls (1 mm. diameter) in a vibratory ball mill. The suspension of broken cells was diluted to 1 litre with phosphate buffer, *p*H 8, and then adjusted to *p*H 4 with dilute acetic acid. The suspension was centrifuged at 2,600 r.p.m. at 0° and the precipitate (cell debris, nucleoproteins, etc.) discarded. The supernatant liquids from the various batches were combined, concentrated to 1 litre by freeze-drying and then mixed with 5 volumes of ethanol. The precipitate so produced was centrifuged off, dissolved in distilled water, dialysed and freeze-dried to give a crude polysaccharide fraction (12 gm.), $[\alpha]_D^{20} + 164°$; N, 0·6 per cent; P, 0·2 per cent. Examination of acidic hydrolysates showed the presence of glucose and small amounts of mannose and arabinose. The presence of glycogen was indicated by the formation of a brown colour with iodine.

The crude polysaccharide (5·9 gm.) was fractionated further by the use of cetrimide ('Cetavlon', Imperial Chemical Industries Pharmaceuticals, Ltd.) in the presence of borate[3]. Four fractions were obtained: fraction I (30 mgm.) was precipitated at *p*H 7·5, and, after acid hydrolysis, contained arabinose, glucose, mannose and amino-acids; fraction II (4·5 gm.) was precipitated at *p*H 9·5 and contained only glucose; fraction III (40 mgm.) was precipitated at *p*H 10 and contained glucose and a trace of mannose, and fraction IV (0·6 gm.) did not precipitate with cetrimide–borate and contained mainly arabinose, a smaller amount of mannose, some glucose and a trace of ribose. Fraction IV was isolated by dialysing the supernatant liquid, freeze-drying and extracting the dry solid with chloroform to remove the cetrimide.

The major fraction (fraction II) was glycogen as shown by the infra-red spectrum (peaks at 930, 845 and 758 cm.$^{-1}$), which was identical with that of an authentic sample of liver glycogen examined at the same time[4], and by the production of a brown colour with iodine. The absorption curve of the iodine complex had $\lambda_{max.}$ 390 mμ. This was lower than that of other glycogens[4], for example, *Mytelus edulis*, 420 mμ, rabbit liver, 490 mμ, and *Bacillus megaterium*, 520 mμ. The intensity of absorption was also lower (E_{390} 0·27 with 0·1 per cent glycogen in 0·01 per cent iodine and 0·2 per cent potassium iodide). The $\lambda_{max.}$ was somewhat variable; another sample had $\lambda_{max.}$ 405 mμ. The glycogen fraction was homogeneous when examined by boundary electrophoresis in borate buffer *p*H 9·2. The polysaccharide also migrated towards the anode in veronal buffer *p*H 9·2, but much more slowly than in borate. This may have been due to the presence of a small number of phosphate groups (phosphorus content, 0·03 per cent).

The second major fraction (fraction IV) contained 3 per cent nitrogen, which was due to the presence of protein and a small amount of nucleic acid; no amino-sugars could be detected. The fraction was heterogeneous when examined by boundary electrophoresis, containing at least two components when examined in borate, *p*H 9·2, glycine, *p*H 9·8, and veronal, *p*H 8·6. The migration in the glycine and veronal buffers could be due to the presence of protein in the fraction. Attempts to remove the protein by precipitation with trichloroacetic acid or by denatura-

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THROMBOANGIITIS OBLITERANS

TREATMENT WITH SODIUM TETRATHIONATE AND SODIUM THIOSULFATE

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Thromboangiitis obliterans is a clinical and pathologic entity characterized by peripheral arterial thromboses of obscure origin.[1] On the basis of our investigative work, we have concluded that thromboangiitis obliterans in the majority of our cases is associated with the following factors: (*1*) changes in the blood; (*2*) disturbed tissue metabolism; (*3*) arterial and venous thromboses, which occur most frequently in the peripheral vessels but may occur in any part of the body, and (*4*) local infection in the involved extremity. Until the complex adjustments which compensate for pathologic conditions of the blood are better understood, it will be difficult to detect the disease before serious circulatory deficiency results from arterial thrombosis. Infection, infarcts or gangrene frequently directs attention to the circulatory disease.

Various changes in the blood have been reported since Koga[2] first, in 1913, reported increased viscosity. In cases of acute involvement we have reported increased viscosity of the blood, rapid sedimentation of the cells, rapid coagulation, increased alkalinity and arterial-like oxygen saturation of the superficial venous blood from the involved extremity.[3] In improved or recovered patients with thromboangiitis obliterans and in patients with other peripheral circulatory diseases these conditions

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of the blood were not present. These findings indicated that there is a relation between the stage of the disease and the complex factors of the pathologic physiology of the blood. To evaluate the importance of changes in the blood, observations should be made while thromboses are forming and during the acute stage of the disease.

Our study of the blood in thromboangiitis obliterans included an investigation of the changes produced by smoking. We reported [4] that smoking by patients with thromboangiitis obliterans generally results in decreased peripheral cutaneous temperatures, increased pulse rate, elevated blood pressure, decreased oxygen saturation of the arterial blood, decreased oxygen saturation of the venous blood in patients with acute thromboangiitis obliterans and increased oxygen saturation in the improved patients and in normal controls. Most of the responses seem to be physiologic protective adjustments to reduced oxygen saturation of the arterial blood. Failure of compensatory adjustment to heavy smoking in some persons may be a cause of thromboangiitis obliterans leading to thrombosis.

Maddock and Coller [5] reported that nicotine is the important ingredient of tobacco and that the deleterious effect of smoking is due to the pharmacologic action of nicotine on the peripheral sympathetic nerves. On the contrary, we found that the specific action of smoking is not entirely on the sympathetic nerves. We observed a decline in temperature of 4 degrees (C.) on the sympathectomized fingers after smoking in a case of Raynaud's disease. Similar response to epinephrine was observed on sympathectomized extremities in cases of Raynaud's disease by Smithwick, Freeman and White.[6] These observations suggest that the effect of smoking on the pulse, blood pressure and peripheral temperatures is due to substances in the circulating blood in addition to sympathetic vasomotor stimulation of the peripheral vessels.

In the treatment of thromboangiitis obliterans we have had considerable clinical success with sodium tetrathionate ($Na_2S_4O_6 . H_2O$) [7] and sodium thiosulfate ($Na_2S_2O_3 . 5 H_2O$). This treatment was directed

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7. Sodium tetrathionate has been prepared for us experimentally and supplied by G. D. Searle and Company, of Chicago.

toward improvement of the condition of the blood. The following facts seem pertinent: 1. Pathologic changes in the blood are present during the acute stage of the disease. 2. Clinically, smoking is associated with an acute attack of the disease, and we believe that heavy smoking may contribute to the changes in the blood. 3. Sodium tetrathionate and sodium thiosulfate are of clinical value in the treatment of the disease. We are reporting the results of our investigation of the effect of these solutions on various changes in the blood and physiologic responses which are affected by smoking. The two solutions are referred to as the inorganic sulfur compounds.

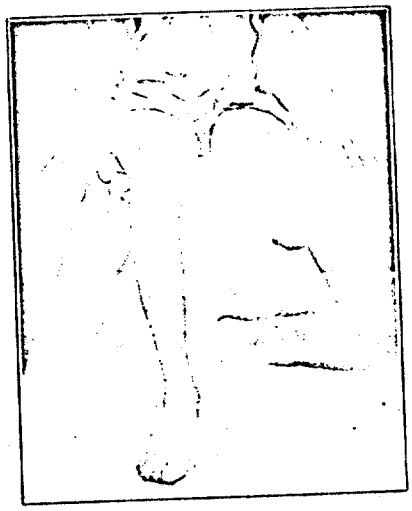

Fig. 1.—Patient with thromboangiitis obliterans. The blood picture in his case is given in table 2. The left leg was amputated in 1935. There was recurrence of the disease in the right foot in 1936.

METHOD OF INVESTIGATION

The methods of obtaining peripheral temperatures, pulse rate and blood pressure and of collecting and analyzing the blood were the same as those used in our previous work.[8] Determinations of glutathione were made by the method of Woodward and Fry.[9]

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After the period of stabilization of the subject at rest in bed and initial observation of peripheral temperatures, pulse rate and blood pressure, specimens of arterial blood from the right radial artery or of venous blood from the right antecubital vein were obtained under oil and immediately transferred to mercury tonometers. Injections of sodium tetrathionate or sodium thiosulfate in 10 cc. of triple-distilled water were given in the left antecubital vein, and ten minutes later specimens of blood were again withdrawn from the right side. The various observations mentioned were repeated under the same resting conditions after the injection.

RESULTS

Peripheral Temperatures.—The average temperature of the fingers and toes was usually increased after intravenous injection of either solution. Figure 2 shows that the inorganic sulfur compounds had

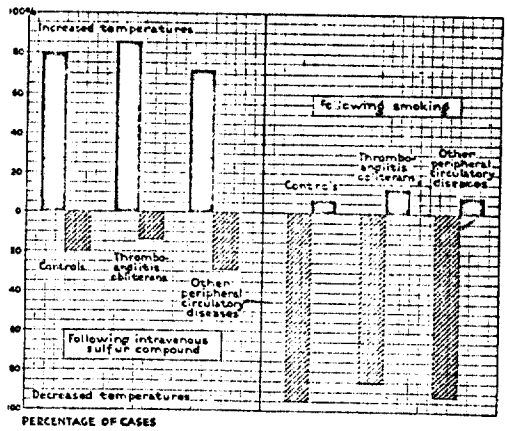

Fig. 2.—Changes in peripheral temperature of the skin. The inorganic sulfur compounds produced the opposite effect on the temperatures to that which occurred after smoking. Increased temperature is indicated by solid black; decreased temperature, by diagonal lines.

an action that was the opposite of smoking. The more rapid and higher elevations in temperatures were produced by the sodium thiosulfate solution, but the effect was of shorter duration than with sodium tetrathionate. Decreased temperatures were occasionally observed, especially in nervous patients, probably as a result of the puncture.

Cutaneous temperatures were more constantly increased when no element of vasoconstriction was present. In completely sympathectomized extremities or when block of the peripheral nerves with procaine hydrochloride had demonstrated organic circulatory disease, the inorganic sulfur compounds still produced an increase in cutaneous temperature of 0.5 to 1 degree (C.). Since vasomotor action was no

longer present in these cases, the increased cutaneous temperatures were produced through the blood stream, probably as a result of increased local tissue metabolism.

Blood Pressure.—The extent of the reduction in blood pressure which frequently followed injection of inorganic sulfur compounds in patients with thromboangiitis obliterans depended on the initial pressure. High initial levels responded with a greater reduction in pressure. In the acute stage of the disease the blood pressure was not elevated; consequently a less marked reduction occurred after injection. The reduction varied from 8 to 40 mm. of mercury in different patients and also in the same patient on different days. The diastolic pressure was less affected than was the systolic, although the two were occasionally affected to the same extent.

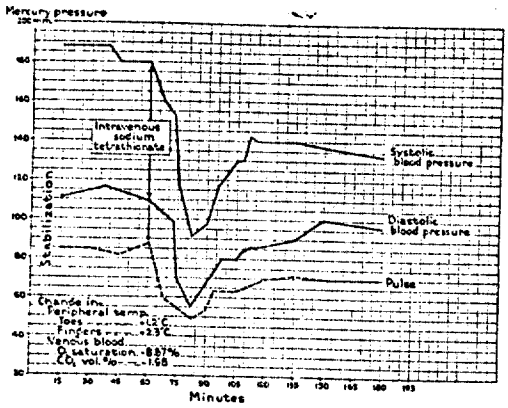

Fig. 3.—Effect of inorganic sulfur on a patient with essential hypertension and peripheral circulatory symptoms. Marked symptomatic relief accompanied reduction in the systolic and diastolic blood pressures and in the pulse rate after intravenous injections of 0.4 Gm. of sodium tetrathionate. Bimonthly and then monthly injections over a period of eighteen months have maintained the subjective and objective improvement.

Hypertension with peripheral circulatory symptoms (fig. 3) may be benefited by intravenous injections of the sulfur compounds to the same degree as the circulatory diseases with hypertension, especially when smoking is a cause of the elevated pressure (fig. 4). The immediate reduction in pressure which followed injection was almost always transitory, but occasionally with subsequent injections the pressure remained at a lower level. Sodium tetrathionate produced a more constant and longer-lasting effect in lowering the pressure than did sodium thiosulfate.

Pulse Rate.—The pulse rate was usually slower after injection of the solutions, especially when the initial rate was higher than normal. With a rate of 80 or 90 per minute, a drop to 70 or less was not uncom-

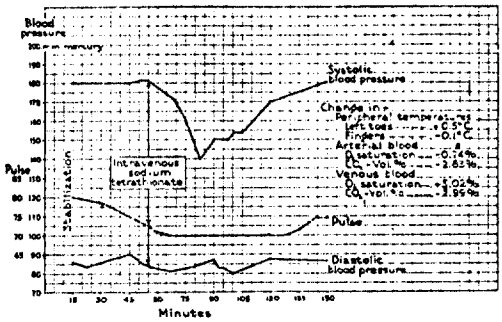

Fig. 4.—Effect of inorganic sulfur on a clinically well patient with thromboangiitis obliterans and hypertension who continued to smoke two or more packages of cigarets daily. The right leg had been amputated. In addition to the marked reduction in blood pressure and slowing of the pulse rate, insignificant changes occurred in the peripheral temperatures and in the arterial oxygenation after injection of 0.6 Gm. of sodium tetrathionate. Clinical improvement has been maintained for four years by biweekly injections, despite heavy smoking.

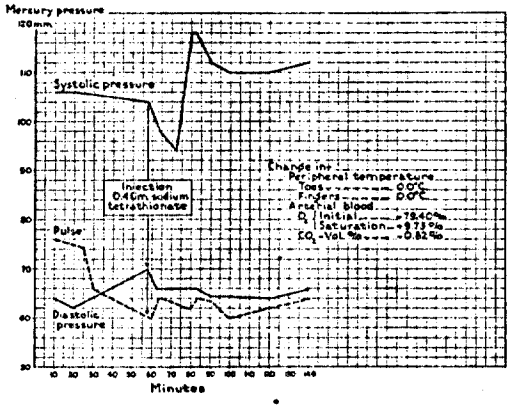

Fig. 5.—Effect of inorganic sulfur on a patient with Raynaud's disease. The initial oxygen saturation of the arterial blood was 79.40 per cent. Normally, in this vicinity the oxygen saturation is from 92 to 95 per cent. After injection of 0.4 Gm. of sodium tetrathionate, elevation of the initial low blood pressure was accompanied by an increase of 9.73 per cent in oxygen saturation of the arterial blood and by a reduction in the pulse rate.

mon. The effect of the inorganic sulfur compounds on the pulse rate was of longer duration than its effect on the blood pressure.

In 2 cases of Raynaud's disease with initial low oxygenation of the arterial blood and low blood pressure, injection of the tetrathionate solution was followed by elevation of the blood pressure to a normal level, slowing of the pulse and increased oxygen saturation of the arterial

TABLE 1.—*Changes in the Oxygen and Carbon Dioxide Content of the Arterial Blood and in the Peripheral Cutaneous Temperatures Ten Minutes After Intravenous Injection of an Inorganic Sulfur Compound*

	Oxygen Capacity, Vols. %	Initial Oxygen Saturation, %	Change in Oxygen Saturation, %	Change in Carbon Dioxide, Vols. %	Change in Temperature	
					Toes	Fingers
Controls (normal)						
Average for 10 patients....	20.24	93.20				
Mr. A.....	20.96	93.91	—2.69	—1.34	—0.9°C.*	—0.5°C.*
Mr. K.....	18.20	94.40	+1.90	—3.40	—0.3°C.*	—0.7°C.*
Peripheral circulatory disease (thromboangiitis obliterans)						
Mr. P.....	19.16	87.50	+3.85	+0.62	—0.4°C.	+1.5°C.
Mr. S.....	16.86	91.89	+2.25	—2.13	+0.2°C.	+0.2°C.
Mr. K. 7/27/39.....	11.00	96.10	—8.70	—1.50	+1.3°C.	+0.4°C.
8/10/39.....	17.60	96.10	—5.80	—0.80	.....	.....
Mr. Z.....	11.68	94.00	.....	.....	.....	.....
Mr. B., 6/17/37.....	20.48	88.14	+1.05	—2.10	+0.4°C.	+1.3°C.
6/23/37.....	19.65	93.44	—3.24	—1.55	+1.0°C.	+0.6°C.
Mr. S., 7/ 8/37.....	19.95	87.80	+1.30	—0.02	.....	.....
11/29/37.....	17.74	88.20	+0.80	—0.88	+0.2°C.	—0.2°C.
Mr. H.....	19.98	80.34	+3.82	—1.66	—1.2°C.	.....
Mr. P.....	17.71	94.82	—0.74	—2.53	+0.5°C.	.....
Mr. M.....	16.30	91.22	—1.58	—1.54	+0.5°C.	+0.2°C.
Mr. B., 4/19/39.....	14.29	81.20	.....	.....	.....	.....
7/22/39.....	20.00	91.40	+2.80	+0.05	+1.4°C.	+0.6°C.
Raynaud's disease						
Miss J., 2/9/39.....	16.64	79.40	+9.73	+0.52	0.0°C.*	0.0°C.*
6/8/39.....	16.68	93.00	+0.30	—0.20	0.0°C.	0.0°C.
Miss P.....	17.36	82.90	+14.00	+1.51	0.0°C.	—0.5°C.
Miss B.....	17.04	95.50	+0.30	—0.59	0.0°C.	+2.0°C.
Mrs. W.....	16.50	84.00	+6.50	.....	—0.9°C.	+3.2°C.
Senile arteriosclerosis						
Mr. T.....	15.75	98.65	—4.91	—0.70	+3.7°C.	+0.2°C.
Mr. R.....	.....	91.64	+0.32	.....	—2.5°C.	—0.3°C.
Unclassified conditions						
Mr. De.....	14.72	89.39	+2.29	—1.05	+0.6°C.	—1.2°C.
Mr. S.....	19.00	95.04	—2.21	+0.96	+3.5°C.	+0.5°C.
Mr. D.....	18.80	88.60	+4.90	+0.90	.....	.....
Mr. S.....	20.00	83.90	+14.80	.....	—0.9°C.	0.0°C.

* Decreased peripheral temperatures usually occurred in nervous patients when difficulty was encountered in performing arteriopuncture.

blood (fig. 5). This indicates a relation between the physiologic mechanism of blood pressure, the pulse rate and the oxygenation of the arterial blood.

Arterial Blood.—The oxygen capacity of normal arterial blood is from 19 to 21 volumes per cent in males and from 17 to 19 volumes per cent in females. In patients with thromboangiitis obliterans the capacity varied from 11 to 20 volumes per cent (table 1). Two patients had an oxygen capacity of only 11 volumes per cent without a reduc-

tion in the erythrocyte count and the hemoglobin index to account for the low capacity. The venous blood of another patient (no arterial blood obtained) had an oxygen capacity of 10 volumes per cent (table 2).

TABLE 2.—*Effect of Sodium Thiosulfate or Sodium Tetrathionate on Blood Withdrawn from the Right Cubital Vein Ten Minutes After Injection in Left Cubital Vein in a Case of Thromboangiitis Obliterans* *

	Oxygen Capacity, Vols.%	Content of Oxygen, Vols.%	Content of Carbon Dioxide, Vols.%	Oxygen Saturation, %	Percentage of Hemoglobin and Blood Counts per Cu.Mm.	Changes in Temperature: Toes	Changes in Temperature: Fingers
6/29/36: sodium thiosulfate, 2 Gm.; new case; no previous treatment							
Before injection	10.31	7.61	45.55	73.82	Hb. 78		
After injection	17.42	17.13	52.81	98.40	R.B.C. 4,080,000		
Change	+7.11	+9.52	−6.27	+24.58	W.B.C. 8,900	.....	.....
7/2/36: three days of sodium thiosulfate (2 Gm.)							
Before injection	10.08	6.81	52.02	67.60	Hb. 70		
After injection	14.70	12.30	45.57	83.67	R.B.C. 3,950,000		
Change	+4.62	+5.49	−6.45	+16.07	W.B.C. 9,900	.....	.....
12/10/36: no treatment for 5 months; improved but still has continuous rest pains							
Before injection (arm)	14.11	9.42	56.52	66.77	R.B.C. 4,100,000		
10 min. later (repeat)	.....	9.28	49.27	65.78			
After injection	13.32	10.50	45.66	78.80	R.B.C. 3,650,000		
Change	−0.79	+1.08	−1.76	−12.03		.....	+0.4
Before injection (leg)	15.22	5.13	47.02	33.70	R.B.C. 3,900,000		
After injection	14.28	6.05	45.27	42.38	R.B.C. 3,650,000		
Change	−1.06	+0.92	−2.25	+8.58		.....	−0.8
Coagulation time, 1¾ min.							
Sedimentation time, 16 min.							
12/23/36: two weeks daily treatment; sodium thiosulfate, 2 Gm. daily							
Before injection	15.10	12.02	59.55	79.60	R.B.C. 3,570,000		
After injection	15.17	12.08	59.15	79.60	R.B.C. 3,770,000		
Change	+0.07	+0.06	−0.40	0.00		+0.1	+0.8
1/6/37: sodium thiosulfate, 2 Gm. daily; marked clinical improvement; no pain							
Before injection	15.27	8.18	51.57	53.60			
After injection	15.25	10.46	47.58	68.00			
Change	−0.02	+2.28	−3.49	+15.00		+0.3	+1.3
4/1/37: improving; no treatment for four months							
Before injection	16.62	9.01	45.51	54.02	R.B.C. 4,070,000		
After injection	16.62	9.75	43.02	58.65	R.B.C. 4,500,000		
Change	0.00	+0.74	−2.79	+4.63		+0.8	+1.0
p_H before, 7.495							
p_H after, 7.548							
1/13/38: injection of 0.6 Gm. sodium tetrathionate; no pain; improving; no treatment since April 1, 1937							
Before injection (arm)	15.78	12.08	37.64	76.54	R.B.C. 3,930,000		
After injection	15.59	8.80	38.62	56.43	R.B.C. 4,110,000		
Change	−0.19	−3.28	+0.98	−20.11		−0.9	+0.5
Before injection (leg)	15.02	4.62	41.30	30.77	R.B.C. 3,160,000		
Sedimentation time, 15 min.							

* There was an acute recurrence of the condition in the left foot. The right leg had been amputated (fig. 1).

The exceptionally low oxygen capacity in such patients may be due to some unknown ingredient of the dark blood which interferes with the analytic reagents or to some inactive form of hemoglobin. Spectroscopic and quantitative chemical tests have not revealed the presence of significant amounts of methemoglobin and carboxyhemoglobin.[3]

The oxygen saturation of normal arterial blood in this locality is from 92 to 95 per cent. In cases of thromboangiitis obliterans as well as of Raynaud's disease the oxygen saturation was found to be as low as 80 per cent. In cases with unusually low oxygen capacity, normal oxygen saturation was noted. Low blood pressure, especially in the presence of Raynaud's disease, may contribute to the low oxygen saturation of the arterial blood.

Treatment in these cases with sodium tetrathionate or sodium thiosulfate was usually followed by return of more nearly normal oxygen

TABLE 3.—*Changes in Oxygen Saturation of the Venous Blood After Injection of an Inorganic Sulfur Compound*

		Percentage of Oxygen Saturation							
		Increase				Decrease			
	No. of Patients Examined	No. of Tests	Maximum	Minimum	Average	No. of Tests	Maximum	Minimum	Average
Normal controls	11	2	9.46	3.26	6.36	9	15.27	0.5	5.5
Patients with peripheral circulatory disease									
Thromboangiitis obliterans	20	38	34.10	0.02	9.44	10	21.76	0.6	9.08
Neurocirculatory disease	7	4	32.94	6.2	14.16	5	6.34	1.26	3.94
Patients with polycythaemia vera (not thromboangiitis obliterans)	2	2	10.4	3.9	7.10	0	.....	....	....
Patients with arteriosclerosis									
Senile	2	2	5.4	1.8	3.6	0	.....	....	....
Diabetic	1	0	.....	....	....	1	19.75	....	....
Patients with frostbite	1	0	.....	....	....	1	6.3	....	....
Patients with thrombophlebitis									
Acute	1	1	2.1	....	....	0	.....	....	....
Recovered	2	0	.....	....	....	2	9.61	5.17	7.49

capacity, saturation of the arterial blood, blood pressure and pulse rate. Clinical improvement accompanied these changes.

Venous Blood.—After injection of the inorganic sulfur compounds the oxygen saturation of the venous blood was decreased in 81 per cent of the normal controls and increased in 79 per cent of patients with thromboangiitis obliterans (table 3). A similar increase in oxygen saturation occurred in 2 cases of Raynaud's disease. The fact that the change in oxygen saturation of the venous blood of patients with thromboangiitis obliterans was opposite to that which occurred in the normal controls is significant. The increased peripheral temperature and the accompanying changes in the venous blood were the reverse of those which occurred after smoking (figs. 2 and 6).

The oxygen capacity of the venous blood of some patients (table 2) with thromboangiitis obliterans was increased after injection of the inorganic sulfur compounds. This has been discussed in connection with the arterial blood and was not due to changes in the erythrocyte counts and hemoglobin content. Since colorimetric hemoglobin standards are based on oxygen capacity, the aforementioned changes in the oxygen capacity of the dark arterial and the venous blood suggested activation of some inactive hemoglobin by the sulfur compounds.[10] An increase in glutathione, which is a sulfur-containing oxidation catalyst, may contribute to this action. The catalyst may be deficient in any of the body tissues, but its deficiency in the blood would be reflected throughout the body.

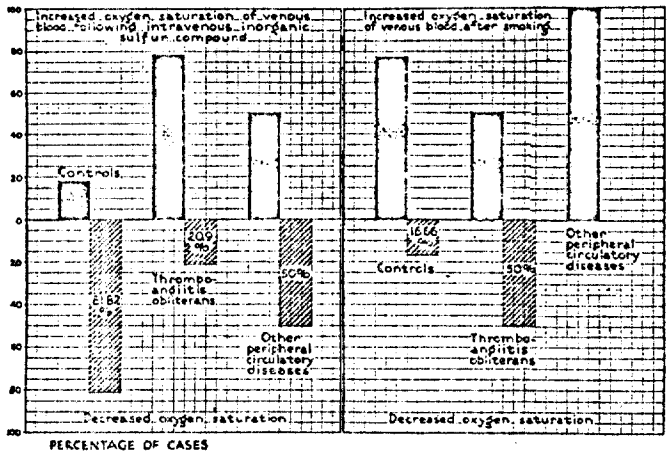

Fig. 6.—Changes in oxygen saturation of the venous blood from the right antecubital vein. Note the opposite effect of the inorganic sulfur to that of smoking.

Glutathione.—The utilization of oxygen by the tissues has been shown to be partly under the control of an oxidation catalyst, glutathione. This substance is found in the blood in two forms, the reduced, active form and the oxidized, inactive form. The results of our study of the glutathione content of the venous blood are contained in table 4. The average content for the normal controls and for patients with arteriosclerotic disease was practically the same.

10. Ammundsen, E.: The Presence of Non-Oxygen-Combining (Inactive) Hemoglobin in the Blood of Normal Individuals, Science **90**:372-373 (Oct. 20) 1939.

In 2 cases of acute thromboangiitis obliterans we found an unusually low content of the reduced form of the catalyst and a high content of the oxidized form. After intensive treatment with the inorganic sulfur

TABLE 4.—*Glutathione Content of the Venous Blood: Effect of Sodium Tetrathionate and Sodium Thiosulfate on Untreated and Treated Thromboangiitis Obliterans and Other Peripheral Circulatory Diseases*

	No. of Patients	No. of Tests	Glutathione, Mg./100 Cc. Total	Reduced	Oxidized	Comment
Controls						
Maximum	..	..	69.0	44.6	34.4	
Minimum	..	..	39.4	25.5	13.9	
Average	5	10	58.23	38.26	21.04	Normal
Peripheral Circulatory Diseases						
Thromboangiitis obliterans						
Acute						
Mr. E. New case: no gangrene or infection						
8/25			69.3	12.7	56.8	No treatment
8/27 Injection of 0.6 Gm. sodium tetrathionate						
Before injection			61.4	11.7	49.7	
After injection			61.3	12.7	48.6	
9/24 Injection of 1 Gm. sodium thiosulfate						
Before injection			83.5	52.6	31.2	Treated 4 weeks; improved
After injection			79.8	56.6	23.2	
Mr. M. Both legs involved previously; hands involved now						
8/20 Injection of 0.6 Gm. sodium tetrathionate						Recurrence
Before injection			61.4	18.1	43.3	
After injection			47.5	17.0	30.5	
9/10 Injection of 1 Gm. sodium thiosulfate						
Before injection			61.3	55.0	5.3	Intensive treatment
After injection			71.4	54.1	17.3	
Mr. S.						
9/9 Injection of 0.4 Gm. sodium tetrathionate						Intensive treatment two months; improved
Before injection			61.30	38.28	23.02	
After injection			61.40	38.20	23.20	
11/15 Injection of 1 Gm. sodium thiosulfate						Serious infection developed on calf of leg
Before injection			53.8	30.8	23.0	
After injection			52.4	36.8	15.6	
11/26 Injection of 0.6 Gm. sodium tetrathionate						
Before injection			48.9	34.6	14.3	
After injection			55.2	37.3	17.9	
11/29 Injection of 0.6 Gm. sodium tetrathionate						Infection clearing up
Before injection			52.1	34.5	17.6	
After injection			56.2	34.8	21.4	
	No. of Patients	No. of Tests				
Improved or recovered						
Maximum	..	..	83.8	52.6	31.2	
Minimum	..	..	37.0	20.1	5.3	
Average	15	30	50.11	33.57	16.54	
Raynaud's disease						
Maximum	..	..	52.0	29.5	31.7	
Minimum	..	..	34.4	11.0	8.0	
Average	3	6	36.77	24.60	13.97	
Senile arteriosclerosis						
Maximum	..	..	69.0	44.6	23.4	
Minimum	..	..	58.0	36.6	18.5	
Average	5	5	61.50	40.08	21.42	

compounds the reduced glutathione as well as the oxidized substance was frequently increased above normal levels. In Raynaud's disease both the reduced and the oxidized form were below normal levels. One patient with Raynaud's disease had an initial reduced glutathione

content of 11 mg. per hundred cubic centimeters of blood, which was the low level found in cases of thromboangiitis obliterans. The clinical similarity between Raynaud's disease in females and thromboangiitis obliterans in males is occasionally observed.[11]

Direct Action of the Sulfur Compounds on the Blood.—Oxygen and carbon dioxide determinations for the venous blood were made before and after addition of a few crystals of the chemical to the blood. The oxygen capacity and content thus examined in vitro were unaffected, but the carbon dioxide content was reduced. In vivo, however, the small amount of the sodium solutions injected intravenously would be insignificant in reducing the carbon dioxide content of approximately 6 quarts of blood.

Reaction of the Blood.—The actual and the theoretic p_H of the blood were frequently increased temporarily by the injection of the sulfur compounds. A rapid increase in p_H from 7.42 to 7.52 as observed in 1 clinically recovered patient with thromboangiitis obliterans could not have been due to the action of the small amount of sodium in the solution in reducing the carbon dioxide content of the blood. Changes in the oxyhemoglobin could be reflected in a shift of hydrogen ion concentration. Activation of some form of inactive oxyhemoglobin would contribute to this effect.[12] The high p_H (7.60 or more) which we reported for patients in the acute stage of thromboangiitis obliterans was found to be reduced to normal levels after intensive treatment with the inorganic sulfur compounds.

Blood Counts.—The immediate effect of the inorganic sulfur compounds on the erythrocyte counts was studied in 30 cases. The results showed no constant change. Polycythemic counts, however, returned to normal after intensive treatment. The high erythrocyte counts and hemoglobin values were probably compensatory adjustments for a deficiency in available oxygen.[13]

	Erythrocyte Counts per Cubic Millimeter of Blood (30 Patients)		
	Maximum	Minimum	Average
Before injection	5,640,000	3,680,000	4,830,000
After injection	5,850,000	3,570,000	4,776,000

11. Reid, M. R.: The General Care of Peripheral Vascular Diseases, Ann. Surg. **96**:733-743 (Oct.) 1932. Homans.[18]

12. Litarczek, G.; Aubert, H., and Cosmulesco, I.: Studies on the Mechanism of the Reaction of Glutathione on the Dissociation Curve of Oxyhemoglobin, Compt. rend. Soc. de biol. **109**:781-783, 1932.

13. Barcroft, J.: The Respiratory Function of the Blood: I. Lessons from High Altitudes, London, Cambridge University Press, 1925.

Excretion of Sulfate in the Urine.—Thiosulfate is rapidly oxidized in the body and largely excreted in the urine as sulfate. Holbøll[14] reported that 60 to 70 per cent is thus eliminated, and Walko[15] found 50 to 70 per cent in the urine. This rapid elimination offers an expla-

TABLE 5.—*Effect of Intravenous Injections of Sodium Thiosulfate and Sodium Tetrathionate on Excretion of Sulfates in the Urine*

	Urine Volume, Cc.	Inorganic Sulfates	Total Sulfates
Twenty-Four Hour Specimens			
Controls			
Mr. M. No injection	1,400	1.0556	1.1592
Mr. O. No injection	1,000	1.2480	1.3429
Composite specimen (aver. of 12 patients; no injection)	2,674	1.2457	1.3589
Mr. Y.			
5/18/37 No injection	510	0.6100	0.6793
5/19/37 2 Gm. sodium thiosulfate	610	1.8950	1.9154
5/20/37 0.885 Gm. sodium tetrathionate	300	0.5112	0.5206
Thromboanglitis obliterans			
Mr. N.			
4/28/37 0.885 Gm. sodium tetrathionate	1,400	1.5344	1.5058
4/29/37 No injections; smoking cigaret	2,200	1.7942	1.7248
4/30/37 2 Gm. sodium thiosulfate	2,500	2.1800	2.3850
5/1/37 No injection; no smoking	3,500	1.5820	1.7150
Mr. B.			
6/16/37 No injection	2,100	.....	2.1570
6/17/37 2 Gm. sodium thiosulfate	3,425	.....	3.2226
6/20/37 2 Gm. sodium thiosulfate	3,850	.....	3.7432
6/23/37 0.885 Gm. sodium tetrathionate	3,375	.....	3.6967
6/24/37 2 amp. of 0.885 Gm. sodium tetrathionate	2,275	.....	3.1300
Individual Specimens			
Thromboanglitis obliterans			
Mr. M. 5/20/37 0.885 Gm. sodium tetrathionate			
Before injection	270	0.0292	0.0442
After injection, ½ hour	255	0.0410	0.0467
2½ hours	465	0.1711	0.2362
Mr. N. 5/4/37 1 Gm. sodium thiosulfate			
Before injection	50	0.0570	0.0672
After injection, 1 hour	50	0.2174	0.2332
5/8/37 1 Gm. sodium thiosulfate			
Before injection	50	0.0504	0.0832
After injection, 1 hour	75	0.2361	0.2463
1½ hours	250	0.1060	0.1150
2 hours	300	0.0996	0.1008
5/12/37 0.885 Gm. sodium tetrathionate			
Before injection	50	0.0834	0.0922
After injection, 1½ hours	50	0.2480	0.2726
3 hours	50	0.2300	0.2324
5/13/37 0.885 Gm. sodium tetrathionate			
Before injection	77	0.1029	0.1141
After injection, 2 hours	66	0.3370	0.3467
Mr. B. 5/20/37 2 Gm. sodium thiosulfate			
Before injection	315	0.1551	0.1915
After injection, ¾ hour	135	0.2084	0.2392
2½ hours	285	0.1915	0.2383

* One gram of the thiosulfate ($Na_2S_2O_3$, $5\ H_2O$) contains 0.258 Gm. of sulfur, and 0.885 Gm. sodium tetrathionate ($Na_2S_4O_6$,H_2O) contains 0.293 Gm. of sulfur.

nation for the transitory effect of the thiosulfate solution on the peripheral temperatures, pulse rate and blood pressure.

14. Holbøll, S. A.: Quantitative Determinations of Sodium Thiosulphate in the Urine, Klin. Wchnschr. 4:1636-1640 (Aug. 20) 1925.

15. Walko, K.: Concerning the Removal of Poisons by Oxidizing Agents, Arch. internat. de pharmacodyn. 4:311-323, 1897-1898.

At the suggestion of William D. McNally, of the department of toxicology, we had prepared for us sodium tetrathionate ($Na_2S_4O_6.H_2O$), which he had found to be more beneficial than sodium thiosulfate ($Na_2S_2O_3.5H_2O$) in cases of metallic poisoning. The intravenous dose (1 Gm.) of sodium thiosulfate contained 0.258 Gm. of sulfur, and the 0.885 Gm. of sodium tetrathionate injected contained 0.393 Gm. of sulfur. The results of sulfate determinations for the urine are shown in table 5.[16] The entire additional elimination after injection was in the free sulfate.[17] The remainder, present as etheral sulfate, was apparently unaffected. The volume of urine in individual specimens had no relation to the amount of sulfate eliminated, although the twenty-four hour volume indicated diuresis. No food was taken for at least four hours before the individual specimens were obtained or during the period of collection of subsequent specimens. This prevented changes in the determinations as a result of diet.

Determinations of the sulfate content of the urine immediately before and at varying intervals after injection showed that the thiosulfate solution was more rapidly eliminated than was the tetrathionate. In cases of thromboangiitis obliterans the excretion of sulfate in the twenty-four hour specimens was greater than that observed for the normal controls; the composite urine from 12 normal subjects averaged approximately the same for free and total sulfate content as did that from 2 individual patients. The more rapid excretion of the thiosulfate in the urine may account for the fact that the effect of sodium thiosulfate was more transitory than that of sodium tetrathionate.

COMMENT

The peripheral arterial occlusion associated with thromboangiitis obliterans is seldom detected until examination for arterial pulsation is made because of infection, infarcts, gangrene or acute circulatory deficiency with impending gangrene. Gross specimens of amputated extremities reveal that the occlusions have been progressive or successive. Although infection has been suspected as the cause of the disease, extensive investigations have failed to establish a bacterial cause. The significance of changes in the blood which disturb tissue metabolism and contribute to thrombosis is difficult to evaluate because of the involved physiologic processes.

16. The determinations of urine sulfate were made by Willard Bergman, assistant to Dr. William D. McNally.

17. Folin, O.: Approximately Complete Analysis of Thirty "Normal" Urines, Am. J. Surg. 21:260-271 (Aug.) 1933.

Naffziger and his co-workers[18] at the University of California reported the results of their investigation of the role of sulfur in the hemorrhagic tendency associated with obstructive jaundice. They found that in obstructive jaundice, either in experimental animals or in man, the sulfur content of the venous blood is increased. They contended that certain sulfur compounds retained in the blood act as anticoagulants. Interesting and important contributions on the sulfur-containing anticoagulant heparin have been made by Best and others.[19] The role of heparin in disease conditions and the effect of therapy on the heparin content of the blood cannot be evaluated until quantitative determinations can be made. Unfortunately, no quantitative test[20] for heparin is available.

To the best of our knowledge, sodium thiosulfate was first used empirically in the treatment of thromboangiitis obliterans by Dr. Robert Johanneson, of the Presbyterian Hospital in Chicago. Although a few striking results were obtained, there was no information as to the modus operandi of the therapy or to the means of selecting patients who may be benefited. Rabinowitz[21] in 1933 and Rabinowitz and Kahn[22] in 1936 reported the presence of abnormal phospholipin metabolism and its effect on utilization of oxygen by the tissues. They were the first to report the use of sodium thiosulfate for thromboangiitis obliterans. Additional metabolic changes associated with the disease have been reported by Friedlander and Silbert[23] for cholesterol metabolism and

18. Naffziger, H. C.; Carr, J. L., and Foote, F. S.: Obstructive Jaundice, Ann. Surg. **106**:745-751 (Oct.) 1937.

19. Best, C. H.; Charles, A., and Cowan, C.: The Administration of Heparin, Proc. Am. Physiol. Soc. **119**:272-273 (May) 1937. Best, C. H., and Taylor, N. B.: The Physiological Basis of Medical Practice, Baltimore, William Wood & Company, 1937. Best, C. H.: Heparin and Thrombosis, Proc. Inst. Med. Chicago **12**:210-218 (Dec. 15) 1938. Murray, D. W. G.; Jacques, L. B.; Perrett, T. S., and Best, C. H.: Heparin and Vascular Occlusion, Canad. M. A. J. **35**: 621-622 (Dec.) 1936. Best, C. H.: Heparin and Thrombosis, Proc. Staff Meet., Mayo Clin. **14**:81-84 (Feb. 8) 1939. Best, C. H., and Murray, D. W. G.: Heparin and Thrombosis of Veins Following Injury, Surgery **2**:163-187 (Aug.) 1937. Best, C. H.; Cowan, C., and MacLean, D. L.: Heparin and Formation of White Thrombi, Science **85**:338-339 (April 2) 1937.

20. Best, C. H.: Personal communication to the authors.

21. Rabinowitz, H. M.: Thrombo-Angiitis Obliterans, Am. J. Surg. **21**:260-271 (Aug.) 1933; The Use of Sodium Iodide Thiosulphate in the Treatment of Thrombo-Angiitis Obliterans, J. Chemotherapy **13**:1-4 (April) 1936.

22. Rabinowitz, H. W., and Kahn, J.: The Relationship of Phospholipin Metabolism to Thrombo-Angiitis Obliterans and Its Treatment, Am. J. Surg. **31**:329-339 (Feb.) 1936.

23. Friedlander, M., and Silbert, S.: Thrombo-Angiitis Obliterans: VI. Chemistry of the Blood, Arch. Int. Med. **48**:500-506 (Sept.) 1931.

by Bernhard[24] for sugar metabolism. Roth, Maclay and Allen[25] found inconstant changes in serum calcium, serum protein, blood urea, serum lecithin, serum phosphorus, cholesterol and hematocrit values. The importance of these observations may be underestimated because of the difficulty in interpreting pathologic blood pictures in different stages of the disease.

Bancroft and his associates[26] have extensively investigated and reported on the use of sodium thiosulfate for thrombophlebitis. The problem studied was directed to determine the effect of sodium thiosulfate on the clotting index of the plasma and the fibrinogen content of the blood, on the assumption that, as there are definite bleeding diatheses, there are also potential "clotters." After using the thiosulfate solution prophylactically for a number of years against postoperative thrombophlebitis, Bancroft concluded that the morbidity may be definitely reduced by injections of sodium thiosulfate combined with a diet low in fats and proteins.

The changes in blood pressure, pulse and peripheral temperature seem to influence or to be affected by the available oxygen in the blood. The occasional low oxygen saturation (80 per cent) of the arterial blood in cases of thromboangiitis obliterans may be increased to normal with an accompanying elevation in blood pressure and the shunting of the peripheral circulation which follows smoking. Subsequently, the oxygen saturation was decreased by continued smoking, and this decrease may have been due to formation of inactive oxyhemoglobin. In 2 cases of thromboangiitis obliterans the oxygen capacity was only 11 volumes per cent, with 80 per cent hemoglobin (by the Dare method) and over 4,400,000 erythrocytes per cubic millimeter. Without significant changes in the hemoglobin or red cell count the oxygen capacity was increased to more than 17 volumes per cent after treatment with inorganic sulfur compounds. The presence of an unidentified form of inactive hemoglobin has just been confirmed by a report from Copenhagen, Denmark.[10]

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24. Bernhard, A.: Summary of the Chemical Blood Findings in Thrombo-Angiitis Obliterans, M. Rec. **97**:430-431 (March 31) 1920.

25. Roth, G. M.; Maclay, E. V., and Allen, E. V.: Blood in Thrombo-Angiitis Obliterans, Arch. Int. Med. **6**:413-422 (Sept.) 1938.

26. Bancroft, F. W.; Kugelmass, I. N., and Stanley-Brown, M.: Evaluation of Blood Clotting Factors in Surgical Diseases. Ann. Surg. **90**:161-189 (Aug.) 1929. Bancroft, F. W., and Stanley-Brown, M.: Postoperative Thrombosis, Thrombophlebitis and Embolism, Surg., Gynec. & Obst. **54**:898-906 (June) 1932. Bancroft, F. W.; Stanley-Brown, M., and Quick, A.: Postoperative Thrombosis and Embolism, Am. J. Surg. **28**:648-668 (June) 1933. Bancroft, F. W.; Stanley-Brown, M., and Chargaff, E.: Postoperative Thrombosis and Embolism, Ann. Surg. **106**:868-879 (Nov.) 1937.

The reports of investigations on bleeding and thrombotic diseases show a relation between the role of sulfur as used in the thiosulfate or the tetrathionate solution and the tendency to thromboses in cases of thromboangiitis obliterans. Further, the inorganic sulfur solutions have the opposite effect on the conditions studied to that of smoking, which is generally accepted as a cause of the disease. The clinical value of the inorganic sulfur solutions may be due to the fact that the effect on blood and tissue metabolism is the opposite to that observed with smoking.

SUMMARY

Sodium tetrathionate and sodium thiosulfate injected intravenously may produce a marked increase in the oxygen capacity and content of the circulating blood in some patients. This was most marked in patients with active thromboangiitis obliterans and dark, thick blood; no significant increase in the erythrocyte count or the hemoglobin value for hemoglobin accompanied the change. The presence of an inactive form of oxyhemoglobin seemed most likely to account for this effect.

In accounting for the marked increase in oxygen capacity and content of the blood of some patients after injection of inorganic sulfur, we considered three possibilities: (1) reduction of methemoglobin or of some unidentified form of inactive hemoglobin; (2) additional supply of oxygen in the sulfate (S_2O_3), or (3) increase in the oxidation catalyst, the reduced form of glutathione. We were unable to establish the presence of a known form of inactive hemoglobin, and the small amount of reducing agent was inadequate to produce the marked increase in oxygen in the approximately 6 quarts of blood. By actual determination, the sulfur compounds did not add oxygen to the blood. All evidence seemed to corroborate our findings that a catalystic action was produced by the injected chemical.

Ammundsen[27] has just reported from Copenhagen, Denmark, the presence of non-oxygen-containing hemoglobin in normal persons. Activation of this hemoglobin occurred on administration of sodium hydrosulfate. The composition of the pathologic hemoglobin and the cause for its appearance are unknown, but it is not to be assumed that all hemoglobin circulating in the blood is capable of freely combining with oxygen.

Sodium thiosulfate has been found to increase the glutathione in the liver and in the blood.[27] Glutathione, a sulfur-containing oxidation catalyst of the blood and tissues, is efficient in reducing methemoglobin,

27. Arnovlevitch, V.: Increase of Glutathione in the Liver and the Blood After Administration of Sodium Thiosulphate, Compt. rend. Soc. de biol. **106**: 681-684, 1931; Physiol. de abstr. **16**:624-626, 1931.

but spectroscopic and quantitative chemical tests have failed to detect methemoglobin as the form of inactive oxyhemoglobin present in patients with thromboangiitis obliterans.

Sodium tetrathionate had a less immediate but more prolonged effect than did sodium thiosulfate. The more rapid oxidation and excretion of the thiosulfate as free sulfate in the urine may account for this difference.

CONCLUSIONS

Intravenous injections of sodium tetrathionate and sodium thiosulfate were usually followed by increased peripheral temperatures, decreased pulse rate and reduction in blood pressure. These physiologic responses were associated with changes in the oxygen capacity and in the oxygen saturation of the arterial and the venous blood.

These effects were opposite to those that occurred with smoking, which is an important etiologic factor in thromboangiitis obliterans. The therapeutic value of sodium thiosulfate and sodium tetrathionate is probably due to the changes produced in the blood and the resulting physiologic responses.

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Biochimica et Biophysica Acta 31: 270-272 (1959).

The initial products of thiosulphate oxidation by *Thiobacillus X*

VISHNIAC[1] has proposed a polythionate pathway for thiosulphate oxidation by Thiobacilli with tetrathionate as the first oxidation product. His conclusions were based mainly on an analysis of the course of oxygen consumption during oxidation of thiosulphate by *Thiobacillus thioparus* and on the facts that tetrathionate is oxidized by a number of Thiobacilli and often accumulates transiently when thiosulphate is being metabolized. The evidence for this hypothesis has been reviewed by VISHNIAC AND SANTER[2].

Recently[3] a soluble enzyme system which oxidizes thiosulphate to tetrathionate was isolated from *Thiobacillus X*[4], a bacterium physiologically similar to *T. thioparus*. In this report are described experiments with intact cells of *Thiobacillus X* which provide direct evidence that tetrathionate is an intermediate in the oxidation of thiosulphate to sulphate.

1-ml samples of a fresh suspension of *Thiobacillus X* (10 mg dry wt.) in 0.1 *M* phosphate, pH 7, were aerated at 23°. Sodium thiosulphate (1 μmole) containing ^{35}S ($3 \cdot 10^5$ counts/min) in each of the inner ($-SO_3H$) and outer ($-SH$) positions was injected into each sample and the reactions stopped at various times by the addition of 1 ml absolute ethanol. (Under these conditions 1 μmole thiosulphate was oxidized completely to sulphate in about 8 min). The cells were removed by centrifuging and samples of the supernatants, which contained virtually all the radioactivity, were chromatographed on Whatman No. 1 paper, developed (descending) for 16–20 h with pyridine–*n*-propanol–water (3:5:5). The radioactive areas were located with a paper-scanning machine and eluted with water. Samples were then plated on aluminium discs and the radioactivity measured with an end-window Geiger-Muller counter.

The results are shown in Fig. 1. After 10-sec incubation about 50% of the thiosulphate had been metabolized, the remaining ^{35}S being almost entirely accounted for by a compound with a similar R_F to that of tetrathionate.

The following tests confirmed that this compound was tetrathionate:

(a) the compound could not be separated from authentic tetrathionate by rear-phase chromatography[5] nor by electrophoresis on paper[6] at pH 5, 7, or 8;

(b) all the ^{35}S exchanged rapidly with the sulphur of unlabelled thiosulphate[7];

(c) when the compound was mixed with an equivalent amount of inactive sulphite[8], two additional radioactive compounds were formed which had the chemical and chromatographic properties of thiosulphate and trithionate respectively. The ratio of ^{35}S between thiosulphate and trithionate was approx. 5:7 which is the ratio expected when exchange[7] between thiosulphate and trithionate is taken into account.

(d) The compound reacted with cyanide[8] to give three compounds with the properties of thiosulphate, thiocyanate and sulphate, which were labelled in the ratio 2:1:1 respectively.

A compound corresponding to trithionate and which contained about 5% of the radioactivity was also present at 10 sec. At 40 sec thiosulphate had largely disappeared, tetrathionate was much decreased, and "trithionate" remained about the same or had increased slightly. The remaining ^{35}S was present in unidentified material moving close to thiosulphate on chromatograms. This material contained at least two compounds which were separated from thiosulphate by paper electrophoresis. The nature of these compounds is at present under investigation. The experiment was also

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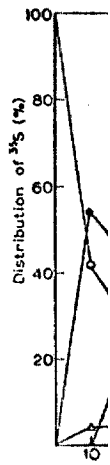

Fig. 1. Products of thiosulphate oxi
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Division of Plant Industry, C.S

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carried out with thiosulphate labelled in either the inner or outer positions: the results were similar to those described above. The distribution of ^{35}S in the tetrathionate formed in each case was determined by the method of VAN DER HEIJDE AND ATEN[9].

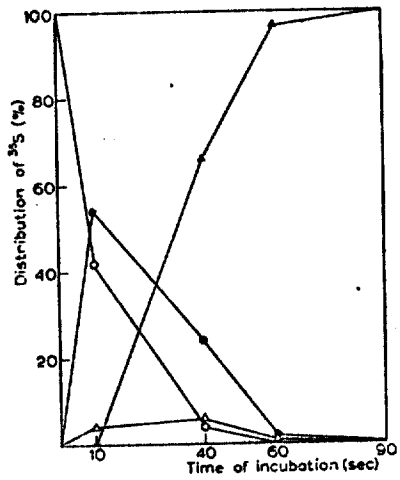

Fig. 1. Products of thiosulphate oxidation (for details see text). Curves: thiosulphate (○), tetrathionate (●), "trithionate" (△), unidentified compounds (▲).

No exchange between the inner and outer S atoms occurred indicating that the reaction proceeded according to the equation:

$$2\,(\overset{1}{S}-\overset{2}{SO_3})^{=} \longrightarrow (\overset{2}{{}_3OS}-\overset{1}{S}-\overset{1}{S}-\overset{2}{SO_3})^{=}$$

These results suggest very strongly that tetrathionate is the first intermediate on the oxidation of thiosulphate to sulphate by *Thiobacillus X* and clearly show that both sulphur groups of thiosulphate are involved.

Recently OSTROWSKI AND KRAWCZYK[10] have concluded that in *T. thioparus* the inner group of thiosulphate is converted at the cell surface directly to sulphate while the outer sulphur atom is metabolized independently, within the cell, to sulphate, cell material and elementary sulphur. However, their conclusions were based on a study of the conversion of the inner and outer sulphur atoms of thiosulphate to elementary sulphur and cell material during the growth of *T. thioparus*. No analyses of the soluble constituents of the medium were reported. Such experiments, while indicating the ultimate fate of the sulphur groups, give little information on the reactions involved in their metabolism. Evidence for the participation of polythionates has been obtained with bacteria from a number of sources (see VISHNIAC AND SANTER[2]) and it is unlikely that the mechanism of oxidation of thiosulphate is radically different in different strains and species of Thiobacilli.

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[1] W. VISHNIAC, *J. Bacteriol.*, 64 (1952) 303.
[2] W. VISHNIAC AND M. SANTER, *Bacteriol. Revs.*, 21 (1957) 195.
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[9] H. B. VAN DER HEIJDE AND A. H. W. ATEN, *J. Am. Chem. Soc.*, 74 (1952) 3706.
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A micro method for the estimation of total sulfur in biological materials

In the course of studies on the sulfur-containing fractions of brain total-lipid extracts, it was found that the Carius procedure[1], *i.e.*, oxidation with HNO_3 in a sealed tube, was unsuitable because (a) prohibitively large samples were required and (b) methionine sulfur was not estimated quantitatively[2]. Digestion in an unsealed tube is open to the objection that, with unknown materials, there is always the possibility that some sulfur may be lost by volatilization. This paper describes a method in which total sulfur in biological materials can be conveniently estimated on samples containing as little as 50 μg S with an error of $\pm$ 2 μg, and in which methionine sulfur is estimated quantitatively. In actual practice, however, results on materials containing less than 0.5 % S appear to yield less accurate results. The method consists of digestion of the sample in a sealed tube with aqua regia followed by removal of $PO_4^{\equiv}$ as magnesium ammonium phosphate, precipitation of $SO_4^{=}$ as a benzidine salt and titration of benzidine sulfate with dilute alkali. The detailed procedure is as follows.

Digestion. A sample containing between 50 and 200 μg S is placed in a heavy-walled pyrex combustion tube (125 mm × 14 mm) and any solvent present removed by appropriate procedures[3]. The size of the sample is limited by the amount of CO_2 produced during digestion since there is the possibility of explosion due to the internal pressure developed at high temperatures. In our experience, samples containing up to 14 mg C can be digested safely. 0.4 ml freshly prepared aqua regia (conc. HNO_3–conc. HCl, 3:1 v/v) is added to the dry sample, the tube sealed, wrapped in asbestos sheeting to protect the sealed tip, put in an iron bomb constructed by fitting a length of pipe with two screw caps, and placed in an oven maintained at 230° for 8 h.

Removal of aqua regia and $PO_4^{\equiv}$. After being allowed to cool, the combustion tube is removed, centrifuged for 1 min at approx. 1000 rev/min to spin down any liquid which may remain in the tip, and the outside of the tube is rinsed with dilute HCl followed by distilled water; the tube is opened by breaking the tip with a hemostat and the opening enlarged to approximately the original diameter of the tube. It is then placed in a beaker two-thirds full of phosphoric acid, the temperature of the acid

slowly raised to 120°, and the a
can no longer be detected. The t
with distilled water. Exactly 3
NH_4Cl mixture (13:5 w/w) are
mixing and centrifuged for 10 mi
into a 5 ml conical centrifuge tu
in a phosphoric acid bath and g
the contents of the tube are dry,
with water and 0.9 ml distilled
bromcresol green indicator dilu
tube is tapped gently to insur
solution adjusted to approx. pH

Precipitation of $SO_4^{=}$. To th
benzidine dihydrochloride dissol
dark) and 0.8 ml 95 % acetone
stirring rod, any ppt. adhering
acetone and the contents of the
a beaker of ice water and kept in
precipitation of benzidine sulfat
the sample has been allowed to
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remove any ppt. which may be
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a micropipette. While bubbling C
NaOH[5] is added from the microl
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boiling for 10 sec is obtained. Th
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Effect of Tissue Slices and Oxidizing Agents on Thyrotrophin

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Noguchi Clinic, Beppu, Japan, and Radioisotope and Medical Services, Boston Veterans Administration Hospital, Boston University School of Medicine and Tufts University School of Medicine, Boston, Massachusetts

ABSTRACT. No inactivation of thyrotrophin (TSH) was observed after incubation with slices of thyroid, liver, spleen, kidney and muscle from steers, guinea pigs, rats and mice. Iodine, peroxide and pancreatin reduced TSH activity. The inactivation of TSH by iodine and peroxide was reversed, whereas the inactivation of TSH by pancreatin was not reversed, by the addition of 1-methyl-2-mercaptoimidazole or methylthiouracil. Complete inactivation of TSH *in vivo* had occurred at 4 hours in the normal mouse and in the mouse pretreated with 1-methyl-2-mercaptoimidazole.

THE ABILITY OF thyroid tissue to reduce the activity of thyrotrophic hormone (TSH) *in vitro* has been reported by various authors (1-3). Other studies, however, have indicated that the thyroid is not important in the *in vivo* inactivation of TSH (5-8). Thyrotrophic hormone has been inactivated by treatment with pepsin, trypsin (9-11) or iodine (12, 13). The more sensitive assay method for thyrotrophin of McKenzie (14) has been used to restudy the inactivation of TSH by chemicals and tissues.

Materials and Methods

The thyroid, liver, muscle and kidney slices used in this study were obtained from steers, guinea pigs, rats and SM mice killed within 2 hours of the beginning of the experiment. Slices were cut freehand (0.5-1.5 mm) and placed (100-300 mg) in 2 ml of modified Krebs-Ringer-phosphate solution ($CaCl_2$ 8×10^{-4} M) at pH 7.4, to which 2-100 mU of TSH (USP Thryotrophin Reference Standard) had been added. Control tubes contained boiled tissue slices. The tubes were placed in a constant temperature (37 C) incubator for 2 hours. After incubation, aliquots of the media only (0.2 ml) were injected intravenously into 6-12 mice previously injected with I^{131}. The thyrotrophic effect was measured by the percentage of increase in circulating radioactivity 2 hours (occasionally 10 or 24 hours) after the injection of the material tested.

The disappearance of subcutaneously administered thyrotrophin was studied in newborn SM mice weighing 2.5-3.0 g. A freshly prepared saline solution of 200 mU of TSH (USP Thyrotrophin Reference Standard) was administered subcutaneously and the animals were killed at intervals varying from 0-4 hours later. The whole body was homogenized in a Waring Blendor with cold saline (5 cc) and centrifuged in the cold at 1,500 rpm. The supernatant was diluted with saline to make a total volume of 100 ml, of which an aliquot (0.2 ml) was assayed for TSH activity.

Results

The effect of bovine thyroid slices on the TSH activity is shown in Table 1. There was no significant reduction in TSH activity by incubation of 2 mU for 2 hours or 20 mU for 24 hours with 300 mg of bovine thyroid; nor was there any significant reduction in TSH activity by incubation of 2 mU for 2 hours with 300 mg of kidney, liver, spleen or muscle slices (Table 2).

The addition of the following substances (10^{-3} M) to the media had no effect on TSH activity: mercuric chloride, cobaltous sulfate, cupric chloride, 1-methyl-2-mercaptoimidazole (Merkazol), potassium iodide, ethylene diamine

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TABLE 1. TSH activity following incubation with bovine thyroid

No. of Exp.	Tissue	Weight of Tissue (mg)	Time of Incubation hr	TSH mU/2 ml	No. of Mice	Blood Radioactivity (as % of initial) Mean	SD‡
1	Control*	300	2	2	12	334	69.5
	Thyroid	300	2	2	12	302	70.2
2	Control	300	2	2	12	310	80.5
	Thyroid	300	2	2	12	280	70.6
3	Control	300	2	2	10	282	40.5
	Thyroid	300	2	2	10	256	32.8
4	Control	300	2	2	9	254	50.2
	Thyroid	300	2	2	9	241	48.6
5	Control	300	24	20†	10	248	60.5
	Thyroid	300	24	20	10	238	62.0

* Control: boiled tissue.
† Assayed after 10-fold dilution.
‡ SD = Standard deviation. The difference between control and thyroid for 11 experiments (not all charted) was not significant at the 5% level.

tetraacetic acid (EDTA), glucose and normal human serum. A reduction in TSH activity was noted when pancreatin, peroxide or iodine (10 mg of iodine to 40 mg potassium iodide in 100 ml aqueous solution) was added to the incubating media (Table 3). No delayed TSH activity (LATS; 15, 16) was found 10 or 24 hours after injection into the assay mice following treatment with pancreatin, peroxide and iodine.

After TSH (2 mU) was incubated for 2 hours with iodine (1 μg), or peroxide (10^{-5} M) or pancreatin (0.1 mg) in KRP solution, the addition of 1-methyl-2-mercaptoimidazole or methylthiouracil (10^{-4} M), followed by incubation for another hour, restored some of the activity of iodine- or peroxide-treated TSH, but the inactivation of TSH by pancreatin was not reversed (Table 4). The addition of large concentrations of other reducing agents also increased the activity of TSH pretreated with iodine (Table 5).

Four hours after the subcutaneous injection of 200 mU of TSH into mice, TSH activity in the whole body had returned to the pre-injection level (Table 6). The

TABLE 2. TSH activity following incubation (for 2 hours) with various animal tissues

Tissue	Weight of Tissue (mg)	TSH mU/2 ml	No. of mice	Blood Radioactivity (as % of initial) Mean	SD*
Kidney (bovine)	300	2	10	335	42.6
Liver (bovine)	300	2	10	335	75.3
Spleen (bovine)	300	2	10	305	87.2
Muscle (bovine)	300	2	10	334	87.5
Control (boiled)	300	2	10	321	45.4
Liver (guinea pig)	300	2	10	381	102.1
Kidney (guinea pig)	300	2	10	351	95.3
Liver (rat)	300	2	10	351	112.5
Kidney (rat)	300	2	10	323	96.2
Liver (mouse)	300	2	10	351	97.7

* SD = Standard deviation.

TABLE 3. Reduction in the activity of TSH by the addition of peroxide, iodine and pancreatin to the incubating media

Substance Added	TSH mU/ml	No. of Mice	Blood Radioactivity (as % of initial) 2 hr* Mean	2 hr* SD	10 hr* Mean	10 hr* SD	24 hr* Mean	24 hr* SD
Control	1	10	340	(52.2)	110	(15.6)	98.5	(16.2
H_2O_2 10^{-2} M	1	10	135†	(43.5)	111	(10.5)	115	(20.6
H_2O_2 10^{-4} M	1	10	171†	(40.2)	105	(20.1)	101	(22.6
H_2O_2 10^{-5} M	1	10	152†	(80.5)	104	(32.1)	100	(16.1
I_2 2.0 μg‡	1	6	111†	(15.1)	110	(10.2)	98	(9.6
I_2 4.0 μg	1	6	105†	(10.1)	106	(10.2)	96	(8.2
I_2 8.0 μg	1	6	101†	(12.3)	101	(9.2)	100	(6.1
I_2 10.0 μg	1	6	92†	(9.6)	98	(9.2)	94	(7.5
Pancreatin 0.1 mg	1	6	146†	(11.5)	110	(10.1)	105	(9.1
Pancreatin 0.1 mg	1	6	128†	(14.1)	102	(9.2)	98	(8.1

* Hours after the TSH was given to the mice.
† The difference between these and the control is significant at the 1% level.
‡ 1 μg, but not 0.5 μg, of I_2 also reduced the TSH activity.

TABLE 4. Effect of 1 methyl-2-mercaptoimidazole and methylthiouracil on the reduction in TSH activity (2 mU) by iodine, peroxide and pancreatin in the absence of thyroid tissue

Pretreatment with	Dosage	MK* MT†	No. of Mice	Blood Radioactivity (as % of initial) Mean	SD
I_2	1 μg		6	110	15.1
I_2	1 μg	10^{-4}*	6	280	36.2
I_2	1 μg	10^{-5}*	6	110	22.3
I_2	1 μg	10^{-4}†	6	228	23.5
I_2	1 μg	10^{-5}†	6	110	10.1
H_2O_2	10^{-5} M		6	150	22.3
H_2O_2	10^{-5} M	10^{-4}*	6	261	25.3
Pancreatin	0.1 mg		6	128	32.2
Pancreatin	0.1 mg	10^{-3}*	6	123	35.5

* MK = 1-Methyl-2-mercaptoimidazole (Merkazol).
† MT = Methylthiouracil.

TABLE 5. Effect of other reducing agents on the reduction in TSH activity (2 mU) by iodine

Pretreatment with	Dosage	No. of Mice	Substance Added	Blood Radioactivity (as % of initial) Mean	SD
I_2	1 μg	6		110	10.2
I_2	1 μg	6	$Na_2S_2O_3$ 10^{-1}	180*	12.3
I_2	1 μg	6	$Na_2S_2O_3$ 10^{-2}	111	10.1
I_2	1 μg	6	$Na_2S_2O_3$ 10^{-3}	115	11.2
I_2	1 μg	6	Ascorbic acid 10^{-2}	175*	19.0
I_2	1 μg	6	Ascorbic acid 10^{-3}	121	11.3

* Significantly different from the control at the 1% level.

TABLE 6. Degradation of exogenous thyrotrophin in newborn mice and the lack of an effect of 1-methyl-2-mercaptoimidazole

TSH Administered (mU USP)	Hours from TSH Injection to Sacrifice	No. of Mice per Group	Merkazol Injected 1 hr Prior to TSH	Blood Radioactivity (as % of initial)	
				Mean	SD
0	0	3		143	25.5
200	0	3		376	92.4
200	1	3		313	62.7
200	2	3		247	75.5
200	4	3		144	20.3
200	4	3	10^{-2} M (0.2 ml)	138	18.7

injection of 1-methyl-2-mercaptoimidazole (0.2 ml of 10^{-2} M) one hour prior to TSH did not reduce this loss of TSH activity (Table 6) at 4 hours.

Discussion

Inactivation of thyrotrophin by incubation with minced guinea pig liver (17), macerated bovine thyroid (1), sliced bovine thyroid (2), cultures of rabbit thyroid and lymphatic tissue (3) and sliced human thyroid tissue (4) has been reported. The present study, however, showed no significant degree of inactivation of TSH (2 mU) after incubation with thyroid (300 mg), liver, spleen, kidney and muscle slices of steers, guinea pigs, rats and mice. It should be emphasized that the amount of TSH used in this study was 10-0.1% the amount of TSH used in the studies noted above. The use of a more sensitive and, possibly, more specific assay technique may explain the discrepancy.

Following a diminution in TSH activity during incubation with iodine and peroxide, the addition of 1-methyl-2-mercaptoimidazole, methylthiouracil and other reducing agents reactivated some of the TSH. These results confirmed the finding (12, 13) that free iodine induced a change in TSH, resulting in a diminution in its activity. The inactivation of TSH by I_2 presumably was due to oxidation (possibly of SH groups) rather than iodination, since it was reversed by the addition of reducing agents. The inactivation of TSH by pancreatin probably was due to proteolysis of the TSH molecule and, therefore, would not be reversed by antithyroid compounds.

The mechanism by which the body inactivates TSH is not known. It has been suggested that the thyroid gland itself plays a major role in the inactivation of thyrotrophin (1, 3, 4). The evidence in support of this suggestion includes the inactivation of TSH by incubation with thyroid tissue. The present study does not confirm such an effect for bovine thyroid and lends support to the thesis (18) that the thyroid itself is not responsible to any large extent for the inactivation of thyrotrophin.

Although it has been suggested that thyroidal inactivation of thyrotrophin may be oxidative (19), the inactivation of thyrotrophin by treatment *in vitro* with oxidants has no necessary pertinence *in vivo*. The failure of antithyroid compounds, which are reducing agents, to reduce *in vivo* the inactivation of thyrotrophin in the mouse confirms a previous finding in the rat (18) and indicates that the reactivation of thyrotrophin (inactivated by rabbit thyroid tissue) by antithyroid reducing agents (19) could not be achieved *in vivo* in the mouse. It should be noted that a failure to find TSH in the assay system used four hours

after injection of thyrotrophin into the mouse does not necessarily mean that the TSH was inactivated for that mouse.

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Untersuchung sei der
der Wissenschaftlichen
..africhtig gedankt.

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Über die Ausscheidung des Thiosulfates und seine Bestimmung im Harn.

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Thiosulfate

Von
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(Eingegangen am 18. April 1935.)

Das Thiosulfat ist nicht nur ein physiologischer Harnbestandteil bestimmter Tiere, sondern vor allem auch toxikologisch und therapeutisch wichtig. Ohne selbst Giftwirkungen auszulösen, hat es die Fähigkeit, gewisse Stoffe zu entgiften. So werden oxydierende Substanzen reduziert (Jod, Jodate, Peroxyde u. a.), ferner Cyanide in Rhodanide übergeführt.

Zur Bestimmung des Thiosulfates neben Sulfiten und anderen reduzierenden Substanzen sind die üblichen Methoden nicht genügend spezifisch. Daher habe ich mich bemüht, eine einfache, dabei aber für biologische Zwecke hinreichend genaue und für Thiosulfat möglichst spezifische Bestimmungsmethode zu suchen. Als solche hat sich mir die für anorganische Lösungen von *Kurtenacker* und *Fritsch*[1], sowie von *Wollak*[2] beschriebene bewährt: Das Thiosulfat wird mit Jod oxydiert:

$$2\,Na_2S_2O_3 + J_2 = 2\,NaJ + Na_2S_4O_6.$$

Das gebildete Tetrathionat wird durch Behandlung mit Cyankalium in Rhodanid, Sulfat und Thiosulfat übergeführt:

$$Na_2S_4O_6 + 3\,KCN + H_2O = 2\,HCN + KCNS + K_2SO_4 + Na_2S_2O_3.$$

Letzteres — die Hälfte der ursprünglich vorhandenen Menge — wird mit Jod titriert. Hierbei stören reduzierende Substanzen nicht, da sie bei der ersten Jodtitration zerstört werden.

Für die Bestimmung im Harn muß diese Methode gewissen Änderungen unterworfen werden. Mit Jod langsam reagierende Substanzen zerstöre ich durch einen Jodüberschuß, welchen ich längere Zeit einwirken lasse. Der Jodüberschuß wird durch Natriumbisulfit gebunden. Weiter stört oft die starke Eigenfarbe. Tierkohle habe ich vermieden, weil viele Kohlen nach *Holbell*[3], wie auch nach eigenen Versuchen Thiosulfat adsorbieren. Als Entfärbungsmittel hat sich das neutrale

[1] *Kurtenacker* u. *Fritsch*, Zeitschr. f. anorg. u. allgem. Chem. 117, 202, 226, 1921. — [2] *Wollak*, Zeitschr. f. anal. Chem. 77, 401, 1929. — [3] *Holbell*, Klin. Wochenschr. 1925, Nr. 34.

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Bleiacetat bewährt, nur darf die Bleifällung wegen der Schwerlöslichkeit des Bleithiosulfats nicht zu Beginn, sondern erst nach der Jodoxydation vorgenommen werden. Basisches Bleiacetat ist ungeeignet. Der Bleiüberschuß wird als Sulfat ausgefällt.

Die Ausführung der Methode gestaltet sich folgendermaßen:

Man mißt den Harn (nicht mehr als $^1/_2$ bis 1 Millimol Thiosulfat) in ein Meßkölbchen von 250 ccm. Falls er alkalisch reagiert, fügt man einige Tropfen Essigsäure hinzu. Hierauf läßt man eine n/10 Jodlösung zunächst tropfenweise unter Umschwenken zufließen. Erst wenn die Reaktion langsam verläuft, fügt man noch einen größeren Jodüberschuß zu und läßt $^1/_2$ Stunde oder länger stehen. Falls die Jodfarbe rasch wieder verschwindet, gibt man noch mehr zu. Dann fällt man mit neutraler Bleiacetatlösung, füllt bis zur Marke auf und schwenkt gut um. Nach kurzem Absetzenlassen filtriert man durch ein trockenes Faltenfilter und fällt das Filtrat mit Kaliumsulfat in Substanz, bis ein weiterer Zusatz nach Absetzen keine Trübung mehr erzeugt. Jetzt läßt man wieder gut absetzen, am besten mehrere Stunden lang. Dann filtriert man durch ein trockenes Faltenfilter und versetzt 200 ccm des Filtrats mit etwas Jodkalium, Stärkelösung und einigen Tropfen Schwefelsäure. Tritt hierbei noch eine Blaufärbung auf — was gewöhnlich nicht der Fall ist, wenn nicht sehr viel Jod zugesetzt wurde —, läßt man aus einer Bürette 1%ige Natriumbisulfitlösung zufließen bis zur Entfärbung unter Vermeidung eines Sulfitüberschusses: eher darf ein *geringer* Jodüberschuß verbleiben, da dieser als Jodcyan gebunden wird. Ein unscharfer Umschlag kann durch zu geringe Azidität oder zu niedrige Jodkalikonzentration bedingt sein und wird durch Zusatz von Jodkalium oder Schwefelsäure behoben. Nun fügt man einen Tropfen Phenolphthaleinlösung zu, hierauf verdünntes (5%iges) Ammoniak bis zu schwacher Rosafärbung und endlich 5 ccm 10%ige Kaliumcyanidlösung (Überschuß), rührt um und läßt 15 Minuten unter dem Abzug stehen. Dann setzt man 25 ccm Schwefelsäure 1 : 3 zu und titriert mit n/100 Jodlösung, wenn nötig unter neuerlichem Stärkezusatz.

Bei der Berechnung ist zu beachten, daß auf diese Weise nur die Hälfte der ursprünglich vorhandenen Thiosulfatmenge bestimmt wird (siehe die obigen Gleichungen). 1 ccm n/100 Jodlösung entspricht also nicht 0,01, sondern 0,02 Millimol Thiosulfat oder 4,96 mg $Na_2S_2O_3 + 5\,H_2O$ = 2,70 mg $Na_2S_2O_3$ = 1,28 mg S. Da jede Volumenänderung vermieden wurde, wird die gefundene Zahl entsprechend dem aliquoten Anteil mit 250/200 = 1,25 multipliziert.

Auch Tetrathionat kann auf diese Weise bestimmt werden. Zur Unterscheidung vom Thiosulfat behandelt man eine zweite Probe erst mit Ammoniak und Cyanid und bestimmt hierauf das entstandene Thiosulfat nach der oben beschriebenen Methode. Liegt Tetrathionat vor, reagiert dieses bei dieser Vorbehandlung nach obiger Gleichung, wobei nur die halbe Menge Thiosulfat entsteht. Infolgedessen verbraucht man bei dieser zweiten Probe nur halb soviel Jod, wie bei der ersten Probe, ohne solche Vorbehandlung. Thiosulfat dagegen wird bei dieser Vorbehandlung nicht verändert, so daß man bei beiden Proben gleiche Werte findet. Bei Vorliegen von Thiosulfat und Tetra-

thionat nebeneinander entspricht die Differenz der beiden Bestimmungen multipliziert mit 2 dem Tetrathionat, der Rest dem Thiosulfat.

Probeanalysen von Harn mit Zusatz bekannter Thiosulfatmengen ergaben eine für biologische Zwecke ausreichende Genauigkeit. Folgende Tabelle gibt einige Analysen von je 100 ccm Menschenharn mit Thiosulfatzusätzen wieder:

Zugesetzt Millimol	Gefunden Millimol	Zugesetzt Millimol	Gefunden Millimol
0,1	0,88 0,90	0,50	0,47 0,475
0,25	0,22 0,225	0,75	0,69 0,71

Mit dieser Methode wurde die Ausscheidung von zugeführtem Natriumthiosulfat nachgeprüft, und ergab etwa übereinstimmende Werte mit den Angaben früherer Autoren (*Walko*[1], *Nyiri*[2]). Je höher die Dosis, um so größer auch der unverändert ausgeschiedene Anteil. Bei intravenöser Applikation wird wesentlich mehr ausgeschieden als bei subcutaner, bei dieser wieder mehr als bei peroraler Verabreichung

Thiosulfatausscheidung bei Kaninchen.

Tag	Thiosulfat-Zufuhr	Thiosulfat-Ausscheidung Millimol	Thiosulfat-Ausscheidung % der Zufuhr	Tag	Thiosulfat-Zufuhr	Thiosulfat-Ausscheidung Millimol	Thiosulfat-Ausscheidung % der Zufuhr
1	—	0,02		9	0,05 Millimol intravenös	0,01	0
2	—	0,017		10	—	0,032	
3	1,0 Millimol intravenös	0,465	45	11	2,0 Millimol intravenös	1,37	69
4	—	0,02		12	—	0,036	
5	—	0,03					
6	1,0 Millimol per os	0,175	14	1	2,0 Millimol subcutan	0,42	20
7	—	0,03		2	—	0,04	
8	0,2 Millimol intravenös	0,043	7	3	8,0 Millimol subcutan	2,30	28

Der im Harn nicht als Thiosulfat wiedergefundene Rest wird im Körper oxydiert und so gut wie quantitativ innerhalb 24 Stunden als Sulfat durch den Harn ausgeschieden. Tetrathionat, welches in vitro bei gelinder Oxydation aus dem Thiosulfat entsteht, konnte im Harn nach Thiosulfatgaben niemals nachgewiesen werden. Nach Schwefelinjektionen (Sufrogel) konnte ebenso wie in den Versuchen *Walkos* keine

[1] *Walko*, Arch. intern. Pharmakodyn. 4, 311, 1898. — [2] *Nyiri*, Klin. Wochenschr. 1923, Nr. 5; diese Zeitschr. 141, 160, 1923.

Vermehrung der Thiosulfatausscheidung beim Kaninchen beobachtet werden. — Beim Menschen wurden nach peroralen Thiosulfatgaben prinzipiell gleiche Verhältnisse gefunden. Die Thiosulfatmenge, welche eben noch quantitativ oxydiert wird, wurde hier zu 2 Millimol gefunden. Bei Zufuhr von 5 Millimol wurden 1 bis 2% im Harn wiedergefunden. Bei weiterer Steigerung der Dosis steigt auch der unverändert ausgeschiedene Prozentsatz an, bei Gaben von 30 Millimol wird etwa die Hälfte unverändert ausgeschieden. Dies ist wichtig, weil dadurch gezeigt werden kann, daß nach Zufuhr größerer Dosen dem Körper die für die entgiftenden Synthesen notwendigen Mengen zur Verfügung gestellt werden können. Die Geschwindigkeit der Entgiftungsreaktionen läßt sich dabei jedoch nicht steigern; deshalb muß auch bei genügendem Thiosulfatgehalt des Organismus die Funktion des Vasomotoren- und Atemzentrums durch geeignete Erregungsmittel bis zum Ablauf der Entgiftungsreaktionen aufrechterhalten werden[1].

[1] *Forst*, Verh. d. Deutsch. pharm. Ges. 1927, S. 150.

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thiosulfate

THE EXCRETION OF THIOSULFATE AND ITS DETERMINATION IN THE URINE

Thiosulfate is not only a physiologic component of the urine of certain animals, it is primarily of toxicologic and therapeutic importance. Without evoking toxic effects itself, it has the ability to detoxify certain substances. Thus, oxydizing substances are reduced (iodines, iodates, peroxides and others), cyanides are transformed to rhodanides.

For the determination of thiosulfate alongside of sulfites and other reducing substances, the commonly used methods are not specific enough. Therefore I have tried to find a method of determination that is simple, but sufficiently exact for biologic purposes and as specific as possible for thiosulfate. As such, the method of KURTENACKER and FRITSCH[1] for inorganic solutions and the one described by WOLLAK[2] have proven effective: thiosulfate is oxydized with iodine:

$$2\ Na_2S_2O_3 + I_2 = 2\ NaI + Na_2S_4O_6\ .$$

The formed tetrathionate is transformed into rhodanide, sulfate and thiosulfate by treatment with potassium cyanide:

$$Na_2S_4O_6 + 3\ KCN + H_2O = 2\ HCN + KCNS + K_2SO_4 + Na_2S_2O_3\ .$$

The latter - half of the original amount - is titrated with iodine. Reducing substances do not interfere here, since they are destroyed during the first iodine titration.

This method has to be subjected to certain changes for the determination in the urine. I destroy substances which react slowly with iodine,

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by an excess of iodine which I let react for some time. The iodine excess is bonded by sodium bisulfate. Furthermore, the strong color is often disturbing. I avoided animal charcoal, because many charcoals , according to HOLBELL[3] as well as to my own experiments, absorb thiosulfate. The neutral lead acetate has proven to be an effective decolorizing agent, but, because of the difficult solubility of the lead thiosulfate, the lead precipitation has to be started not at the beginning, but after the iodine oxydation. Basic lead acetate is not suitable. The excess lead is precipitated as sulfate.

THE PROCEDURE OF THE METHOD is as follows:

250 ccm of urine is poured into a buret (not more than ½ to 1 millimole of thiosulfate). If it reacts alkaline, a few drops of acetic acid are added. Then, while shaking, a N/10 iodine solution is added, in drops at first. When the reaction procedes slowly, a greater excess of iodine is added and then it is left to stand for ½ an hour or longer. If the iodine color disappears rapidly, more is added. Then one precipitates with neutral lead acetate solution, refills up to 250 ccm and shakes well. After a short sedimentation time it is filtered through a dry fluted filter and the filtrate is precipitated in substance with potassium sulfate, until further addition does not result in cloudiness after sedimentation. Now it is left to settle again, for several hours for best results. Then it is filtered through a dry fluted filter and 200 ccm of the filtrate is is combined with a little potassium iodide, starch solution and a few drops of sulfuric acid. If a blue coloration still appears - which is usually not the case when not much iodine has been added -, one adds from a buret a 1‰ solution of sodium bisulfate until discoloration, while avoiding an excess of sulfite; a minute excess of iodine may remain, since this is bonded as cyanogen iodine. A change that is not clear could be caused by

insufficient acidity or too low a concentration of potassium iodide and is remedied by addition of potassiun iodide or sulfuric acid. Now a drop of phenophthalein is added, after that diluted (5 %) ammonia up to slightly pink coloration, and finally 5 ccm of a 10 % potassium cyanide solution (excess); this is stirred and left under the hood for 15 minutes. Then 25 ccm of sulfuric acid 1:3 is added and it is titrated with N/100 iodine solution, if necessary with another addition of starch.

During calculation one has to take into consideration that only half of the originally present amount of thiosulfate is determined in this manner (see the above equations). 1 ccm of N/100 iodine solution therefore does not correspond to 0.01 but 0.02 millimole of thiosulfate or 4.96 mg $Na_2S_2O_3 + 5\ H_2O$ = 2.70 mg $Na_2S_2O_3$ = 1.28 mg S . Since every alteration of volume has been avoided, the resulting number is multiplied with 250/200 = 1.25, according to the aliquot portion.

Tetrathionate can be determined in this manner as well. In order to differentiate from thiosulfate, a second sample is first treated with ammonia and cyanide, and the resulting thiosulfate is then determined in the method described above. If tetrathionate is present, it reacts, with this pretreatment, in the above equation, whereby only half the amount of thiosulfate develops. Therefore, the amount of iodine used in this second sample is only half of that used in the first sample without such pretreatment. However, thiosulfate is not changed by this pretreatment, so that the resulting values are the same in both samples. When thiosulfate and tetrathionate are present next to each other, the difference between both determinations, multiplied by 2, corresponds to the tetrathionate, the remainder to the thiosulfate.

Sample analyses of urine with addition of known amounts of thiosulfate resulted in accuracy sufficient for biologic purposes. The following table

reproduces several analyses of @100 ccm of human urine with additions of thiosulfate:

added Millimole	found Millimole	added Millimole	found Millimole
0,1	0,88 0,90	0,50	0,47 0,475
0,25	0,22 0,225	0,75	0,69 0,71

With this method, the excretion of added sodium thiosulfate was examined and the resulting values generally concurred with those attained by previous authors (WALKO[4], NYIRI[5]). The higher the dosage, the greater the portion excreted unchanged. With intravenous application, considerably more is excreted than with subcutaneous application, and with the latter more than with oral administration.

Thiosulfate excretion in rabbits

day	Thiosulfate administration	Thiosulfate excretion Millimole	Thiosulfate excretion % of adm.	day	Thiosulfate administration	Thiosulfate excretion Millimole	Thiosulfate excretion % of adm.
1	—	0,02		9	0,05 Millimole intravenous	0,01	0
2	—	0,017		10	—	0,032	
3	1,0 Millimole intravenous	0,465	45	11	2,0 Millimole intravenous	1,37	69
4	—	0,02		12	—	0,036	
5	—	0,03					
6	1,0 Millimole per os	0,175	14	1	2,0 Millimole subcutaneous	0,42	20
7	—	0,05		2	—	0,04	
8	0,2 Millimole intravenous	0,043	7	3	8,0 Millimole subcutaneous	2,30	28

The remainder, not found as thiosulfate in the urine, is oxydized in the body and is excreted through the urine as sulfate within 24 hours in about the same quantity. Tetrathionate which develops in vitro with mild oxydation from thiosulfate, could never be demonstrated in the urine after administration of thiosulfate. After sulfur injections (Sulfrogel) as well

as in WALKOS' experiments, an increase of thiosulfate excretion could not be observed in rabbits. - In humans, basically equal proportions were found after oral administration of thiosulfate. The thiosulfate which is oxydized quantitatively, was found in the amount of 2 millimole. After administration of 5 millimole, 1 to 2 % were found again in the urine. On additional increase of dosage, the percentage of the unchanged excretion increases as well; with administration of 30 millimole, about half the amount is excreted unchanged. This is important because it demonstrates that, after administration of larger doses, the body can be supplied with the amounts necessary for the detoxifying syntheses. However, the speed of the detoxifying reaction cannot be changed by this; therefore, the vasomotor and respiratory functions must be sustained by suitable stimulants until completion of the detoxifying reaction, even in the presence of sufficient thiosulfate in the organism[6] .

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AN EVALUATION OF THE SINGLE INJECTION THIOSULFATE METHOD FOR THE MEASUREMENT OF EXTRACELLULAR WATER [1]

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The purpose of this paper is to examine the validity of the single injection thiosulfate method, proposed by Cardozo and Edelman (1) for the measurement of the extracellular fluid volume. The conclusion is that the method does not give a valid estimate of the extracellular fluid volume and probably does not even measure the volume of distribution potentially available to thiosulfate.

Cardozo and Edelman gave an accurately known amount of sodium thiosulfate (about 10 gm. of $Na_2S_2O_3 \cdot 5\ H_2O$ in 10 per cent solution) by intravenous injection over a period that varied from 7 to 16 minutes. They then collected venous blood samples at intervals and plotted the logarithms of the serum thiosulfate concentrations against time. The points usually fell on a straight line; this was extrapolated back to zero time, which arbitrarily was set as the moment the infusion had been started. If the points did not fall on a straight line, the test was discarded because of the variable clearance rate of the thiosulfate. The extrapolated value for serum concentration of thiosulfate (P_o) is presumed to be what the concentration would be if the thiosulfate were instantaneously injected and evenly distributed in its final volume of dilution. This extrapolated P_o is then divided into the total amount of thiosulfate injected to calculate the volume of distribution.

Aside from the fact that virtually none of the thiosulfate has been injected at this zero time, the method is based upon rather questionable assumptions, which will be discussed later.

In some of the evaluations of the single injection method that follow, comparisons were made between the volumes of distribution of thiosulfate and sucrose as calculated by different methods. The other procedures used were the infusion/slope method devised by Schwartz (2) and the calibrated infusion (IV minus UV) method of Deane, Schreiner, and Robertson (3).

MATERIAL AND METHODS

All experiments were done with hospitalized women. While they were not "normal," they were selected. Patients with any discernible cause for disturbance in hydration, such as fever, renal disease or heart disease, were excluded. All patients were less than age 50 years and most were in the third and fourth decades of life. Nearly all patients were fasting the day of the test. The few exceptions are those patients in whom simultaneous measurements were made of the volumes of distribution of sucrose and thiosulfate.

Reagent grade sodium thiosulfate ($Na_2S_2O_3$) was made up as a 6.7 per cent solution in either isotonic saline or 5 per cent dextrose. This gives the same thiosulfate concentration as Cardozo and Edelman used in their 10 per cent solutions of the hydrated salt. The solution was sterilized by Seitz filtration and given intravenously from a calibrated buret. The dose was roughly 0.5 ml. per pound of body weight and was given over a period of from 8 to 14 minutes. Collections of venous blood were begun 25 minutes after the end of the infusion and five samples were taken over the next 75 minutes. When the volume of distribution was measured by the infusion/slope method, the priming dose was followed by constant infusion of more of the same solution at the rate of 1.05 to 1.10 ml. per minute for periods of 2 to 3 hours. The constant infusion was given by a Bowman pump, which was recalibrated after each use.

The sucrose solutions, used in measuring the volume of sucrose distribution, were made up in 8 per cent concentration in either isotonic saline or 5 per cent dextrose. (The latter gives an appreciable blank in the chemical method for measuring sucrose).

In the calibrated infusion method this solution was given intravenously at the rate of 1.05 to 1.10 ml. per minute. Half-hourly collections of venous blood and of urine were begun 2 hours after the start of the infusion. After the collection of 3 or 4 blood and urine samples the thiosulfate was given for the single injection procedure.

In measuring the volume of sucrose distribution by the

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infusion/slope method, a priming dose of 8 per cent sucrose was followed by more of the same solution, as described for the administration of thiosulfate. Usually, simultaneous measurements were made of the volumes of sucrose and thiosulfate distribution, with sucrose and thiosulfate combined in the same solution. In the infusion/slope procedure, a minimum of three venous blood samples were taken during the constant infusion, the first being obtained about 90 minutes after the beginning of the infusion. Five more venous blood samples were collected over the 100-minute period following the end of the infusion. In both the single injection method and the infusion/slope method the test was discarded in any case where the logarithms of the serum thiosulfate or sucrose concentrations, plotted against time, were not closely fitted by a straight line. Also no calculations were made from the data obtained by the infusion/slope method unless the serum concentrations of thiosulfate and sucrose had attained constancy.

The solutions injected were hypertonic, but the volumes were small in comparison to the volumes of distribution, being about 1 per cent in the single injection procedure. In the constant infusion methods the rate of administration was 1.07 ml. per minute, so that in 3 hours the total volume given was of the order of 2 to 3 per cent of the volume of distribution. Meanwhile renal excretion and metabolism of the solutes (except for sodium chloride) kept pace with the infusion rate in the cases studied by the infusion/slope method. Furthermore, the procedure was the same in all experiments except that solutions were sometimes given in 0.9 per cent saline and sometimes in 5 per cent dextrose.

For chemical analyses, two samples of the infusion solution were diluted (1:1,000 for thiosulfate and 1:2,000 for sucrose); urine samples were suitably diluted (usually 1:500 or 1:1,000) and serum samples were prepared by precipitating the proteins. For thiosulfate the serum was diluted 5 times in the protein precipitation by tungstic acid and for sucrose it was diluted 10 or 20 times in precipitating the proteins by zinc hydroxide. All samples were analyzed in duplicate or quadruplicate. Thiosulfate was measured by the iodine titration method of Brun (4) and sucrose by the resorcinol method of Roe, Epstein, and Goldstein (5), using a Beckman DU spectrophotometer. Blood, urine, water and reagent blanks were analyzed with all measurements. When sucrose infusions were given in 5 per cent dextrose, an aliquot of the original dextrose solution was diluted 1:2,000, as was the infusion solution. These dextrose blanks were carried through the sucrose analyses and their optical densities were substracted from the optical densities of the infusion samples. This correction factor averaged about 4 per cent of the readings of the infusion samples.

RESULTS

Experimental alteration of the renal clearance rate

The first experiments with the single injection method were designed to find what effect changing the slope of the exponential disappearance rate of serum thiosulfate would have upon P_o and, therefore, upon the calculated volume of thiosulfate distribution. The slope of the exponential curve was changed in two ways: (*a*) by pre-medication with carinamide, which partially blocks the renal tubular excretion of thiosulfate (6), and (*b*) by intravenous theophylline ethylenediamine, which increases the glomerular filtration rate (7) and therefore the renal excretion rate of thiosulfate. More than half of the experiments could not be used because of the irregular effect of the drugs over the 100-minute period following the infusion of the thiosulfate. (This irregularity is reflected in a non-linear plot of the logarithms of the decreasing serum thiosulfate concentrations against time. Cardozo and Edelman wrote that such cases can not be used, for obvious reasons). By trial and error the best dose of carinamide was found to be 4 gm. given at 6:00 A.M. and 2 gm. given at 9:00 A.M., with the thiosulfate infusion given at 10:00 A.M. In the theophylline ethylenediamine experiments, 0.25 gm. of the drug was given intravenously about 10 minutes before the thiosulfate infusion and this was followed with a continuous infusion of another 0.25 gm. in 250

TABLE I

Experimental alteration of the clearance rate of serum thiosulfate by carinamide or theophylline, showing the times at which the experimental curves intersect with the curves found without medication and the percentage difference in calculated thiosulfate space resulting from the change in extrapolated P

Experiment:	1a	1b	2	3	4	5	6	7	8	9
Minutes after zero time at which curves cross:	29	30	2	No	18	21	27	29	16	36
Percentage difference in thiosulfate space resulting from changed excretion rate:	10	24	7	7	19	18	11	13	17	30

ml. of 5 per cent dextrose over the 100-minute period of the test.

Nine successful experiments were done in fasting women selected with special care as to "normality." In all cases the thiosulfate was given in 5 per cent dextrose. The order in which the experiments were done was varied from case to case and the tests were done on successive days in any one patient. All exponential disappearance curves for serum thiosulfate were fitted by the method of least squares to ensure objectivity. If the single injection method is valid, the disappearance curves should intersect at zero time, that is, P_o should be constant because the amount of thiosulfate given was constant and the potential volume of distribution should not vary much from test to test (see Discussion).

Table I shows that the disappearance curves did not intersect at zero time; in one case the lines did not cross at all and in the majority of cases the intersections occurred at 20 to 30 minutes after zero time. The experimental alteration of the renal clearance rate of thiosulfate changed the calculated volume of distribution in every case, the change being from 7 to 30 per cent. One of the experiments is shown in Figure 1, where the lines cross at 29 and 30 minutes. In this particular case, the thiosulfate space calculated from P_o with carinamide is 138 per cent of that calculated from P_o with theophylline ethylenediamine; the calculated volumes were 11.7 and 8.5 liters, respectively. This is the only experiment, in more than 20, in which three good disappearance curves were obtained. In the other eight cases reported there were two good curves, but the third with either carinamide or theophylline ethylenediamine departed from linearity on the semilog plot. Of the eight, five compare curves with no medication to curves with carinamide and three compare curves with no medication to those with theophylline ethylenediamine.

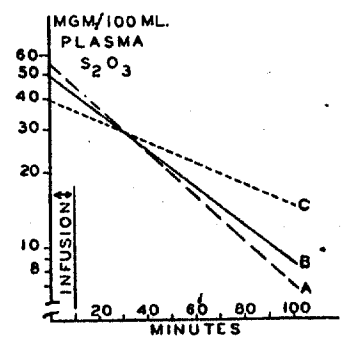

FIG. 1. THE EFFECT OF ALTERING THE RENAL EXCRETION OF THIOSULFATE UPON THE VALUE OF EXTRAPOLATED P_o

A = pre-medication with theophylline ethylenediamine, B = basal measurement with no medication, C = pre-medication with carinamide. Note that P_o is a function of the clearance rate of serum thiosulfate.

It would appear that the value for P_o is a function of the clearance rate of thiosulfate. Experimental alteration of the renal clearance rate changes P_o and results in changes in a predictable direction of the calculated volume of thiosulfate distribution. Decreasing the slope of the curve increases the calculated volume of distribution while increasing the slope of the curve decreases the calculated volume.

Comparison of thiosulfate spaces measured by the infusion/slope method to those calculated from the single injection method

The single injection method was compared with the infusion/slope method in six fasting patients, with alternation of the method first applied. In any one patient the tests were done on successive days. All solutions were given in 0.9 per cent saline.

The pairs of measurements did not check. In five of the six cases the volume of distribution for thiosulfate was considerably greater as measured by the infusion/slope method than as estimated from the single injection method. The results, in Table II, show that the average discrepancy was 30 per cent and ranged from 0 to 53 per cent.

Comparison of the thiosulfate space as estimated by the single injection method to the volume of sucrose distribution

In 18 patients the sucrose space was measured by the calibrated infusion method and immediately thereafter a single injection of sodium thiosulfate was given for the estimation of the volume of thiosulfate distribution. No experiment is included unless equilibrium of sucrose was established (that is, essentially constant volumes of

TABLE II

Comparison of measurements of the thiosulfate space by the methods of Cardozo and Edelman and of Schwartz and of the slopes of the serum thiosulfate disappearance curves in the two methods

Case:	1	2	3	4	5	6	Mean
Body weight, *Kg.*:	55.0	65.0	69.1	63.7	56.8	50.9	
Thiosulfate space							
Cardozo and Edelman:	9.8	7.8	7.9	8.2	8.8	10.6	8.85
Schwartz:	12.0	11.9	11.5	11.5	11.6	10.6	11.50
Ratio, Schwartz to Cardozo and Edelman methods:	1.22	1.53	1.46	1.40	1.32	1.00	1.30
Ratio, disappearance curve slopes, Cardozo and Edelman to Schwartz methods:	1.60	1.96	2.14	1.86	1.67	1.87	1.85

distribution in successive periods). In all but two cases the solutions were given in 5 per cent dextrose. The calculations from the data for the single injection method gave apparent volumes of distribution for thiosulfate that averaged about 35 per cent less than the sucrose space, as also was found by Ikkos (8). The apparent volume of thiosulfate distribution, as calculated by the single injection method, bore no constant relationship to the volume of distribution of sucrose, as the scattering of points in Figure 2 shows. Of course, sucrose may not measure the extracellular fluid volume but under the conditions of the experiments a steady state had been attained and a reproducible space had been measured. (This often was not

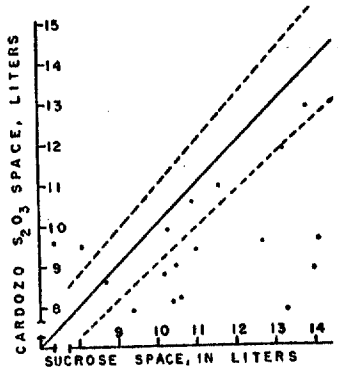

FIG. 2. COMPARISON OF THE THIOSULFATE SPACE, AS CALCULATED BY THE CARDOZO AND EDELMAN SINGLE INJECTION METHOD, TO THE SUCROSE SPACE MEASURED ALMOST SIMULTANEOUSLY

The solid line is the line of identity and the broken lines represent plus and minus 10 per cent.

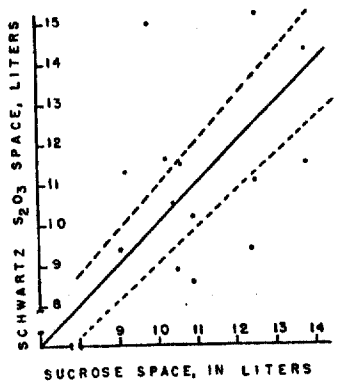

FIG. 3. COMPARISON OF THE THIOSULFATE SPACE, AS MEASURED BY THE SCHWARTZ INFUSION/SLOPE METHOD, TO THE SUCROSE SPACE MEASURED SIMULTANEOUSLY

The solid line is the line of identity and the broken lines represent plus and minus 10 per cent.

true in Ikkos' experiments, for in about 80 per cent of his cases the apparent volume of sucrose distribution increased steadily with time. Arbitrarily, he took the apparent volume of distribution at 150 minutes for comparison with apparent volumes of thiosulfate distribution.)

Comparison of the thiosulfate space measured by the infusion/slope method with the volume of distribution of sucrose

Measurements of the volume of distribution of thiosulfate, made with the infusion/slope method, were compared with simultaneous measurements of the volume of sucrose distribution in 14 patients. In nine of these the volume of sucrose distribution

TABLE III

Comparison of thiosulfate and sucrose spaces measured simultaneously by the infusion/slope method of Schwartz

Case:	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sucrose space, *l.*	10.4	13.7	9.0	10.9	10.6	12.5	10.2	10.5	13.8	12.5	10.9	9.2	12.4	9.8
Thiosulfate space, *l.*	10.5	14.3	9.4	10.2	11.5	11.1	11.6	8.9	11.5	15.1	8.6	11.3	9.4	15.1
Percentage deviation of thiosulfate space from sucrose space	1	4	5	6	8	11	14	15	16	21	21	23	24	54

was measured both by the infusion/slope method and by the calibrated infusion method; in the other five, one or the other of the methods was used.

Figure 3 shows that there was no consistent relationship between the thiosulfate and sucrose volumes of distribution, although the average difference was very small. Relative to the volume of sucrose distribution, the thiosulfate space varied from plus 54 to minus 24 per cent, as shown in Table III. While the average ratio is close to 1.0, the wide scatter of the points in Figure 3 indicates that this may be fortuitous.

Comparison of volumes of sucrose distribution measured simultaneously by the infusion/slope and calibrated infusion methods

Inasmuch as the calculations of the volume of thiosulfate distribution did not agree when the single injection and the infusion/slope methods were applied successively in individual patients, it is of interest to compare the infusion/slope method to some other procedure. Therefore simultaneous measurements of the volume of sucrose distribution were made by the infusion/slope and the calibrated infusion methods. Here the sucrose was given in 0.9 per cent saline and the priming dose of sucrose was added in the "IV" factor of the IV minus UV formula of the calibrated infusion method.

As Figure 4 shows, these methods, quite different in principle, gave very similar volumes for the sucrose distribution. All but 1 of the 10 pairs of measurements checked within 10 per cent. The points shown in the graph are symmetrically distributed about the line of identity and the average difference between the simultaneous measurements is zero. Here, the average ratio of 1.0 may not be fortuitous for, in contrast to the comparison of volumes of thiosulfate and sucrose distribution, the pairs of measurements check closely.

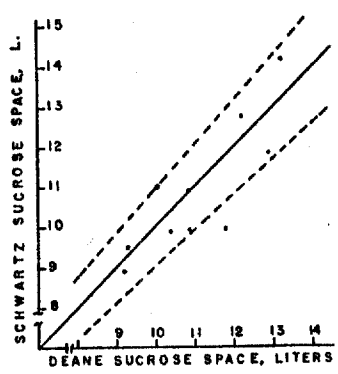

FIG. 4. COMPARISON OF SIMULTANEOUS MEASUREMENTS OF THE SUCROSE SPACE MADE BY THE SCHWARTZ INFUSION/SLOPE METHOD AND BY THE DEANE, SCHREINER, AND ROBERTSON CALIBRATED INFUSION (IV MINUS UV) METHOD

The solid line represents identity and the broken lines plus and minus 10 per cent.

DISCUSSION

The apparent advantage of the single injection method lies in its circumvention of the constant infusion and urine collections. However, its theoretic bases do not seem sound. Thiosulfate is known to enter the red blood cells (1) and if it enters these cells it may enter others. Gilman, Philips, and Koelle (9) found that in dogs about one-fourth of the injected thiosulfate cannot be recovered and they concluded, on good evidence, that most of the irretrievable fraction disappeared during and just after the infusion. The incomplete recovery was confirmed, for man, by Cardozo and Edelman. This together with the odor of the urine after thiosulfate injection suggest that

thiosulfate is metabolized in the body. Inasmuch as it is relatively stable in blood, *in vitro*, such metabolism probably occurs in cells.

The extrapolation of the decremental curve back to zero time implies questionable assumptions. One assumption is that thiosulfate disappears at the same exponential rate from the beginning of the infusion to the end of the blood collections (100 minutes). The assumed starting point for this exponential decrement in plasma concentration is based not upon the amount already injected at zero time, but upon the total amount to be injected over the next 10 or more minutes. This does not seem reasonable. Also, even when such injections are given within seconds, the early portion of the decremental curve is not adequately described by the course of the exponential limb, as shown by Sheppard, Overman, Wilde, and Sangren (10) and by Sapirstein, Buckley, and Ogden (11). Another assumption is shown graphically in the first figure in the paper by Cardozo and Edelman. The early portion of the decremental curve lies above the extrapolated exponential limb (as in all such cases); Cardozo and Edelman factor this early portion of the curve into two straight (exponential) lines by the standard procedure of subtracting the extrapolated limb from the observed points. From this they draw the conclusion that two processes are going on in the early minutes: (*a*) equilibration of the thiosulfate throughout plasma and interstitial fluid, and (*b*) clearance of thiosulfate at a uniform exponential rate, by renal excretion and metabolism. However, the rapid component of the decremental curve, in man, may represent the immediate loss of thiosulfate, which Gilman, Philips, and Koelle found in dogs. If the steep curve represents equilibration of thiosulfate throughout all of extracellular water, as Cardozo and Edelman suggest, the distribution must occur with almost incredible rapidity—within 10 minutes, as they say. In view of the time (30 to 120 minutes) required for sucrose to attain its maximum volume of distribution in normal subjects, it does not seem likely that thiosulfate would become evenly distributed in so short a time. The diffusion coefficients can not be specified exactly because of concentration effects. Cardozo and Edelman cited 0.68 cm.^{2} per day for thiosulfate and Raisz, Young, and Stinson (12) cited 0.55 cm.^{2} per day for sucrose; the source in both cases is the International Critical Tables.

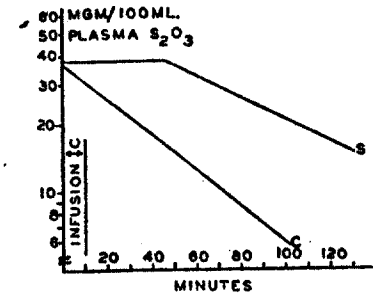

FIG. 5. COMPARATIVE RATES OF CLEARANCE OF SERUM THIOSULFATE IN THE SCHWARTZ INFUSION/SLOPE METHOD (S) AND CARDOZO AND EDELMAN SINGLE INJECTION METHOD (C)

The constant infusion of the Schwartz method had gone on for 2 hours before the beginning of the time shown in the graph, allowing time for the establishment of a steady state. "Infusion C" refers to the period of infusion in the Cardozo and Edelman method.

One observation is consistent with the interpretation that the steep component may represent immediate loss of thiosulfate. Figure 5 and the last line in Table II show that the slope of the serum thiosulfate disappearance curve was nearly twice as great in the single injection procedure as in the infusion/slope method. This suggests that the prolonged infusion of the latter method may have "saturated" some space not so "saturated" in the much shorter period of the single injection procedure, where thiosulfate may still be leaving the blood stream by diffusion into extravascular fluid throughout the whole course of observation. Possibly this "saturation" occurring in the prolonged period of constant infusion represents the attainment of concentration equilibrium between plasma and extravascular fluid available to thiosulfate. There are other possible factors in the slower rate of clearance from the plasma, after prolonged infusion. One might be increased feedback from extravascular sources; this, again, would suggest that in the single injection method the extravascular sources had not been brought into equilibrium with plasma water. Another possibility is that the rate of metabolism of thiosulfate slows down after prolonged infusion.

If a large proportion of the injected thiosulfate does disappear quickly, in man, then obviously the

dividend in the equation for the single injection method is too great and, of itself, should give calculated volumes of distribution that are too large. However, the volume calculated from this equation averages 30 per cent less than the measurements of the thiosulfate space by the infusion/slope method. This suggests that the divisor (P_0) in the equation for the single injection method is too large and in even greater error than is the dividend. This could result from the failure of thiosulfate to diffuse into and attain equilibrium in its potential total volume of distribution within the short period of time encompassed in the test. Thus, at least two variable and partially compensatory errors fortuitously result in calculations of an apparent thiosulfate space that often falls within the range described as normal for the volume of distribution of inulin.

The experimental alteration of the renal clearance of thiosulfate changes P_0 and therefore the apparent volume of thiosulfate distribution as calculated in the single injection method. Since this work was completed, Peterson, O'Toole, and Kirkendall (13) have reported marked variations in the sucrose space of man, during a 24-hour period. Inasmuch as the present experiments, in which the renal clearance rate of thiosulfate was varied, were done on successive days, the assumption that the space should be nearly constant from day to day may be in error. In the three patients studied in regard to such reproducibility there was less than 5 per cent variation in the calculated volume of the thiosulfate distribution, from day to day. No more cases were studied because of the relative constancy reported for repeated estimations by Cardozo and Edelman (1), Ikkos (8), Raisz, Young, and Stinson (12) Becker and Joseph (14) and others. The reproducibility seems to be within the limits of analytic error. Even if it were not, changing the renal clearance rate of thiosulfate has invariably changed the calculated volume of distribution in a predictable direction.

SUMMARY

Experimental alteration of the renal excretion rate of thiosulfate, by pre-medication with carinamide or theophylline ethylenediamine, changed significantly the volume of thiosulfate distribution as calculated by the single injection method.

The volume of thiosulfate distribution, as measured by the infusion/slope method, averaged 30 per cent greater than the space calculated by the single injection method.

The thiosulfate space, measured by the infusion/slope method or calculated by the single injection method, bore no constant relation to the volume of sucrose distribution.

Simultaneous measurements of the sucrose space by the infusion/slope and by the calibrated infusion methods gave consistent results.

The failure of the single injection method to give thiosulfate spaces even approximating those measured by the infusion/slope method strongly suggests that the single injection method does not measure the volume of thiosulfate distribution.

It is concluded that the single injection method is not sound theoretically and that apparent volumes of distribution calculated from P_0 have no significance as spaces. They only fortuitously come close to volumes measured by sucrose or inulin because of multiple, variable and partially compensatory errors.

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LA PRÉSENCE CONSTANTE DU THIOSULFATE DANS L'URINE DES ANIMAUX SUPÉRIEURS ET SA SIGNIFICATION PHYSIOLOGIQUE

S2
Thiosulfate

PAR

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(6—VII—1944)

Le thiosulfate de l'urine des animaux supérieurs a fait déjà l'objet d'un certain nombre d'investigations; tout d'abord, en ce qui concerne sa présence, celle-ci a été constatée, de façon plus ou moins régulière, chez les espèces suivantes:

le Chat: dès 1867, SCHMIEDEBERG [1]) a observé que l'urine du chat renferme constamment du thiosulfate, en quantité relativement abondante; cette observation a été fréquemment confirmée dans la suite, notamment par MEISSNER [2]) qui a précisé que, d'une façon générale, l'urine du chat mâle est sensiblement plus riche en thiosulfate que l'urine de la chatte, la comparaison étant faite sur des animaux soumis au même régime alimentaire.

le Chien: d'après SCHMIEDEBERG [1]), l'urine du chien renferme souvent, mais pas toujours, du thiosulfate. Cette inconstance de la présence de thiosulfate dans l'urine du chien ressort également des expériences de SALKOWSKI, lequel tantôt n'y trouve pas de thiosulfate ([3]) et tantôt en trouve ([4]).

le Lapin: la présence de thiosulfate dans l'urine du lapin dépend, d'après SALKOWSKI [5]) considérablement du régime auquel il est soumis: inexistant chez l'animal nourri avec des carottes, le thiosulfate apparait en quantité notable chez l'animal ingérant des choux. Une observation analogue a été faite par DEZANI [6]).

l'Homme: d'après les investigations de SCHMIEDEBERG [1]), de KÜLZ [7]), de PRESCH [8]), l'urine de l'homme ne renferme pas normalement de thiosulfate en quantité appréciable; PRESCH précise en disant que s'il en existe, sa quantité, exprimée en thiosulfate de sodium, est inférieure à 10 mg par litre, quantité qui correspond à la limite de sensibilité de la méthode de détection utilisée par cet Auteur. Toutefois, la présence de thiosulfate en quantités notables a été mise en évidence dans deux cas particuliers: celui d'un homme cystinurique, cité par SPIEGEL [9]) et celui d'un typhique étudié par STRÜMPELL [10]); d'autre part, VAN EEKELEN [11]) attribue au thiosulfate une partie de l'aptitude de l'urine de l'homme à réduire le 2,6-dichlorophénol-indophénol en milieu acide.

En ce qui concerne la source du thiosulfate de l'urine, il est rapidement apparu que celle-ci doit être finalement constituée par les dérivés sulfurés ingérés avec leur nourriture par les animaux. On a vu que l'absence ou la présence de thiosulfate dans l'urine du lapin dépend de son alimentation: carottes ou choux blancs; or la teneur en soufre du chou blanc est, d'après SALKOWSKI, cinq fois plus élevée que celle des carottes *), et cet Auteur a préparé ([12]) un extrait aqueux de chou blanc, riche en soufre, et qui provoque chez le lapin, comme le chou blanc, une abondante excrétion de thiosulfate. D'autre part, un lapin soumis à un régime qui normalement ne produit pas l'apparition de thiosulfate dans l'urine, se met à en excréter des quantités importantes si l'on ajoute à ce régime de la taurine ([14]) ou de la cystine ([15]). Enfin, d'après EMMERIE et VAN EEKELEN ([16]), VAN EEKELEN et EMMERIE [17]), HEINEMANN [18, 19]), tout accroissement dans l'ingestion de protéines, donc de soufre organique, provoque, chez différents animaux supérieurs (chat, chien, homme), un accroissement parallèle de l'excrétion du thiosulfate urinaire.

En ce qui concerne l'origine du thiosulfate de l'urine, malgré l'observation faite par

*) Des analyses récentes de MASTERS et Mc CANCE [13]) indiquent pour les teneurs en soufre total de ces aliments des valeurs qui, exprimées en mg pour 100 g de matière fraîche, sont de 100 à 200 pour le chou, et de 5 à 10 pour la carotte.

L'intérêt de cette manière de voir est d'ailleurs accru par l'aptitude que possèdent les animaux supérieurs à oxyder le thiosulfate en sulfate ([29, 30, 31]), oxydation que Pirie [32]), a, d'autre part, mise en évidence avec des coupes de tissus.

Il convient de remarquer ici qu'un tel mécanisme de la genèse des sulfates à partir du soufre organique diffère profondément de celui qu'imagine Pirie [33]), ou de celui qu'admettent Medes et Floyd [34]); en effet, alors que dans l'hypothèse actuelle le soufre n'est oxydé qu'après avoir été détaché de la molécule organique à laquelle il était lié, ces Auteurs pensent que l'oxydation du soufre se fait, en partie au moins, sur l'atome de soufre toujours attaché à la molécule organique, donnant ainsi naissance à une série de corps intermédiaires comme l'acide cysteine sulfénique, l'acide cystéine sulfinique et l'acide cystéinique. Ces différents mécanismes ne s'excluent d'ailleurs pas, et on peut très bien imaginer qu'ils puissent fonctionner ensemble et qu'ils aient des réactions communes.

Pour décider de la validité du mécanisme discuté ici, il nous a paru important de chercher à connaître, avec plus de sécurité que cela ne l'avait jamais été fait jusqu'ici, l'origine du thiosulfate de l'urine des animaux supérieurs. En effet, dans ce mécanisme, le thiosulfate apparait comme corps intermédiaire; et quoique normalement, la brièveté de son existence implique la faiblesse de sa concentration dans les tissus, donc dans l'urine, il n'est pas impossible que cette concentration, qui dépend des vitesses respectives des réactions C et D, ne soit suffisante pour pouvoir être mesurée avec précision, et ne soit susceptible d'être augmentée ou diminuée par des modifications judicieuses des conditions physiologiques dans lesquelles l'animal en expérience se trouve placé.

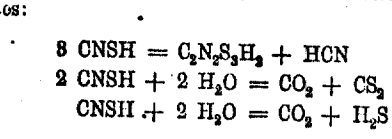
Mais, avant d'entreprendre ce genre de recherches, il était indispensable de disposer d'une méthode convenable de dosage du thiosulfate urinaire. Le présent travail comporte ainsi les divisions suivantes:

I. Mise au point d'une microméthode spécifique de dosage du thiosulfate dans l'urine.
II. Dosage du thiosulfate dans l'urine de diverses espèces animales.
III. Etude de l'origine du thiosulfate urinaire.

1. Méthode de dosage du thiosulfate dans l'urine des animaux supérieurs.

Si on néglige les anciennes méthodes de dosage du thiosulfate dans l'urine, dont le manque de finesse est la cause de la discordance apparente entre les résultats anciens et certains de ceux que nous apportons ici, on ne trouve guère que les méthodes indiquées par Zörkendorfer [31]) et par Dezani [6]) qui semblent pouvoir être utilisées pour le but que nous poursuivons. Toutefois, étant donné leurs divers inconvénients, nous avons employé ici la microméthode que nous avons décrite précédemment ([35]), après l'avoir adaptée aux cas actuel. Cette méthode est basée sur la décomposition des thiosulfates en soufre et en hydrogène sulfuré, en milieu acide; son adaptation était rendue nécessaire par la présence fréquente de sulfocyanure dans l'urine des animaux supérieurs.

On sait en effet ([36]) que l'acide sulfocyanhydrique en solution aqueuse se décompose facilement par chauffage; selon sa concentration, la réaction correspond à l'une ou à plusieurs des équations suivantes:



$$3\ CNSH = C_2N_2S_3H_2 + HCN$$
$$2\ CNSH + 2\ H_2O = CO_2 + CS_2 + 2\ NH_3$$
$$CNSH + 2\ H_2O = CO_2 + H_2S + NH_3$$

et l'hydrogène sulfuré susceptible d'être ainsi formé, venant s'ajouter à celui qui provient de la décomposition des thiosulfates est une cause d'erreur importante dans le dosage des thiosulfates. Nous avons tout d'abord cherché quelle est la fraction d'hydrogène sulfuré produite à partir de quantités déterminées de sulfocyanure, lorsque l'on opère avec notre appareil dans des conditions identiques à celles où nous nous plaçons pour effectuer le dosage du thiosulfate.

SCHMIEDEBERG [1]) que chez le chat soumis à un jeune de cinq jours, puis à une alimentation pauvre en substances sulfurées pendant quatorze jours, la chute de l'excrétion urinaire du thiosulfate ne se manifeste que tout à fait vers la fin de l'expérience, observation qui a conduit SCHMIEDEBERG à la conclusion que le thiosulfate devait provenir ici de la dégradation de constituants des tissus de l'animal, on admet généralement avec ROTHERA [20]) que cette origine est due plus ou moins directement à des actions bactériennes s'exerçant, dans le tube digestif, sur les différents dérivés sulfurés apportés par les aliments, soit que ces actions bactériennes aboutissent elles-même à la formation de thiosulfate, plus ou moins absorbé par l'organisme, soit qu'elles engendrent de l'hydrogène sulfuré qui passe, lui aussi, dans l'organisme et dont celui-ci se débarrasse en l'oxydant en thiosulfate; le thiosulfate est alors plus ou moins oxydé en sulfate, et l'animal excrète ce qui en reste par voie urinaire. Cette manière de voir, soutenue récemment encore par DEZANI [6]), repose essentiellement d'une part sur des observations de SALKOWSKI [21]) d'après lequel le thiosulfate se rencontre non seulement dans l'urine du chien, mais aussi dans ses fæcès, les variations de la teneur en thiosulfate étant à peu près parallèles dans ces deux matières, d'autre part sur celles de WOHLGEMUTH [22]) qui, ayant ajouté de la cystine à de la viande hâchée puis soumise à l'action des bactéries de la putréfaction, a constaté la formation de quantités notables de thiosulfate, et de NEUBERG et RUBIN [23]) qui ont également observé la formation de thiosulfate par les bactéries de la putréfaction exerçant leur action sur la taurine ou sur l'acide chondroïtine sulfurique, d'autre part enfin sur l'aptitude que possèdent d'assez nombreuses bactéries intestinales à provoquer la formation d'hydrogène sulfuré à partir de la cysteine ou de la cystine.

L'ensemble des données généralement admises, à l'heure actuelle, concernant le thiosulfate de l'urine des animaux supérieurs, se résume donc ainsi: présence constante chez le chat, irrégulière chez le chien, et plus encore chez le lapin; absence, sauf exception, chez l'homme; origine essentiellement bactérienne.

Des faits nouvellement découverts plaident toutefois en faveur d'une possibilité de formation du thiosulfate au sein même des tissus des animaux supérieurs, s'accordant ainsi avec les conclusions anciennes de SCHMIEDEBERG: ce sont, d'une part l'existence, dans le foie et à un moindre degré dans le pancréas et le rein, de deux ferments, la cystéinedésulfurase ([24]) et l'homocystéinedésulfurase ([25]) qui provoquent la formation abondante d'hydrogène sulfuré à partir des deux aminoacides sulfurés correspondants; et d'autre part la facilité avec laquelle l'hydrogène sulfuré est oxydé en thiosulfate par l'oxygène, soit par un catalyseur organique présent dans le foie et quelques autres organes des animaux supérieurs ([26]), soit même par des catalyseurs minéraux ([27]). On peut, en outre, rapprocher de ces faits la formation de thiosulfate par oxydation de la cystéine au moyen de l'eau oxygénée ([9]).

On est ainsi conduit à supposer que, contrairement à l'opinion admise jusqu'ici, la formation de thiosulfate chez les animaux supérieurs est, pour une part importante au moins, d'origine animale et fait partie d'un mécanisme d'ensemble assurant l'oxydation en sulfates du soufre organique ingéré, soufre organique qui consiste, pour la plus grande part, en cystine, cystéine et en méthionine. Ce mécanisme, encore hypothétique, pouvant se représenter de la façon suivante:

$$\text{A)}\quad \begin{array}{lll} \text{Cystine} & + \; XH_2 & \longrightarrow \text{Cystéine} \\ \text{Méthionine} & + \; YH & \longrightarrow \text{Homocystéine} \end{array}$$

$$\text{B)}\quad \left.\begin{array}{ll} \text{Cystéine} & \xrightarrow{\text{Cystéinedésulfurase}} \\ \text{Homocystéine} & \xrightarrow{\text{Homocystéinedésulfurase}} \end{array}\right\} \rightarrow H_2S$$

Une partie de H_2S produit ainsi participe probablement aux réactions de transsulfuration (synthèse de l'homocystéine à partir de la cystéine), discutée par FROMAGEOT et DESNUELLE [28]); le reste réagit selon:

$$\text{C)}\quad H_2S \longrightarrow S_2O_3^{=}$$

$$\text{D)}\quad S_2O_3^{=} \longrightarrow SO_4^{=}$$

Pour ce faire, nous introduisons dans un ballon jaugé de 50 cc un volume connu d'une solution de CNSK 0,02 N; nous ajoutons 20 cc d'eau distillée et 5 cc de la solution de sulfate de zinc et de sulfate de cadmium, puis nous complétons au trait avec de l'eau distillée. Il est inutile d'éliminer le léger précipité d'hydroxyde apporté par la solution des sels de zinc et de cadmium. Le contenu du flacon jaugé est alors versé quantitativement dans le ballon A de l'appareil servant au dosage des thiosulfates. Nous faisons arriver un courant d'azote, puis chauffons à l'ébullition. Nous introduisons alors, à l'aide de l'entonnoir à robinet *b*, 20 cc de la solution d'acide sulfurique (environ 18 N); nous maintenons l'ébullition 30 minutes et dosons, après centrifugation et lavage du précipité des sulfures de zinc et de cadmium formés dans le lavour F, l'hydrogène sulfuré provenant de la décomposition du sulfocyanure. Les résultats obtenus sont donnés dans le tableau I.

TABLEAU I

A = quantité de S en γ correspondant à la quantité de CNSK traitée.
B = quantité de S en γ obtenu sous forme d'H_2S.

Volume de la solution de CNSK 0,02 N traitée (cc)	A	B	$\frac{B.100}{A}$
0,25	160	22	14
0,50	320	33	10
1,00	640	77	12
1,50	960	97	10
2,00	1280	115	9
5,00	3200	317	10
7,50	4800	475	9,9
10,00	6400	672	10,5

Ces chiffres montrent que, dans les conditions réalisées ici, 10 % environ du soufre initialement présent sous forme de sulfocyanure sont transformés en hydrogène sulfuré.

Le dosage des thiosulfates en présence de sulfocyanures repose sur le principe suivant: On dose en premier lieu les thiosulfates et la fraction des sulfocyanures décomposés, en opérant selon le mode habituel; on obtient ainsi (calculée en thiosulfate de sodium et en sulfocyanure de potassium), la somme $S_2O_3Na_2$ + f. CNSK, f étant le facteur de decomposition des sulfocyanures, égal à 0,10 environ. On traite ensuite une autre partie du mélange primitif par une solution d'acétate de plomb légèrement acidifiée per l'acide acétique *); les thiosulfates sont ainsi précipités sous forme de thiosulfate de plomb, peu stable, qui se décompose en sulfure de plomb et en sulfate de plomb, tous deux insolubles; au contraire, le sulfocyanure de plomb est soluble dans ces conditions. On filtre, on élimine l'excès de plomb du filtrait à l'état de sulfate par l'acide sulfurique; on filtre de nouveau et traite le liquide limpide dans l'appareil à doser les thiosulfates. La quantité d'hydrogène sulfuré que l'on trouve provient alors uniquement de la décomposition des sulfocyanures: il suffit alors de la retrancher de la quantité totale trouvée dans le premier dosage.

Voici un exemple de mode opératoire et de résultats: On place dans un ballon jaugé de 200 cc un volume déterminé d'une solution titrée de thiosulfate de sodium et un volume également connu d'une solution titrée de sulfocyanure de potassium; on ajoute environ 20 cc d'eau distillée, puis 5 cc de la solution de sulfate de zinc et de sulfate de cadmium, puis on complète le volume au trait par de l'eau distillée. On filtre, puis on prélève 50 cc du mélange et l'on dose l'hydrogène sulfuré provenant de la décomposition du thiosulfate et du sulfocyanure, ainsi que le soufre provenant du thiosulfate. Soit A la quantité totale d'hydrogène sulfuré trouvée.

On prélève ensuite 50 cc du mélange soufré et on y ajoute 5 cc de la solution d'acétate de plomb; on agite et laisse reposer 15 minutes; on filtre, et recueille 50 cc de liquide clair, auxquels on ajoute 5 cc d'acide sulfurique normal. On laisse reposer 15 minutes, puis on filtre de nouveau; on prélève 50 cc du liquide limpide obtenu; ce volume correspond à 41,6 cc du mélange initial. On traite cet échantillon dans l'appareil et recueille l'hydrogène sulfuré provenant de la décomposition partielle du sul-

*) Cette solution est obtenue en dissolvant 300 g d'acétate neutre de plomb dans la quantité d'eau suffisante pour obtenir un litre de solution, et en ajoutant quelques gouttes (une dizaine) d'acide acétique pour redissoudre le précipité d'hydroxyde de plomb formé.

focyanure; soit B la quantité d'hydrogène sulfuré trouvée dans ces conditions. La différence A-B est égale à la quantité d'hydrogène sulfuré correspondant au thiosulfate seul; c'est ce que montre le tableau II.

TABLEAU II

Dosage de thiosulfate en présence de sulfocyanure.

Quantités de S exprimées en γ, correspondant à:
A: $S_2O_3Na_2$ + CNSK. B: CNSK seul C: $S_2O_3Na_2$ seul (A—B)

Solution de CNSK 0,02N introduite		Solution de $S_2O_3Na_2$ 0,02N introduite		A	B	C	$\frac{\text{S trouvé}}{\text{S théorique}} \cdot 100$
cc	S	cc	S				
1	640	1	1280	1490	70	1240	110
5	3200	1	1280	1655	330	1325	105
10	6400	1	1280	1947	615	1332	104

II. Dosage du thiosulfate dans l'urine de différents animaux supérieurs.

L'application de la méthode qui vient d'être décrite à la détermination du thiosulfate dans l'urine de différentes espèces animales, a donné les résultats présentés par le tableau III.

TABLEAU III.

Teneur en thiosulfate de l'urine de différents animaux.

Les chiffres placés sur une même ligne horizontale correspondent à l'urine d'un même individu, mais prélevée à des jours différents.
Les lettres désignent des individus différents.

Espèce		Thiosulfate exprimé en $S_2O_3Na_2$ (mg/litre)		Régime alimentaire
Herbivores				
Cobayes	Groupe I (2 animaux)	2,93	3,01	Mie de pain, carottes, rutabagas
	Groupe II (2 animaux)	2,44	2,46	id.
Lapins:	A	12,60		Carottes, épluchures de pommes de terre.
	B	9,40		
	C	31,80	30,0	Même régime + choux, rutabagas et pain.
Vache:	A	8,1	7,7	Fourrage sec
	B	7,9		
Cheval:		38,6	54,8	Fourrage sec + avoine
Carnivores				
Chat:	A	64,0	67,0	
	B	60,4	52,9	Lait, viande, poisson
	C	32,2	34,0	
Omnivores				
Chien:	A	8,1		Soupe au pain, carottes et pommes de terre.
Homme:	A	7,0		
	B	8,1		
	C	8,0		
	D	7,1		Alimentation variée.
	E	5,4		
	F	5,8		
	G	5,9		
	H	5,4		

Ces premiers chiffres, qui ne font pas intervenir les volumes urinaires, n'ont qu'une valeur indicative; néanmoins, ils montrent que:

1°. Les thiosulfates existent toujours dans l'urine, quelle que soit l'espèce ou l'individu considéré; en particulier, l'urine de l'homme normal contient une quantité de thiosulfate qui, quoique faible, n'est pas négligeable.

2°. Les teneurs en thiosulfates de l'urine des individus d'une même espèce, soumis au même régime alimentaire, sont à peu près du même ordre de grandeur.

3°. Les teneurs en thiosulfate de l'urine d'individus d'une même espèce mais soumis à des régimes alimentaires différents, peuvent varier dans des proportions considérables.

Certains de ces résultats peuvent paraître en contradiction avec ceux obtenus autrefois; mais il ne s'agit là que de contradictions apparentes, dues, comme on s'en rend compte facilement, à l'emploi d'une méthode dont la sensibilité et la précision sont très supérieures à celles des méthodes anciennes. En ce qui concerne les thiosulfates de l'urine de l'homme, par exemple, on constate que, comme l'avait prévu PRESCH [8]), leur teneur, dans des conditions physiologiques normales, est inférieure à 10 mg par litre.

III. Origine du thiosulfate urinaire.

La constance de la présence du thiosulfate dans l'urine des animaux supérieurs n'apprend rien sur son origine; nous avons vu plus haut que cette origine peut être, a priori, attribuée soit à des réactions plus ou moins directes des bactéries du tube digestif, soit au métabolisme propre de l'organisme. Pour en décider, nous avons cherché à réaliser des conditions expérimentales telles que toute décomposition de dérivés sulfurés dans le tube digestif par action bactérienne soit suspendue. Pour ce faire, nous avons maintenu les différents animaux en expérience à un jeune complet; on sait que dans ces conditions, les animaux font appel, pour assurer leur métabolisme, à leurs substances de réserve, parmi lesquelles leurs propres protéines interviennent pour une certaine part; ils métabolisent donc les dérivés sulfurés constitutifs de ces protéines, et les produits de ce métabolisme propre à l'animal ne peuvent être confondus avec ceux du métabolisme intestinal, si celui ci n'existe plus faute de matériaux sur lesquels il puisse s'exercer; pour nous rapprocher de ces conditions, et dans le cas où cela était possible, nous avons soumis les animaux, avant de les maintenir à l'état de jeune, à un régime ou à un traitement préliminaires destinés à s'opposer avec efficacité suffisante aux actions bactériennes intestinales.

Les expériences sont faites sur le chat, le cobaye, le lapin et le rat.

Les animaux sont placés dans des cages en fer galvanisé, munies de dispositifs destinés à recueillir l'urine aussi quantitativement et proprement que possible. Toutes les 24 heures, on note soigneusement le volume d'urine recueillie, puis à l'aide d'un fin jet d'eau distillée, on rince le grillage formant le fond des cages, réunit les eaux de lavages à l'urine et ramène ainsi le volume de celle ci à un volume donné qui contient la quantité totale de thiosulfate excrétée en 24 heures par l'animal. Le poids total du thiosulfate excrété en 24 heures se calcule en tenant compte du volume total du liquide recueilli (urine + eau de lavage) alors que la concentration en thiosulfate par litre d'urine se calcule à partir de la quantité émise et mesurée avant le lavage des cages et de leurs accessoires.

Chat.

L'animal est tout d'abord alimenté normalement pendant quelques jours, son régime consistant en viande cuite, poisson et lait. Le dosage journalier du thiosulfate nous ayant montré que la quantité de ce composé éliminée en 24 heures était sensiblement constant, nous avons soumis l'animal au jeune total. Ce jeune a été prolongé pendant 8 jours. Pendant cette période, l'animal, quoiqu'ayant de l'eau à sa disposition, n'a pas voulu boire; aussi, pour obtenir la diurèse nécessaire, avons nous dû lui faire des injections intrapéritonéales d'eau salée à 9 g de NaCl par litre: tous les jours, à 16 heures, nous avons anesthésié le chat sous une cloche à chloroforme et

lui avons injecté dans l'abdomen 80 cc d'eau salée en prenant les précautions d'usage pour éviter toute infection du péritoine. Après 72 heures de jeûne, l'animal a été alimenté normalement et nous avons continué pendant trois jours à doser le thiosulfate journellement excrété par l'urine. Les résultats obtenus sont donnés dans le tableau IV, complété par la figure 1.

TABLEAU IV

Thiosulfate excrété par le Chat.

Temps (jours)	Régime	Volume d'urine émis en 24 h. (cc)	H_2S (γ) formé à partir de CNSK	Thiosulfate ($S_2O_3Na_2$) mg/litre d'urine
1	Normal	60	23,8	32
2	Normal	60	23,8	34
3	Jeûne	60	30,6	114
4	Jeûne	80	37,4	92
5	Jeûne	96	34,0	102
6	Normal	100	27,2	73
7	Normal	65	27,2	30
8	Normal	60	23,8	35

Ces résultats sont très nets: à l'état de jeûne, le chat excrète par l'urine une quantité de thiosulfate près de cinq fois supérieure à celle qu'il élimine lorsqu'il est soumis à une alimentation normale.

Une autre expérience, faite dans les mêmes conditions avec un autre chat, a donné des résultats analogues.

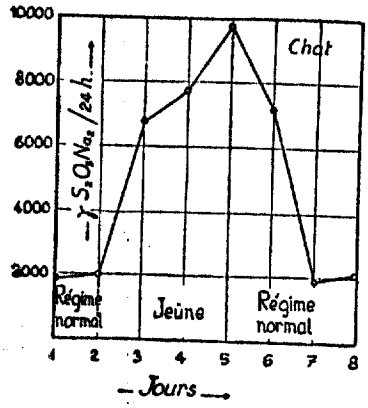


Fig. 1.

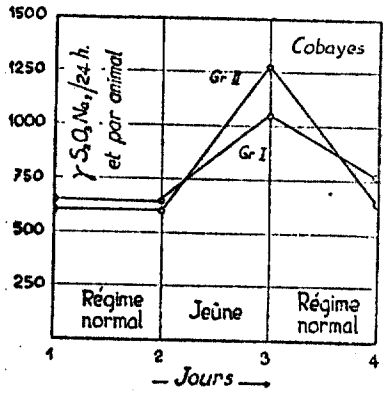


Fig. 2.

Cobaye.

Afin de disposer d'un volume d'urine plus grand et de pouvoir ainsi exécuter deux dosages simultanés donnant plus de sécurité, nous avons fait porter nos expériences sur quatre cobayes. Nous avons placé ces animaux par groupes de deux, et avons recueilli séparément l'urine de chacun de ces groupes. Les résultats obtenus pour chacun des échantillons d'urine ont été à leur tour divisés par deux, afin de ramener les quantités trouvées à celles excrétées par un animal.

Les animaux sont tout d'abord alimentés normalement pendant quelques jours, leur régime consistant en carottes, choux, rutabagas et mie de pain. La quantité de thiosulfate excrété en 24 heures ayant été trouvée sensiblement constante, nous avons soumis les animaux à un jeune

total. Ce jeûne n'a pu durer que 24 heures, car au bout de ce temps, les cobayes présentaient des signes de faiblesse tels que nous avons du les alimenter de nouveau normalement pour éviter de les voir périr. Les résultats obtenus dans cette expérience sont donnés dans le tableau V, complété par la figure 2.

TABLEAU V

Thiosulfate excrété par le Cobaye.

Temps (jours)	Régime	Volume total d'urine émis en 24 h. (cc).	H_2S (γ) formé à partir de CNSK	Thiosulfate ($S_2O_3Na_2$) mg/litre d'urine
		Groupe I		
		Poids initial des animaux: 720 g et 480 g.		
1	Normal	225	23,8	5,9
2	Normal	220	23,8	6,0
3	Jeûne	41	27,2	51,2
4	Normal	220	27,2	6,9
		Groupe II.		
		Poids initial des animaux: 575 g et 740 g.		
1	Normal	250	23,8	4,9
2	Normal	250	23,8	4,9
3	Jeûne	46	37,4	55,7
4	Normal	245	27,2	5,4

Les résultats sont ici également très nets: comme chez le chat, la quantité de thiosulfate excrétée par le cobaye est nettement accrue par le jeûne, cet accroissement étant ici du double environ.

Lapin.

Un lapin est tout d'abord alimenté normalement pendant quelques jours, son régime consistant en carottes, rutabagas, choux verts, épluchures de pommes de terre et pain. Le dosage journalier du thiosulfate urinaire nous ayant montré que la quantité de ce composé éliminée en 24 heures était sensiblement constante, nous avons soumis l'animal aux traitements suivants: en premier lieu, nous lui avons administré, à l'aide d'une sonde oesophagienne, une solution purgative constituée par:

Sulfate de magnésium . . . . . 5 g.
Eau tiède . . . . . 80 g.

24 heures après, nous avons introduit dans son estomac, toujours à l'aide de la sonde, une suspension de ferments lactiques dans une bouillie de lactose étant composée par:

Lactéol liquide „Boucard" . . 80 cc.
Lactose pur . . . . . 20 g.
Eau tiède . . . . . 80 g.

L'urine prélevée 24 heures après l'ingestion de la bouillie de lactose, correspond donc à un jeûne partiel (lactose) de 48 heures. L'animal est alors soumis à un jeûne total pendant 24 heures, au cours desquelles on lui injecte, devant son refus de boire, par voie intrapéritonéale, 80 cc d'eau salée, sans anesthésie, afin d'obtenir une émission d'urine. Au bout de ce temps, c'est-à-dire 72 heures après la cessation de l'alimentation normale, nous avons sacrifié l'animal afin de nous rendre compte de l'état de son tube digestif. Nous avons constaté que l'estomac lui même et l'intestin contenaient encore des matières de couleur verdâtre de la consistance d'une bouillie; ces matières à réaction nettement acide, ne présentaient aucune odeur. Le tableau VI, complété par la figure 3, indique les variations de la teneur en thiosulfate de l'urine, au cours de cette expérience.

TABLEAU VI

Thiosulfate excrété par le Lapin.

Temps (jours)	Régime	Volume d'urine émis en 24 h. (cc)	H_2S (γ) formé à partir de CNSK	Thiosulfate ($S_2O_3Na_2$) mg/litre d'urine
1	Normal	280	85	32
2	Normal	300	92	30
3	Jeûne avec purgation	"	"	"
4	Jeûne sauf ferm. lact. + lactose	100	116	101
5	Jeûne total	125	180	187

On retrouve ainsi chez le lapin le même phénomène que nous avons déjà rencontré chez le chat et le cobaye: à l'état de jeûne, le lapin produit du thiosulfate en quantité nettement supérieure à celle qu'il forme lorsqu'il est soumis à une alimentation normale, l'accroissement observé étant ici de deux fois et demi environ.

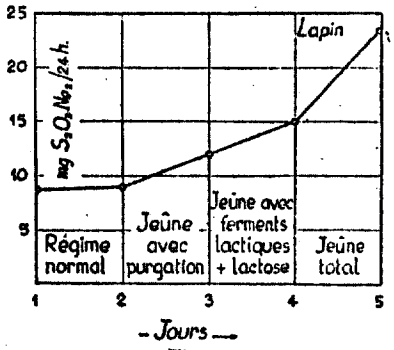


Fig. 3.

Rat.

Quatre rats sont placés chacun dans une cage séparée: les urines émises par chacun d'eux sont réunies de façon à obtenir un volume suffisant pour effectuer les dosages avec la sécurité nécessaire.

Les animaux sont tout d'abord alimentés normalement pendant quelques jours, leur régime consistant en fécule de pomme de terre, grains de blé, grains d'orge, pain et carottes. Le dosage journalier du thiosulfate nous ayant montré que la quantité de ce composé éliminée en 24 heures était sensiblement constante, nous avons soumis les animaux à un régime leur apportant à peu près tout ce qui leur est nécessaire; mais rigoureusement privé de soufre.

Ce régime est constitué de la façon suivante:

Fécule de pomme de terre	14 g.
Sciure de bois lavée	4 g.
Mélange d'acides aminés	0,8 g.
Mélange salin	1,5 g.
Huile de foie de morue R.P.	3 gouttes.

Le mélange d'acides aminés est formé par des poids égaux des substances suivantes: glycocolle; l(+) alanine; dl-sérine; l(+)valine; l(—) leucine; l(+)isoleucine; l(—)tyrosine; l(—)tryptophane.

Le mélange salin présente la composition suivante:

Chlorure de sodium	11,6
Chlorure de potassium	10,4
Phosphate dipotassique	23,2
Citrate de calcium	34,8
Citrate de fer	6,8
Bicarbonate de sodium	2,9

Quatre jours après le début de ce régime, nous avons constaté que, après une baisse sensible, la teneur en thiosulfate de l'urine était de nouveau constante. Les animaux ont été alors soumis au jeûne total pendant lequel, devant leur refus de boire, nous avons dû leur injecter 10 cc d'eau distillée salée à 0,9 %. Ce jeûne a duré trois jours; les dosages de thiosulfate faits sur l'urine recueillie les deux derniers jours ayant fourni des chiffres voisins, le deuxième jour du jeûne nous avons sacrifié l'un des animaux: son tube digestif s'est montré vide de tout résidu alimentaire.

Après ces trois jours de jeûne, les rats ont été soumis de nouveau au régime „sans soufre" pendant trois autres jours, laps de temps suffisant pour que la quantité de thiosulfate de l'urine redevienne constante.

Finalement les animaux ont été replacés dans des conditions d'alimentation normale. Les résultats obtenus au cours de cette expérience sont donnés par la figure 4. Ces résultats sont rapportés à un animal moyen, les valeurs fournies par les dosages ayant été divisées par le nombre de sujets en expérience. Signalons que la quantité de sulfocyanure de l'urine de ces animaux est ici constamment trop faible pour nécessiter une correction.

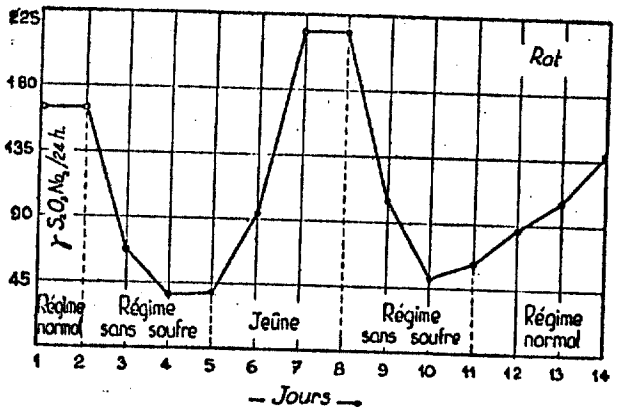


Fig. 4.

Le phénomène, tel qu'il se manifeste par la courbe de la figure 4, est particulièrement net: comme les animaux étudiés précédemment, le rat à l'état de jeûne excrète par l'urine une quantité de thiosulfate supérieure à celle qu'il élimine lorsqu'il est soumis à une alimentation normale, et très supérieure, environ six fois, à celle qu'il élimine lorsqu'il est maintenu à un régime privé de soufre, les variations de la quantité de soufre excrété étant réversibles, comme dans les cas précédents.

Discussion.

Les expériences précédentes montrent toutes que la quantité de thiosulfate excrété par l'urine est considérablement plus forte chez un animal, carnivore ou herbivore, maintenu à l'état de jeûne, que chez le même animal alimenté normalement, le phénomène étant parfaitement réversible. Un tel phénomène correspond vraisemblablement au fait que l'animal à l'état de jeûne consommant ses propres protéines, catabolise une quantité de soufre organique assez élevée; il est connu ([37]), en effet, que la quantité de soufre total excrété par le lapin par exemple, à l'état de jeûne, est très supérieure à ce qu'elle est chez le même animal nourri normalement. Toutefois, on conçoit a priori que la différence entre les quantités de soufre total métabolisé par un animal à l'état de jeûne ou soumis à un régime donné, puisse dépendre des quantités respectives de soufre apportées par ce régime et de celles apportées par les protéines propres utilisées au cours du jeûne. On peut expliquer ainsi que chez le porc, le jeûne ne semble pas accroître la quantité de soufre urinaire total excrété par l'animal nourri normalement ([37]). Il est intéressant de souligner à ce point de vue les résultats obtenus ici sur le chat: on a vu plus haut que chez cet animal, pourtant soumis à un régime particulièrement riche en soufre: viande, poisson, lait, l'excrétion de thiosulfate urinaire est accrue environ cinq fois par le jeûne.

L'expérience sur le rat est particulièrement intéressante; elle permet d'écarter définitivement l'objection que l'accroissement du thiosulfate urinaire observé au cours du jeûne ne correspondrait pas à un accroissement du métabolisme du soufre par l'animal, mais à une augmentation de l'intensité des putréfactions bactériennes s'exerçant sur les aliments qui, au cours du jeûne, peuvent subsister dans le tube digestif et s'y trouver immobilisés. Gauthier et Hervieux ([38]) ont montré, en effet, que dans certains cas, les fermentations intestinales sont accrues au cours du jeûne; d'autre part, rien ne prouve que le tube digestif du chat, même après trois jours de

jeûne, ou celui du cobaye après 24 heures, ait été vidé des aliments qu'il contenait; l'observation faite sur le lapin dont le tube digestif est particulièrement long il est vrai, montre, même après une purge énergique, suivie 24 heures après de l'ingestion de bactéries lactiques dans une bouillie de lactose, suivie elle-même d'un jeûne de 48 heures, qu'il reste, non seulement dans l'intestin mais aussi dans l'estomac, une masse de matières ne présentant d'ailleurs, comme nous l'avons vu plus haut, aucun signe de putréfaction. Les expériences faites sur le chat, le cobaye et le lapin, si elles apportent des arguments favorables à la thèse de l'origine essentiellement animale du thiosulfate, ne sont donc pas absolument démonstratives.

Au contraire, les conclusions des expériences faites sur le rat nous paraissent à l'abri de toute objection; avant d'être soumis à un jeûne total, les animaux ont été en effet maintenus à un régime leur apportant tout ce qui leur est nécessaire sauf le soufre; ils présentent dans ces conditions une dépense sulfurée endogène minimum ([30]) à laquelle correspond vraisemblablement la faible excrétion de thiosulfate observée; d'autre part, l'évacuation des aliments ingérés avant ce traitement se poursuit normalement. Cette évacuation est tout à fait terminée, et les aliments ordinaires sont remplacés par les aliments sans soufre quand on soumet les animaux au jeûne total; à ce moment, il n'y a donc plus, comme source de soufre dans le tube digestif, que les cellules de desquamation de l'épithélium intestinal, et les liquides secrétés par les différentes glandes ou organes, et se déversant dans l'intestin (bile, etc.); les possibilités d'origine bactérienne du thiosulfate se trouvent donc réduites au minimum. Rappelons à ce point de vue que le tube digestif de l'un des animaux en expérience, sacrifié à la fin de la période de jeûne, s'est trouvé parfaitement vide.

L'ensemble des expériences précédentes renforce ainsi l'hypothèse que la thiosulfate excrété par l'urine provient bien du métabolisme propre de l'animal, et que le thiosulfate représente, dans ce métabolisme, un produit intermédiaire de l'oxydation, en sulfate, du soufre organique des protéines consommées au cours du jeûne. D'autre part, si l'on joint aux résultats précédents le fait que la proportion de thiosulfate urinaire dépend étroitement, chez l'animal soumis à une alimentation normale, de la quantité de soufre organique ingéré, on peut également supposer que le thiosulfate est un produit intermédiaire de l'oxydation par l'animal supérieur, des dérivés sulfurés apportés par sa nourriture.

Remarquons enfin que le sulfocyanure de l'urine, tel qu'il peut être approximativement évalué par l'hydrogène sulfuré prenant naissance à partir de cette substance au cours du dosage, varie dans le même sens que le thiosulfate. Ce fait indique que le sulfocyanure comme le thiosulfate, provient du métabolisme propre de l'animal et d'autre part, correspond au mode de formation montré par LANG [40]) qui attribue sa naissance à l'action de l'acide cyanhydrique sur le thiosulfate en présence de rhodanèse.

Résumé.

La méthode de dosage des thiosulfates indiquée précédemment ([35]) a été adaptée à l'urine des animaux supérieurs; cette adaptation, rendue nécessaire par la présence fréquente de sulfocyanure dans l'urine, consiste à faire un premier dosage selon la technique décrite antérieurement, puis à éliminer le thiosulfate par le plomb, et à exécuter un second dosage. La différence entre les deux dosages correspond au thiosulfate. Dans les conditions réalisées, environ 10 % du soufre des sulfocyanures apparait sous forme de H_2S. Cette methode montre la présence constante de thiosulfate dans l'urine des animaux suivants: Cobaye, lapin, vache, cheval, chat, chien, homme.

D'autre part, des expériences faites sur le chat, le cobaye, le lapin et le rat, montrent toutes que la quantité de thiosulfate excrété par l'urine est considérablement plus forte chez un animal, carnivore ou herbivore, maintenu à l'état de jeûne, que chez le même animal alimenté normalement, le phénomène étant parfaitement réversible. Ces expériences conduisent à la conclusion que le thiosulfate excrété par l'urine provient bien du métabolisme propre de l'animal, et qu'il représente, dans ce métabolisme, un produit in-

termédiaire de l'oxydation en sulfate, du soufre organique des protéines consommées a cours du jeûne. Il semble que l'on puisse conclure également que le thiosulfate est u produit intermédiaire de l'oxydation par l'animal supérieur, des dérivés sulfurés appo tés par sa nourriture.

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THE CONSTANT PRESENCE OF THIOSULFATE IN THE URINE OF HIGHER ANIMALS AND ITS PHYSIOLOGICAL SIGNIFICANCE

By Claude Fromageot and Antonin Royer

Thiosulfate of the urine of higher animals has already been the object of numerous investigations; its presence, has been confirmed more or less regularly in the following species:

The cat: in 1867 Schmiedeberg (1) observed that the urine of the cat constantly contained relatively abundant quantities of thiosulfate; this observation has frequently been confirmed, notably by Meissner (2) who stated that generally the urine of the male contains higher quantities of thiosulfate than the female, the comparison being made on animals submitted to the same diet.

The dog: according to Schmiedeberg (1) the urine of the dog often, but not always, contains it. This inconsistency in the presence of thiosulfate in the urine also occurs in the work of Salkowski who found it in some cases (3) but not in others (4).

The rabbit: according to Salkowski the presence of thiosulfate in the urine of the rabbit depends considerably on its diet; not being present when the animal is fed carrots but occurring when it is fed cabbage. An analagous observation has been made by Dezani (6).

Human: according to the investigations of Schmiedeberg (1), Kulz (7) and Presch (8), the urine of the human does not normally contain appreciable quantities of thiosulfate; Presch states that if it is present it must be less than 10 mg/liters, which corresponds to the limit of detectability of the method used by that author. However, important quantities have been discovered in two particular cases: that of a cystinuric man reported by Spiegel (9) and in a study by Strumpell (10); on the other hand Van Eekelen (11) attributes the ability of human urine in acid milieu to reduce 2,6-dichlorophenol-indophenol to thiosulfate, in part.

It rapidly appeared that the source of the urinary thiosulfate must be sulfur derivatives in the diet. It was shown that the presence of thiosulfate from the rabbit depended on the diet: carrots or cabbage; the sulfur content of white cabbage is five times higher than that of carrots, according to Salkowski and this author has prepared (12) an aqueous extract of white cabbage, rich in sulfur, which induces an abundant excretion of thiosulfate, just as the white cabbage itself. On the other hand, a rabbit submitted to a diet which normally does not produce thiosulfate excretion passes large quantities if taurine (14) or cystine (15) is added to the diet. Also, according to Emmerie and Van Eekelen (16), Van Eekelen and Emmerie (17) and Heinemann (18,19) every increase in the protein content of the diet and thus of organic sulfur causes a parallel increase in the excretion of thiosulfate in different higher animals (cat, dog, man).

In spite of the observation of Schmiedeberg that when a cat was fasted for five days and then was fed a diet low in sulfur for 14 days a drop in the level of thiosulfate excretion manifested itself only toward the end of the experiment, an observation which led Schmiedeberg to the conclusion that thiosulfate must arise here from the degradation of animal tissue. Some conclude, with Rothera (20), that this origin is due more or less directly to the action of intestional bacteria on the sulfur-containing constituents of the diet, whether these bacteria form thiosulfate which is more or less absorbed or that they form hydrogen sulfide which is absorbed and then oxidized by the animal itself to thiosulfate; thiosulfate is then more or less oxidized to sulfate and the animal passes whatever remains into the urine. This view, held recently also by Dezani (6) rests in one part on the observations of Salkowski (21) who holds that thiosulfate is found not only in the urine of the dog but also in the feces with the variations of thiosulfate content being almost equal in both; it rests on the other hand on the results of Wohlgemuth (22) who having added cystine to minced meat submitted to bacterial action of putrefaction, noticed the formarion of notable quantities of thiosultate. Neuberg and Rubin (23) have also observed the formation of thiosulfate by putrefying bacteria using taurine or chondroitin sulfate, and numerous intestinal bacteria possess the aptitude to form hydrogen sulfide from cysteine or cystine.

At the present time, the total of this data concerning thiosulfate of the urine of higher animals is summarized as follows: it is constantly present in the cat, sometimes in dogs and very irregular in rabbits; only exceptionally is it present in humans and its source is bacterial.

Some newly discovered facts, however, argue in favor of the possibility of formation of thiosulfate in the tissues of higher animals, which is in accord with the conclusions of Schmiedeberg: there are in the pancreas and the kidney two enzymes, cysteinedesulfurase (24) and homocysteinedesulfurase (25), which induce the formation of hydrogen sulfide from the corresponding sulfur-containing amino acid; and also, hydrogen sulfide is oxidized to thiosulfate by oxygen, either by an organic catalyst present in fat and some other organs of higher animals (26) or by mineral catalysts (27). Besides, from these facts one can reconcile the formation of thiosulfate by oxidation of cysteine with oxygenated water (9).

One is thus led to suppose, contrary to previous opinion, that the formation of thiosulfate in higher animals is, at least in great part, of animal origin and is part of the mechanism assuring the oxidation of organic-sulfur into sulfates and this organic sulfur ingested consists mostly of cystine, cysteine and methionine. This hypothetical mechanism can be represented as follows:

A) Cystine + XH ----> Cysteine

Methionine + YH -----> Homocysteine

B) Cysteine --Cysteinedesulfurase--┐
Homocysteine --Homocysteinedesulfurase--┘→ H_2S

A part of the hydrogen sulfide produced participates in transsulfuration reactions (synthesis of homocysteine from cysteine), discussed by Fromageot and Desnuelle (28); the rest reacts as follows:

C) hydrogen sulfide ---------> thiosulfate

D) thiosulfate ----------> sulfate

The interest in this scheme is increased by the aptitude that higher animals possess of oxidizing thiosulfate to sulfate (29-31), an oxidation that Piroe (32) showed in tissue slices.

It is suitable to remark here that such a mechanism for the genesis of sulfate from organic sulfur differs profoundly from the mechanism of Piroe (33) or Medes and Floyd (34); in fact, in the present hypothesis sulfur is oxidized only after having been detached from the organic molecule to which it is attached but, these authors think that the oxydation is accomplished, in part at least, on a sulfur atom which is still attached to the organic molecule from which it is derived, giving birth to such intermediates as cysteine sulfinate, cysteine sulfenate and cysteinic acid. These different mechanisms are not exclusive and they are able to function together and to share common reactions.

In order to decide about the validity of the mechanism discussed here, it appears important to investigate the origin of the thiosulfate of the urine of higher animals. In this mechanism thiosulfate appears as an intermediate and although its brief existence implies a low concentration in tissue and thus in the urine, it is impossible that this concentration, which depends on the speed of reactions C and D, is not sufficient to be measured with precision and not susceptible to be augmented or diminished by modifications of the physiologic conditions in which the experimental animal is placed.

Before enterprising such investigations it is indespensible to possess a convenient method to analyze urinary thiosulfate. The present paper comprises the following divisions:

I. Development of a micromethod which is specific for the analysis of thiosulfate in the urine.

II. Analysis of the urine of diverse animals for thiosulfate

III. Study of the origin of urine thiosulfate

1. METHOD OF ANALYSIS OF THIOSULFATE IN THE URINE OF HIGHER ANIMALS

If one neglects older methods of analysis of thiosulfate in the urine, whose insensitivity is the cause of the discrepency between former results and those that we are presenting here, one finds only the methods indicated by Zorkendorfer (31) and by Dezani (6) which seem to be able to be utilized for the goal we are pursuing. Nevertheless, because of the inconveniences of these two methods we have employed here a micromethod that we have described previously (35), after having modified it for the present work. This method is based on the decomposition of thiosulfate into sulfur and hydrogen sulfide in acid; its adoption is rendered necessary by the frequent presence of thiocyanate in the urine of higher animals.

It is known (36) that thiocyanate in aqueous solution is decomposed easily by heating; according to the concentration the reaction corresponds to one or several of the following equations:

$$3\ HCNS = (HCNS)_2 + HCN$$

$$2\ HCNS + 2\ H_2O = CO_2 + CS_2 + 2\ NH_3$$

$$HCNS + 2\ H_2O = CO_2 + H_2S + NH_3$$

The hydrogen sulfide which may be thus formed is added to that which arises from the decomposition of thiosulfate and is the cause of a serious error in the analysis. First, we have investigated what fraction of hydrogen sulfide is produced by known quantities of HCNS when our apparatus is used under conditions identical to those which will be used in the analysis of thiosulfate.

To do this we introduce a known volume of 0.02 N KCNS into a 50 ml volumetric flask; we add 20 ml of distilled water and 5 ml of solutions of zinc sulfate and of cadmium sulfate, and then fill to the mark with distilled water. It is not useful to eliminate the precipitate of hydroxide formed by the salts of zinc and cadmium. The contents of the volumetric flask is then poured quantitatively into a beaker of the apparatus serving for the analysis of thiosulfate. We pass a current of nitrogen, then, heat to boiling. We introduce then, with the aid of a buret, 20 ml of sulfuric acid (approximately 18 N); we maintain the boiling for 30 min. and analyze, after centrifugation and washing of the precipitate of the zinc and cadmium sulfides, the hydrogen sulfide arising from the decomposition of thiocyanate. The results obtained are presented in table I.

TABLE 1

A=mcg of S corresponding to KCNS treated
B= mcg of S as hydrogen sulfide

volume of 0.02 N KCNS	A	B	$\frac{B.100}{A}$
0,25	160	22	14
0,50	320	33	10
1,00	640	77	12
1,50	960	97	10
2,00	1280	115	9
5,00	3200	317	10
7,50	4800	475	9,9
10,00	6400	672	10,5

These numbers show that in the conditions employed here 10% approximately of the thiocyanate is transformed into hydrogen sulfide.

The analysis of thiosulfate in the presence of thiocyanate rests on the following principal: one analyzes thiosulfate and the fraction of thiocyanate decomposed, while working as usual; one obtains the sum of sodium thiosulfate + KCNS x f, f being the fraction of the thiocyanate which has decomposed and is equal to approximately 0.10. One treats another part of the crude mixture with lead acetate, acidified with acetic acid; the thiosulfate is thus precipitated as lead thiosulfate which is very unstable and decomposes into lead sulfide, also insoluble; however, lead thiocyanate

is soluble in these conditions. One filters and eliminates the excess of lead from the filtrate with sulfuric acid; one filters again and treats the filtrate in the thiosulfate analyzer. The quantity of hydrogen sulfide that one finds arises totally from the decomposition of thiocyanate: it suffices then to subtract this from the total found initially.

Here is an example of the method and results: one places a known solution of sodium thiosulfate and an equal volume of a known solution of KCNS in a 200 ml volumetric flask; approximately 20 ml of distilled water is added and then 5 ml of a solution of zinc sulfate and cadmium sulfate and the volume is brought to the mark with distilled water. One filters, and then removes 50 ml of the mixture and analyzes the hydrogen sulfide arising from the decomposition of thiosulfate and thiocyanate, just as the sulfur arising from thiosulfate. Let A be the quantity of hydrogen sulfide found.

One removes 50 ml of the mixture and adds 5 ml of lead acetate, stirs and lets stand for 15 minutes; one filters and collects 50 ml of clear liquid to which one adds 5 ml of normal sulfuric acid. One lets this stand for 15 minutes and filters again; one removes 50 ml of the liquid obtained; this volume corresponds to 41.6 ml of the initial mixture. One treats this sample in the apparatus and collects the hydrogen sulfide arising from the partial decomposition of thiocyanate; let B be the quanity of hydrogen sulfide found under these conditions. The difference of A minus B is equal to the quantity of hydrogen sulfide corresponding to the thiosulfate alone; this is shown in Table II.

TABLE II

ANALYSIS OF THIOSULFATE IN THE PRESENCE OF THIOCYANATE

Quantities of S expressed in mcg correspond to:

A: $Na_2S_2O_3$ + KCNS. B: KCNS alone. C: $Na_2S_2O_3$ alone (A-B)

0.02 N KCNS introduced		.02N S_2O_3= introduced		A	B	C	$\frac{\text{S found}}{\text{S theory}} \cdot 100$
cc	S	cc	S				
1	640	1	1280	1490	70	1240	110
5	3200	1	1280	1655	330	1325	105
10	6400	1	1280	1947	615	1332	104

II ANALYSIS OF THIOSULFATE IN THE URINE OF DIFFERENT ANIMALS

The application of the method which has been described for the determination of thiosulfate in the urine of different species, has given the results presented in Table III.

These first numbers, which do not take into account the urine volume, have only a qualitative value; nevertheless they show that:

1. Thiosulfate always exists in the urine, whatever the species or individual; in particular, the urine of humans contains a low but not negligible amount.

2. The content of thiosulfate in the urine of individuals of the same species and consuming the same diet is of the same order of magnitude.

3. The content of thiosulfate in the individuals of the same species but receiving different diets can vary considerably.

Some of these results appear to be in contradiction with those obtained previously; but these are only apparent contradictions due to the use of a method of analysis whose sensitivity and precision are far superior to that of former methods. Concerning the thiosulfate in the urine of man, one can varify as Presch (8) has done, that the value in normal individuals is less than 10 mg/liter.

III ORIGIN OF THIOSULFATE IN THE URINE

The constant presence of thiosulfate in the urine of higher animals tells nothing of its origin; we have seen, as stated above, that this origin may be, a priori, attributed to reactions more or less directly due to bacteria in the gastro-intestinal tract or to the metabolism of the animal itself. To decide this we set up conditions to eliminate the effect of bacterial action in the intestine. To do this we maintained the animals used in a complete fast; it is known that in these conditions the animals use reserve material to insure their metabolism, among which their own proteins are included; they metabolise the sulfur derivatives of these proteins and the products of this metabolism can not be confused with that of metabolism in the intestine because there is no material for these bacteria to metabolise; to obtain these conditions where possible we submitted the animals to a regime or treatment to oppose bacterial action in the intestine before submitting the animals to a total fast.

TABLE III

THIOSULFATE CONTENT OF THE URINE OF DIFFERENT ANIMALS

Species		Thiosulfate in mg/liter		Diet
Herbivores				
Guinea pig	2 animals	2,93	3,01	bread crumbs, carrots, rutabaga
	2 animals	2,44	2,46	same + cabbage, carrots + apples
rabbits	A	12,60		
	B	9,40		
	C	31,80	30,0	same + cabbage
cows	A	8,1	7,7	dry forrage
	B	7,9		
horse		38,6	54,8	dry forrage + oats
Carnivores				
cat	A	64,0	67,0	milk, meat, fish
	B	60,4	52,9	
	C	32,2	34,0	
Omnivores				
dog	A	8,1		bread, carrots, apples
man	A	7,0		varied diets
	B	8,1		
	C	8,9		
	D	7,1		
	E	5,4		
	F	5,8		
	G	5,9		
	H	5,4		

The experiments are made on the guinea pig, cat, rabbit and rat.

The animals are placed in cages of galvanized steel fitted with apparatuses to collect urine as quantatively and purely as possible. Every 24 hrs the volume of urine collected is noted, then with the aid of a jet of distilled water the grill of the bottom of the cage is rinsed, the water collected with the urine and the volume of the total is adjusted. The total weight of thiosulfate excreted by the animal in 24 hrs is taken into account when the concentration of thiosulfate per liter of urine is calculated from the amount measured before the washing of the cages.

CAT

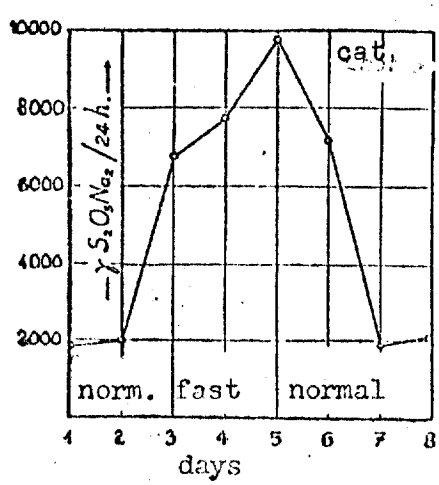
The animal is at first nourished normally for several days, his diet consisting of cut meat, fish and milk. Daily analysis of thiosulfate has shown us that the quantity of this compound eliminated in 24 hrs is relatively constant; we then submitted the animal to a total fast for three days. During this period the animal did not choose to drink, although he had water at his disposal; therefore, to obtain the necessary diuresis we had to administer physiological saline i.p.; every 16 hrs we anesthetized the cat with chloroform and injected 90 ml while taking precautions to avoid infection of the peritoneum. After 72 hrs the animal was fed normally again and we continued to analyze the thiosulfate output for another three days. The results obtained are given in Table IV and Figure 1.

TABLE IV

THIOSULFATE EXCRETED BY THE CAT

days	diet	24 hr urine volume ml	mcg H_2S from KCNS	$Na_2S_2O_3$ mg/liter
1	Normal	60	23,8	82
2	Normal	60	23,8	84
3	fast	60	30,6	114
4		80	37,4	92
5		96	34,0	102
6	Normal	100	27,2	73
7	Normal	65	27,2	80
8	Normal	60	23,8	85

Figure 1



GUINEA PIG

In order to obtain a larger volume of urine and to be able to make two simultaneous determinations for more accurate results we have used 4 guinea pigs. We have placed these animals in groups of two and gathered the urine of each group. The results obtained for each of the samples has in turn been divided by two to give the output per animal.

The animals were at first fed normally for several days; their diet consisted of carrots, cabbage, rutabagas and bread crumbs. The quantity of thiosulfate excreted in 24 hours was constant and the animals were then submitted to a total fast. This fast lasted for only 24 hrs because at the end of this time they presented signs of weakness; we again had to feed them normally so that they would not perish. The results obtained in this experiment are given in Table V and Figure 2. The results here are clear, just as in the cat; the quantity of thiosulfate excreted by the guinea pig clearly increased during the fast, the increase being approximately double.

TABLE V
THIOSULFATE EXCRETED BY THE GUINEA PIG

days	diet	24 hr urine volume ml	mcg H_2S from KCNS	urine $Na_2S_2O_3$ mg/liter
		Group I initial weights 720 g et 480 g.		
1	Normal	225	23,8	5,9
2	Normal	220	23,8	6,0
3	fast	41	27,2	51,2
4	Normal	220	27,2	6,9
		Group II. initial weights 575 g , 740 g.		
1	Normal	250	23,8	4,9
2	Normal	250	23,8	4,9
3	fast	46	37,4	55,7
4	Normal	245	27,2	5,4

Figure 2

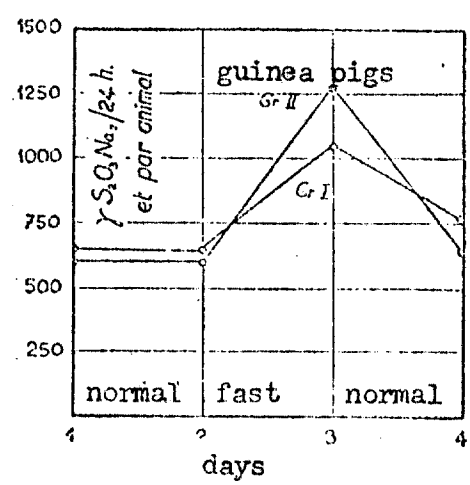


Fig. 2.

RABBIT

A rabbit was first fed normally for a few days, his diet consisting of carrots, green cabbage, rutabagas, apple pealings and bread. After daily analysis of thiosulfate in the urine showed that the output was relatively constant we submitted the animal to the following treatment: first, we administered, with the aid of a tube, a purgative solution consisting of 5 g of magnesium sulfate in 30 g of warm water. Twenty four hours later we introduced into his stomach with the same tube a suspension of lactic enzymes in a broth composed of 30 cc of Lacteol liquid, 20 g of pure lactose and 30 g of warm water.

The urine sample taken 24 hrs after the ingestion of the lactose broth corresponds to a partial fast of 48 hrs. The animal was then submitted to a total fast for 24 hrs, during the course of which he received 80 cc of saline water without anasthesia because of his refusal to drink, in order to obtain a urine sample. At the end of this time, that is 72 hrs after the cessation of the normal diet, the animal was sacrificed in order that the digestive tract may be examined. We verified that the stomach and the intestine contained some green liquid material still; this material was acid but, gave no odor. Table VI and Figure 3 indicate the variations of the thiosulfate content of the urine during the course of this experiment.

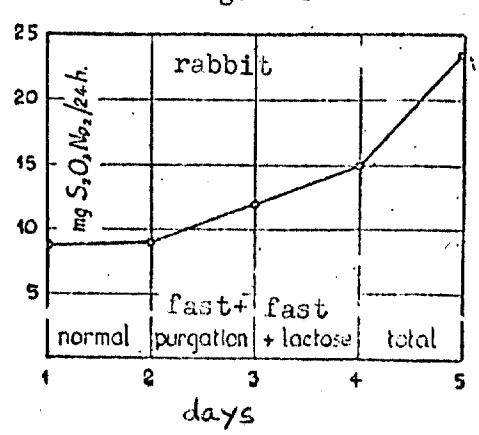
Thus, one finds for the rabbit the same phenomenon observed in the cat and guinea pig; during fasting the rabbit excretes larger quantities of thiosulfate than when the animal receives a normal diet; in this case the increase was approximately 2.5 times.

TABLE VI

THIOSULFATE EXCRETED BY THE RABBIT

days	diet	24hr urine volume ml	mcg H_2S from KCNS	urine $Na_2S_2O_3$ mg/liter
1	Normal	280	85	32
2	Normal	300	92	30
3	fast + purge	"	"	"
4	partial fast	100	116	101
5	total fast	125	180	187

Figure 3



RATS

Four rats were each placed in separate cages. The urine excreted by each was collected in a manner to obtain a volume sufficient to conduct accurate analyses.

The animals were fed normally for several days, their diet consisting of potato starch, wheat, barley, bread and carrots. When daily analyses indicated to us that the output of thiosulfate in the urine was relatively constant we submitted the animals to a diet which was normal except for the absence of sulfur. It consisted of the following:

potato starch	14 g
wood shavings	4
amino acid mixture	0.8
saline solution	1.5
cod liver oil	3 drops

The amino acid mixture is formed by equal weights of the following: glycine, l(+)alanine, dl-serine, l(+)valine, l(-)leucine, l(+)isoleucine, l(-)tyrosine and l(-)tryptophane.

The salt solution has the following composition:

NaCl	11.6 g
KCl	10.4
K_2PO_4	23.2
Ca citrate	34.8
Fe citrate	6.8
$NaHCO_3$	2.9

Four days after the beginning we noted that the thiosulfate output again became constant, at a lower level. The animals were then submitted to a total fast and because they refused to drink we had to inject 10 cc of physiological saline. This fast lasted three days; the analyses of the thiosulfate of the urine furnished almost equal values for the last two days, and so the second day of the fast we sacrificed one of the animals; his digestive system was completely empty.

After these three days of fast the remaining rats were again submitted to a diet very low in sulfur content for three days, a time sufficiently long for the thiosulfate output to again become constant.

Finally the animals were fed normally again. The results obtained from this experiment are presented in Figure 4. These results represent an average animal, the total values obtained in this experiment being divided by the number of animals. Notice that the quantity of thiocyanate in the urine here is too low to necessitate a correction.

The results are very clear cut: just as with other animals, rats excrete a higher level of thiosulfate when they are in a state of fast than when they are fed normally. The thiosulfate level during fasting is 6 times the output when the animals are on a low sulfur diet and the variations in the quantities excreted are reversible, as in preceding experiments.

Figure 4

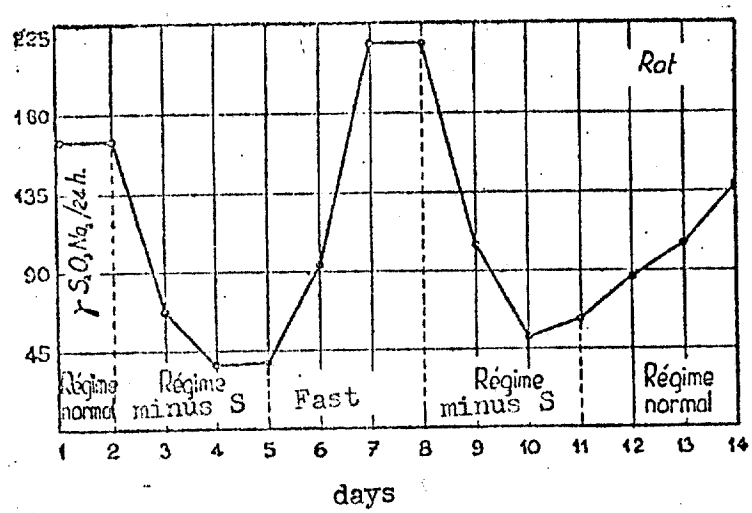


Fig. 4.

DISCUSSION

The preceding experiments indicate that the quantity of thiosulfate excreted in the urine of both carnivores and herbivores is considerably greater when the animals are in a state of fast than when they are fed normally and the phenomenon is completely reversible. This phenomenon probably corresponds to the fact that the fasting animal consumes his own proteins, catabolizing a rather high quantity of organic sulfur; it is known that (37) the total quantity of sulfur excreted by a fasting animal is much greater than when the same animal is fed normally. One can conceive, a priori, that the difference between the total quantities of sulfur metabolized by an animal fasting or submitted to a particular diet must depend on the sulfur brought by this diet or that from his own proteins during a fast. It should be noted that in the pig the quantity of thiosulfate excreted when the animal is fasted is not much greater than when the animal is fed normally. (37) It is interesting here to underline the results that we obtained on the cat: with this animal the excretion of thiosulfate is approximately 5 times greater when the animal is fasted than when it is submitted to a regime rich in sulfur (meat, fish and milk).

The experiment on the rat is particularly interesting; it allows one to remove definitively the objection that the increase in urinary thiosulfate during fasting does not correspond to an increase in metabolism of sulfur by the animal but to an augmentation of the action of putrefactive bacteria using material still remaining in the digestive tract. Gauthier and Hervieux (38) have shown that in certain cases the intestinal enzymes are increased in the course of fasting; on the other hand nothing proves that the digestive tract of a cat, even after three days of fasting or the guinea pig after 24 hrs, had been emptied of the food that it contained; the observation made on the rabbit who, it is true, has a particularly long digestive tract, shows that even after an energetic purge following 24 hrs after the ingestion of lactic enzymes in a lactate broth and then a fast of 48 hrs, there remains in the intestine and the stomach a mass of material which has been undigested. The experiments made on the cat, guinea pig, and rabbit are not absolutely demonstrative, although they furnish favorable arguments to the thesis that thiosulfate originates essentially from the animal itself.

On the contrary, the conclusions from experiments made on rats exclude all objection; before being submitted to a total fast the animals were maintained on a diet deprived of sulfur; they present, in this condition, a minimum output of sulfur (39) to which corresponds, probably, the low output of thiosulfate observed; on the other hand, the evacuation of food ingested before this treatment takes place normally. This evacuation is completely terminated and the usual food is replaced by food without sulfur when one submits the animals to the total fast; at this moment there is no longer any source of sulfur in the digestive tract than from desquamation of epithelial cells of the intestine and liquids secreted by different glands or organs into the intestine (bile, etc.); the possibility of a bacterial origin for sulfur is therefore at a minimum. Recall at this point that the digestive system of the animal sacrificed at the end of the fasting period was completely empty.

The sum of the preceding experiments reinforce the hypothesis that thiosulfate excreted in the urine arises from the metabolism proper of the animal and that the thiosulfate represents in this metabolism an intermediate product in the oxidation of sulfur from proteins consumed during the fast. On the other hand, if one adds to the preceding results the fact that the proportion of urine thiosulfate depends directly in animals fed normally, on the quantity of sulfur ingested, one can claim equally that thiosulfate is an intermediate product of the oxidation of sulfur derivatives from food by metabolism of the animal proper.

Let us note finally that thiocyanate in the urine varies in the same manner as thiosulfate itself. This fact indicates that thiocyanate, just as thiosulfate, arises from the metabolism proper of the animal and corresponds to the mode of formation shown by Lang (40), who attributes its formation to the action of HCN on thiosulfate in the presence of rhodanese.

RESUME

The method of analysis of thiosulfate reported previously (35) has been adapted to the urine of higher animals; this adaptation, made necessary by the presence of thiocyanate in the urine, consists of making a preliminary analysis according to techniques described previously and then eliminating thiosulfate with lead and carrying out a second analysis. The difference between the two values corresponds to the amount of thiosulfate. In the conditions used approximately 10% of the sulfur from thiocyanate appears as H_2S. This method shows the constant presence of thiosulfate in the urine of the following animals: guinea pig, rabbit, cow, horse, cat, dog and man.

On the other hand, the experiments made on the cat, guinea pig, rabbit and rat show that the quantity of thiosulfate excreted in the urine is considerably higher when the animal is fasted than when the animal is fed normally; this occurs in both carnivores and herbivores and is completely reversible. These experiments lead to the conclusion that thiosulfate excreted in the urine arises from the metabolism proper of the animal and that it represents, in this metabolism, an intermediate product in the oxidation of organic sulfur from proteins consumed in the fast. It seems that one may conclude as well that thiosulfate is an intermediate product in the metabolism of sulfur derivatives from alimentation.

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Using a standard bond length[5] $a(=1.40\times10^{-8}$ cm), we find at once from (1) that each perturbation δ_k gives a vector contribution

$$ea\delta_k/3=2.24\delta_k \text{ Debye} \qquad (2)$$

to the dipole moment of the π-electrons. The total dipole moment of the compound is then found by adding also the contribution from the σ-electrons, calculated by vector addition from the bond moments. Each heteroatom A is generally assumed to give rise to

TABLE II. Dipole moments of some cyclic compounds containing methyl in Debye units.

Compound	Dipole moment calculated	Dipole moment observed[a]	Compound	Dipole moment calculated	Dipole moment observed[a]
			Toluene	0.25	0.37
α-picoline	2.49	1.72	o-xylene	0.41	0.52
β-picoline	2.25	2.30	m-xylene	0.25	0.37
γ-picoline	2.61	2.57	p-xylene	0	0

[a] See reference a, Table I.

a perturbation δ_A in its position and to smaller perturbations δ_A' in the adjacent positions, where δ_A' equals about $\delta_A/8$. This means, according to (2), that each heteroatom A gives a vector contribution of about $2.52\delta_A$ Debye to the π-moment.

On the whole, we have therefore theoretically found that the vector addition rule for calculating dipole moments holds even for the contribution from the *mobile* π-electrons in conjugated systems having the character of a perturbed benzene ring.

If, in pyridine, the value $\delta_N=2$, recommended by Wheland and Pauling is used, one finds from (2) the π-moment 5.04 D or from a more exact solution of the secular equation 4.48 D, which values both are much too high. Consequently, δ_N and then the whole δ-scale must be essentially reduced in value in order to give the correct dipole moments. We have found a value of $\delta_N=0.6$ more reasonable, and Table I gives the result for pyridine and the diazines. These results are supported by a recent paper by Orgel

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S3

The Acid Decomposition of Sodium Thiosulfate

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(Received August 13, 1951)

THE acid decomposition of thiosulfate leads to the formation of sulfite and sulfur which after an induction period precipitate as colloidal sulfur. The induction period, designated as t_B,

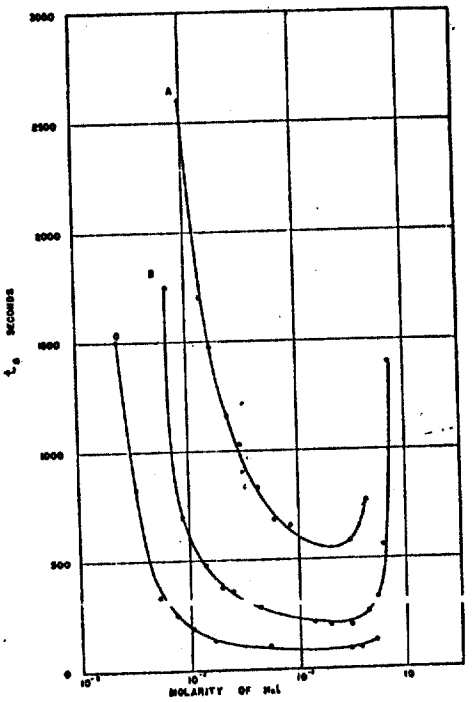

FIG. 1. Induction time *versus* acid concentration. A. 0.00203 M $Na_2S_2O_3$. B. 0.00520 M $Na_2S_2O_3$. C. 0.0124 M $Na_2S_2O_3$.

is due to the time necessary for sulfur to exceed its critical supersaturation concentration.[1]

t_B decreases with increasing thiosulfate concentration but goes through a minimum with respect to initial acid concentration. This leads to the interesting result that in concentrated hydrochloric acid, sodium thiosulfate does not produce a precipitate of colloidal sulfur even after several days. Basset and Durant[2] have determined t_B qualitatively for various thiosulfate and acid concentrations in both hydrochloric and phosphoric acids.

In order to obtain quantitative data upon which to formulate the kinetics of this reaction, the author has redetermined t_B as a function of initial concentrations. This was done by observing the increase in optical density of the reaction mixture at 3000A° as a function of time. The onset of turbidity at t_B is accompanied by a sharp increase in the slope of the optical density *versus* time curve. This technique has been used previously by La Mer, Kenyon, and Zaiser,[3,4] and we have confirmed its validity by light scattering measurements. The Beckmann Model DU quartz spectrophotometer with fused silica cells was used. The reaction was maintained at 25°C. The variation of t_B with concentration is shown in Fig. 1. The characteristic minimum was observed for each of the sodium thiosulfate concentrations used.

It was noted with great interest that the optical density of the solution at t_B varied with initial concentration. This is shown in Fig. 2. The optical densities are based on a 10-cm light path and vary almost 1000-fold over the range investigated.

Zaiser and La Mer[4] have previously reported that for the limited range of reactant concentrations employed in the preparation of mono-dispersed sulfur sols, the optical density at t_B is constant. They assumed that at 3000A° absorption is due only to molecularly dispersed sulfur, and they interpreted the constant optical density as a measure of the critical supersaturation concentration of molecular sulfur.

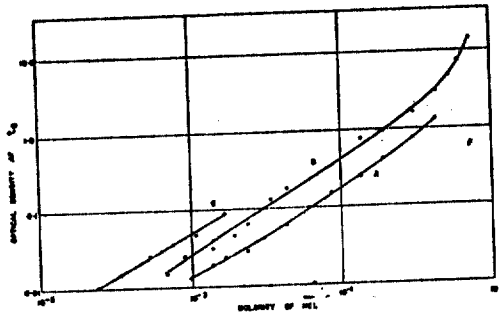

FIG. 2. Optical density at t_B *versus* HCl concentration. A. 0.00203 *M* $Na_2S_2O_3$. B. 0.00520 *M* $Na_2S_2O_3$. C. 0.0124 *M* $Na_2S_2O_3$.

Our results indicate that even for the dilute range, the optical density at t_B is not constant. The variation could be interpreted as due to a change in the critical supersaturation concentration of sulfur with reactant concentration. However, it seems unlikely that this could account for the tremendous range of optical densities observed at t_B. It is more likely that molecular species other than sulfur which absorb at 3000A° are preferentially formed at the higher acid concentrations.

The polythionates have been identified among the acid decomposition products of thiosulfate.[5] Lorenze and Samuel[6] have measured the absorption spectra of the polythionates. They give for the molar extinction coefficient of tetrathionate and pentathionate at 3000A°, 40, and 320, respectively. On the other hand, La Mer and Kenyon[3] found no appreciable absorption by tetrathionate at 3000A° and it was on the basis of this that they concluded that the transmission of their solutions during the homogeneous reaction was only a function of sulfur concentration. Messrs. Robert Penn and Carleton Hommel of this laboratory [illegible] the absorption spectra of tetrathionate and pentathionate and confirm the results of Lorenze and Samuel. This indicates that the optical density at 3000A° measures both polythionates and molecular sulfur.

This interpretation is consistent with the minimum in t_B. Both polythionates and sulfur are among the acid decomposition products of thiosulfate. The formation of polythionate is favored at higher acid concentrations so that although the rate of decomposition increases monotonically with acid concentration (as attested by the high optical densities atrained) the net rate of formation of sulfur becomes low, hence the large values of t_B.

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The Polarization of Rayleigh Scattering As an Aid to the Determination of Molecular Orientation in Solids

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ATTENTION[1] has been drawn by D. H. Rank to the value of the polarization of Rayleigh scattering ρ as an aid to the determination of molecular configuration in liquids. It may be of interest to point out that this constant can also be used to determine the approximate orientation of molecules in crystals. In the case of a non-associated molecule having two of its three principal polarizabilities equal or approximately so, the values of ρ and R, the molecular refraction, suffice to determine the numerical values of the polarizabilities; knowledge of the space-group, density, and refractive indices of the substance in the crystalline state then enables the approximate molecular orientation to be deduced. The results are not so accurate as those based on measurement of magnetic anisotropy, but the method is likely to be convenient for relatively simple substances of low melting point on which magnetic measurements are less easily made and for which the values of ρ are more likely to be available. A simple example is provided by benzene where it was found possible in this way to confirm the molecular orientation deduced by x-ray methods;[2] details of the calculation were not published at the time but are quoted by Hartshorne and Stuart.[3]

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R-Branch Heads of Some CO_2 Infrared Bands in the $CO+O_2$ Flame Spectrum

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SOME time ago the authors observed the presence of several small peaks on the short wavelength side of the CO_2 fundamental ν_3 in the emission spectrum of the $CO+O_2$ flame. These observations were made with a spectral slit width of ~ 8 cm^{-1}. Recently the spectrum was re-examined with a Perkin-Elmer spectrometer using a LiF prism, a sensitive Golay detector, and slit widths of 40 microns which give a resolving power of ~ 2.2 cm^{-1}. At this resolution these peaks were recognized as the *R*-branch heads of some of the vibration-rotation bands of the CO_2 molecule. The emission spectrum is shown in Fig. 1 together with the atmospheric transmission band of CO_2 at $\sim 4.2\mu$. A comparison of the observed and calculated positions of the *R*-branch heads found is given in Table I. The calculated values were obtained from well-known formulas[1,2] using a set of constants which were obtained in the course of a recent effort to fit all available data. These constants which differ slightly from previously published values[1] are given in cm^{-1} as follows: $\nu_1=1342.9$, $\nu_2=667.3$, $\nu_3=2349.3$, $X_{11}=3.06$, $X_{22}=0.67$, $X_{33}=22.5$, $X_{12}=-3.20$, $X_{13}=20.50$, $X_{23}=11.75$, $X_{11}=-1.17$, $b^2=51.69$ $(1-0.039\ V_3)$, $\alpha_1=0.00044$, $\alpha_2=-0.00072$, $\alpha_3=0.00307$, $B_0=0.3904$, and $D_0=1.8\times 10^{-7}$. The calculated value of the rotational quantum number at the *R*-branch heads of all the bands is J(head) = 127. The existence of moderately prominent heads is to be expected since in the $CO+O_2$ flame employed the rotational temperature has been determined from the CO overtone bands to be ~ 2700°K.[3]

TABLE I.[a]

Band	Transition	ν_0(cm^{-1})	ν (*R*-branch head) (cm^{-1}) Observed	Calculated
a	001 → 000	2349.3	2396.7	2397.1
b	$01^11 \rightarrow 01^10$	2337.6	2385.3	2385.4
c	{101} → {100}	2327.4	2377.1	2375.3
d	{02^21} → {02^20}	2326.3	2374.1	2373.9
	$02^21 \rightarrow 02^20$	2325.8	...	2373.6
e	002 → 001	2324.2	2372.4	2372.0

[a] Wave numbers are referred to vacuum (see Fig. 1).

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STUDIES ON THE ELIMINATION OF RADIOACTIVE STRONTIUM

4. Effects of the Administration of Various Compounds

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In the previous studies (Ogawa et al. 1961a, b, 1962) we reported on efficacy of various chelating agents and salts in eliminating Sr^{90}, and in the present investigation we performed further experiments on the elimination of radioactive strontium, using Sr^{85} and Sr^{90}.

Material and Methods

Male mice of ddN strain, weighing 20-27 g. were used. Each animal was housed individually in a separate metal metabolic cage, and bred at an environmental temperature of 20±2°C with commercial solid diet (Oriental Co.) and water.

The used nuclides were carrier-free Sr^{85} and Sr^{90} nitrate. These were neutralized and diluted with 0.9% sodium chloride solution, and 1 μc 0.1 ml solution was subcutaneously given to a mouse. Immidiately after the administration of Sr^{90}, the first treatments were made with drugs shown in the table, and the subsequent treatments were given once daily for 2 days.

The doses and modes of administration of the drugs were shown in the table. Each group consisted of 5 mice.

Seventy two hours after Sr^{85} injection, all the animals were sacrificed. The radioactivity of Sr^{85} in excrements and femur was determined with a scintillation counter. In the case of Sr^{90} experiment, all samples were stored at least for 4 weeks in order to obtain equilibrium between Sr^{90} and Y^{90}, and then the activity was measured with a G-M counter.

Differences between averages for various groups were tested for the statistical significance.

Results

1. Effect of chelating agents:

As shown in Table 1, Na Sr citrate and aspartic Mg K in combination with NH_4Cl displayed Sr-eliminating effect. ATP Na and tetracycline increased only

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Table 1. Effect of chelating agents on the excretion and retention of Sr^{85}

Groups	Daily dose (mg/day)	Mode of administ.	Body wt. (g)	No. of mice	Sr^{85} Excretion (%) Urine	Feces	Total	Sr^{85} Retention in skeleton (%/g)
Control			25	5	24.5	21.2	45.7	33.0
Na Sr citr.1)		i.p. & subc.	25	5	37.2*	16.9	54.1*	13.8**
Na Sr citr.2)		〃	23	5(2+)	41.6	9.6	51.2	13.6
Control			25	5	22.9	30.8	53.7	40.7
Aspartic Mg K	10	subc.	25	5	28.2	21.8	50.0	31.4*
〃	5	〃	25	5	25.9	22.9	48.8	31.7*
Control			24	5	23.1	25.1	48.2	37.9
Aspartic Mg K	1	subc.	24	5	26.4	25.7	52.1	38.3
Control			20	5	15.5	28.5	44.0	36.2
Aspartic Mg K + NH_4Cl	10 1.8	subc.	20	5	28.3*	24.4	52.7*	30.7*
Control			27	5	26.8	28.0	54.8	30.0
ATP Na	0.25	subc.	27	5	34.4	32.1	66.5*	28.9
Control			24	5	17.3	27.7	45.0	45.1
ATP Na	0.5	subc.	24	5	38.7*	22.8	61.5*	42.0
Control			24	5	23.1	25.1	48.2	37.9
Tetracycline	1	i.m.	24	5	34.3*	23.4	57.7*	32.2
Tetracyline + $SrCl_2$	1 +1	i.m. subc.	24	5	27.7	22.6	50.3	32.9
Control			23	5	21.1	22.2	43.3	31.4
BADE	1	i.p.	24	5	21.6	15.3	36.9	36.9
BADE + $SrCl_2$	1 +2	i.p. subc.	24	5	29.7*	14.4*	44.1	33.9

1) : Na Sr citr./Na_3citr.=0.5 (citr.: 13 mM/Kg/day)
2) : 〃 =2.0 (〃)
* : $P<0.05$, ** : $P<0.01$ 2+ Cases died during the period of study

the excretion without diminishing the retention in bone. BADE alone or in combination with $SrCl_2$ were not effective.

2. Effect of salts:

As shown in Table 2, Na thiosulfate alone or in combination with $SrCl_2$ was effective in eliminating Sr^{85}, and $SrCl_2$ alone, Ca gluconate, Na polyphosphate and chondoroitin sulfuric acid exerted no effect.

3. Effect of diuretics:

As shown in Table 3, among diuretics, there were no effective drugs.

4. Effect of hormones, vitamins and metabolic inhibitors:

As shown in Table 4, out of hormones, glucocorticoids had Sr-eliminating effect. TSH increased only the excretion without diminishing the retention in bone. ACTH, DOCA, thyroxine, parathyroid extracts, adrenaline were not

Table 2. Effects of salts on the excretion and retention of Sr^{85}

Groups	Daily dose (mg/day)	Mode of administ.	Body wt. (g)	No. of mice	Sr^{85} Excretion (%) Urine	Feces	Total	Sr^{85} Retention in skeleton (%/g)
Control			24	5	20.8	20.9	41.7	33.6
$Na_2S_2O_3$	16	subc.	24	5	30.1*	22.3	52.4*	27.2*
$SrCl_2$	2	〃	23	5	18.3	18.4	36.7	30.2
$Na_2S_2O_3$	16	〃	24	5	27.7*	21.9	49.6*	19.8**
$+SrCl_2$	+1	〃						
Control			22	5	25.5	14.5	40.0	33.0
Ca gluconate	10	subc.	21	5	18.5	13.9	32.4	33.3
〃	5	〃	22	5	18.9	12.6	31.5	40.5
Control			26	5	24.5	21.2	45.7	31.5
Ca gluconate	1	subc.	26	5	18.2	18.2	36.4	27.2
Control			24	5			45.7	31.2
Na polyphosph.	2	subs.	24	5			41.1	36.8
〃	4	〃	24	5			43.1	28.7
Control			23	5	28.2	22.1	50.3	43.7
Chondor. S.A.	2	subc.	24	5	24.8	17.0	41.8	37.7
〃	4	〃	24	5	18.7*	18.0	36.7*	40.3

* : $P<0.05$ ** : $P<0.01$

Table 3. Effect of diuretics on the excretion and retention of radioactive strontium

Treatment	Daily dose (mg)	Mode of administ.	Excretion of Sr* 1)	Retention of Sr* in skeleton 2)	Nuclide
Diuretin	5	i.p.	88	111	Sr^{90}
Caff. Na benz.	1	subc.	114	108	〃
Na ferrocyan.	25	i.p.	109	121	〃
〃	5	〃	108	93	Sr^{85}
〃	10	〃	101	99	〃
Melamin	1	〃	99	106	〃
Mannitol	15	subc.	78	96	〃
〃	30	〃	82	92	〃
Salyrqan	0.01	i.p.	79	105	〃
Chlorothiazide	2	subc.	99	114	〃
G-Strophanthine	0.038	i.p.	83	101	〃

1) 2) Values expressed as a percentage of control

effective. Also anti-thyroidal agents were not effective, and methylthiouracil conversely increased Sr retention. Out of vitamin, cocarboxylase increased Sr excretion, but did not decrease the retention in bone. Vitamin B_2 and K_1 were not effective. Also antimetabolic agents were ineffective, and NaF conversely increased Sr retention in bone.

5. Effect of thiol drugs and weak chelating agents:

Table 4. Effects of hormones, vitamins and metabolic inhibitors on the excretion and retention of radioactive strontium

Treatment	Daily dose (mg)	Mode of administ.	Excretion of Sr* 1)	Retention of Sr* in skeleton 2)	Nuclide
ACTH	0.25	subc.	99	113	Sr^{90}
Hydrocortisone	0.5	"	113*	79*	"
Prednisolone	0.1	"	113*	81*	"
TSH	0.5	"	121*	82*	"
Thyroxine	0.05	"	102	92	"
Na thiocyanate	20	"	94	87	"
Methylthiouracil	2	"	98	120*	Sr^{85}
Methylmercapto-imidazol	1	"	107	113	"
DOCA	0.5	"	116	116	"
Vit. B_2	0.5	"	108	96	"
"	1	"	115	93	"
Vit. K_4	1	"	107	102	"
Cocarboxylase	0.25	"	131*	100	"
"	1	"	118*	96	"
Na azide	0.05	"	101	99	Sr^{90}
Na pentobarbiturate	1.25	"	93	100	"
Na F	0.5	"	102	127*	Sr^{85}
Parathyroid extract	10 U	"	132	103	"
Adrenaline	0.01	"	107	93	"

1) 2) Values expressed as a percentage of control * $P<0.05$

Table 5. Effects of SH-drugs and weak chelating or complexing agents on the excretion and retention of radiostrontium

Treatment	Daily dose (mg)	Mode of administ.	Excretion of Sr* 1)	Retention of Sr* in skeleton 2)	Nuclide
Thioctic acid	0.5	subc.	92	97	Sr^{90}
Dihydrothiotic acid	0.5	"	96	97	Sr^{85}
"	1	"	82	97	"
Mercaptoacetic acid	3	"	77	88	"
α-Thiolactoylglycine	5	"	87	118*	"
Na sulfosalicylate	7.4	"	96	82	Sr^{90}
Na salicylate	5	"	94	95	"
PAS	1.65	"	102	113	"
INAH	1.25	"	97	96	"
Na sulfacetamide	0.5	"	80	97	"

1) 2) Values expressed as a percentage of control * $P<0.05$

As shown in Table 5, among thiol drugs and weak chelating agents, there were no effective drugs, and α-thiolactoylglycine conversely increased Sr retention in bone.

Discussion

Many attempts have been made to eliminate radioactive substances from the body using appropriate chelating agents. For elimination of Sr*, however, chelating agents are not always effective. Since Sr is similar to Ca both chemically and biologically it is difficult to find out such chelating agents as can eliminate Sr prior to Ca. It was reported in the previous paper (Ogawa et al. 1961a) that EDTA and DTPA have effect to eliminate Sr*. Catsch (1961) had considerable success in eliminating Sr^{85} from rats by the use of Sr-salt of BADE. We administered BADE and BADE + $SrCl_2$ to mice, but these had no eliminating effect. We plan to repeat experiments on this point. Ito (1958a) obtained good result in rats by joint administration of Na citrate and Ca salt, and in the previous paper we reported on our reduplicating experiments with mice. In the present experiments we obtained still better effect by joint administration of Na citrate and Sr-salt. This can probably be attributed to prevention of hypocalcemia and isotope dilution by Sr and chelating action by citric acid. Tetracycline group showed considerably high chelate stability constant against Ca, namely the log K of the 1:1 calcium oxytetracycline complex was 3.04, compared with 3.19 for the 1:1 complex of citric acid and calcium (Urist et al. 1962). On account of this they are expected to be highly useful for the elimination of Sr. Furthemore they will serve for the prevention of secondary infection in radiation damage since they are antibiotics. In the previous paper we reported on efficacy of tetracycline for elimination of Sr^{90}, but in the present experiment on elimination of Sr^{85}, tetracycline produced rather inferior effect. This occurred assumedly because in the previous experiments it was given concurrently with Sr^{90} whereas in the present experiments it was given 10 minutes after the administration of Sr^{85}. In fact it had no effect when given at 24 hours and one week after the administration of Sr* (unpublished datum). This was also clarified by duplicating experiment by Catsch (1963). Thus, reports on the effect of tetracycline on Sr* elimination are varying by experimental conditions (Rechards, 1961, Schmid and Zipf, 1963, Smith, 1963). It was also found out that in general chelating agents produced widely varying effect according to the time of their administration with regard to Sr* administration (unpublished datum). Aspartic acid is a weak chelating agent (Lumb and Martell, 1953), but nevertheless a large amount of its Mg- and K-salt had Sr-eliminating effect, and more satisfactory result was obtained by either of them alone than in association with ammonium chloride. It is well known that aspartic acid reduces toxicity of ammonium chloride, and that acido-

sis induced by ammonium chloride is effective for elimination of Sr^*. If these two can be combined in an appropriate way, an effective means for Sr elimination will be obtained. Also ATP has high stability constant for Ca and Sr (Nanninga, 1961; Samachson and Lederer, 1961). In view of this we performed experiment with it, and it was found to increase excretion of Sr. However, we could not observe any decrease in Sr retention in bone.

In a series of studies by Spencer et al. (1961), it is shown that, Ca gluconate is effective for the elimination of Sr^{85} from human body. In the present experiments, carried out with mice, this salt could not produce any satisfactory results. Catsch and Melchinger (1959) reported that thiosulfate was effective in diminishing Sr^{90} retention in rat bone. This was confirmed in the present experiments with Sr^{85} as well as in the previous experiments with Sr^{90} in mice.

Experiments were also performed with diuretics that were shown in the literature to influence excretion of Ca, but none of them were found effective for the elimination of Sr^*.

Out of hormones, parathyroid extract has been most intensively studied for the eliminating effect. However, reports on its effect on Sr^* elimination are varying (Posin, 1942, 1943; Tweedy, 1945; Ito et al., 1958b; Khomutovskii, 1958; Torizuka, 1960; Graul et al., 1961; Schmid and Zipf, 1961; Lengemann, 1963a; Takeuchi et al., 1963). Under the present experimental condition, satisfactory results could not be obtained by this hormone. Glucocorticoids were found effective for Sr elimination. Also we obtained results which indicated that thyroidal function may have some bearing on Sr metabolism.

There is a report (Ida, 1961) that V.K is effective in association with vitamin. In the present experiment, however, it did not produce any alteration in Sr elimination. Cocarboxylase increased Sr excretion, but did not diminish its retention in bone. No effects were exerted on Sr elimination by metabolic inhibitors, and NaF conversely augmented its retention. This is in agreement with Lengemann's result (Lengemann and Comar, 1963b).

None of SH drugs produced satisfactory results as was expected from ineffectiveness of BAL on Sr elimination (Kisielski et al., 1951). Nor was obtained favorable result with weak chelating or complexing agents.

Summary

Mice were subcutaneously injected with Sr^{90} or Sr^{85}, and effects of various drugs on their 3-day excretion and retention on the 4th day were investigated.

Out of chelating agents, Na Sr citrate and aspartic Mg K in combination

with NH_4Cl displayed Sr^{85}-eliminating effect. ATP Na and tetracycline increased only the excretion without diminishing the retention in bone. BADE, either alone or in combination with $SrCl_2$, was not effective.

Out of salts, Na thiosulfate alone or in combination with $SrCl_2$ was effective in eliminating Sr^{85}, and $SrCl_2$, Ca gluconate, Na polyphosphate and chondoroitin sulfuric acid exerted no effect.

Among diuretics, there were no effective drugs.

Out of hormones, glucocorticoids had Sr-eliminating effects. TSH was effective, and antithyroidal drugs conversely seemed to have excretion-diminishing effects.

Out of vitamins, cocarboxylase increased Sr^{85} excretion, but did not decrease the retention in bone.

Also metabolic inhibitors were ineffective, and NaF conversely increased bone deposition of Sr^{85}.

Among SH-drugs and weak chelating or complexing agents, there were no effective drugs.

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