In a number of tests, cultures in Medium E without rotation have proved satisfactory for primary isolation of virus and for the performance of neutralization tests with the three types of poliomyelitis virus. Poliomyelitis viruses have been isolated from infected monkey cord, mouse cord, and human stools. One of the stool specimens was negative by monkey inoculation, but positive results were obtained by this tissue culture method. The fluid phase of this tissue culture, produced paralysis in two monkeys inoculated with it, and poliomyelitis was confirmed by histopathologic findings.

Neutralization tests, with the Mahoney strain as the test virus, have been performed by this method on a group of 80 human serum specimens with quite satisfactory results. In non-neutralized mixtures first evidence of cellular degeneration was usually observed on the second day after inoculation with the virus. This became fairly definite by the third day, and the final readings were possible on the fourth day.

References

1. ENDERS, J. F., WELLER, T. H., and ROBBINS, F. C. *Science*, **109**, 85 (1949).
2. ROBBINS, F. C., *et al. Am. J. Hyg.*, **54**, 286 (1951).
3. SMITH, W. M., CHAMBERS, V. C., and EVANS, C. A. *Proc. Soc. Exptl. Biol. Med.*, **76**, 696 (1951).
4. SYVERTON, J. T., SCHERER, W. F., and BUTORAC, G. *Ibid.*, **77**, 23 (1951).
5. YOUNGNER, J. S., WARD, E. N., and SALK, J. E. *Am. J. Hyg.*, **55**, 291 (1952).
6. LEDINKO, N., RIORDAN, J. T., and MELNICK, J. L. *Ibid.*, 323.
7. SCHERER, W. F., and SYVERTON, J. T. *J. Exptl. Med.*, **96**, 369 (1952).
8. MELNICK, J. L., and RIORDAN, J. T. *Proc. Soc. Exptl. Biol. Med.*, **81**, 208 (1952).
9. ENDERS, J. F., and ROBBINS, F. C. Personal communications.

Manuscript received October 31, 1952.

On a New Derivative of Thiamine with Cysteine

Taizo Matsukawa and Shojiro Yurugi

Research Laboratory, Takeda Pharmaceutical Industries, Ltd., Osaka, Japan

The mechanism of thiamine in the metabolism in the living body is not yet clear. Zima and Williams (*1*) previously proposed a redox system between the thiol form of thiamine and thiamine disulfide.

The present authors have recently hypothesized a mechanism of thiamine in a medium that contains cystine, cysteine, glutation, or proteins having thiol groups, and have studied as a model experiment the reactions between thiamine and cystine, and between thiamine disulfide and cysteine by paper partition chromatography.

(1) Thiamine and equivalent cystine were dissolved in a phosphate buffer solution (pH 7.5), left standing at 37° C for 20 min, and then subjected to paper partition chromatography using the solvent prepared by shaking a mixture of alcohol, acetic acid, butanol, and water (1:1:4:10) and then drawing off the lower layer (Table 1).

The five paper strips on which the specimen was developed under the same conditions were dried in air and sprayed separately with the reagents indicated in Table 1.

The zones of Rf 0.04–0.05, 0.28–0.29, 0.29–0.31, and 0.36–0.38 correspond to cystine, cysteine, thiamine, and thiamine disulfide (VI), respectively. Besides these a new zone of Rf 0.14–0.15 was clearly detected by the reagents D, N, and I between the zones of cystine and thiamine.

When the paper strip sprayed with the reagent B was heated at 70–80° C for 5–10 min and then sprayed with the reagent A, the zone corresponding to thiamine showed strong fluorescence of thiochrome under the quartz mercury lamp, while the zones of Rf 0.14–0.15 and thiamine disulfide (VI) did not. However, when the paper strip was sprayed with the reagent C and heated at 70–80° C for 15–30 min in order to reduce the substances before spraying with the reagents B and A, all the three zones showed strong fluorescence.

From the result it seems evident that the new zone of Rf 0.14–0.15 means a new compound having the structure (IV) which was produced from the thiol form (II) of thiamine and cystine (III).

TABLE 1

Reagents	Cystine	Thiamine cysteine (IV)	Cysteine	Thiamine	Thiamine disulfide (VI)
D	—	0.14–0.15	—	0.29–0.31	0.36–0.38
N	0.04–0.05	0.14–0.15	(0.28–0.29)	—	—
I	0.04–0.05	0.15	—	0.30	0.37
B + A	—	(0.15)	—	0.30	—
C + B + A	—	0.14–0.15	—	0.29–0.31	0.36–0.38

D = the Kraut-Dragendorff reagent (*2*).

N = 0.2% solution of ninhydrin in the water saturated with butanol.

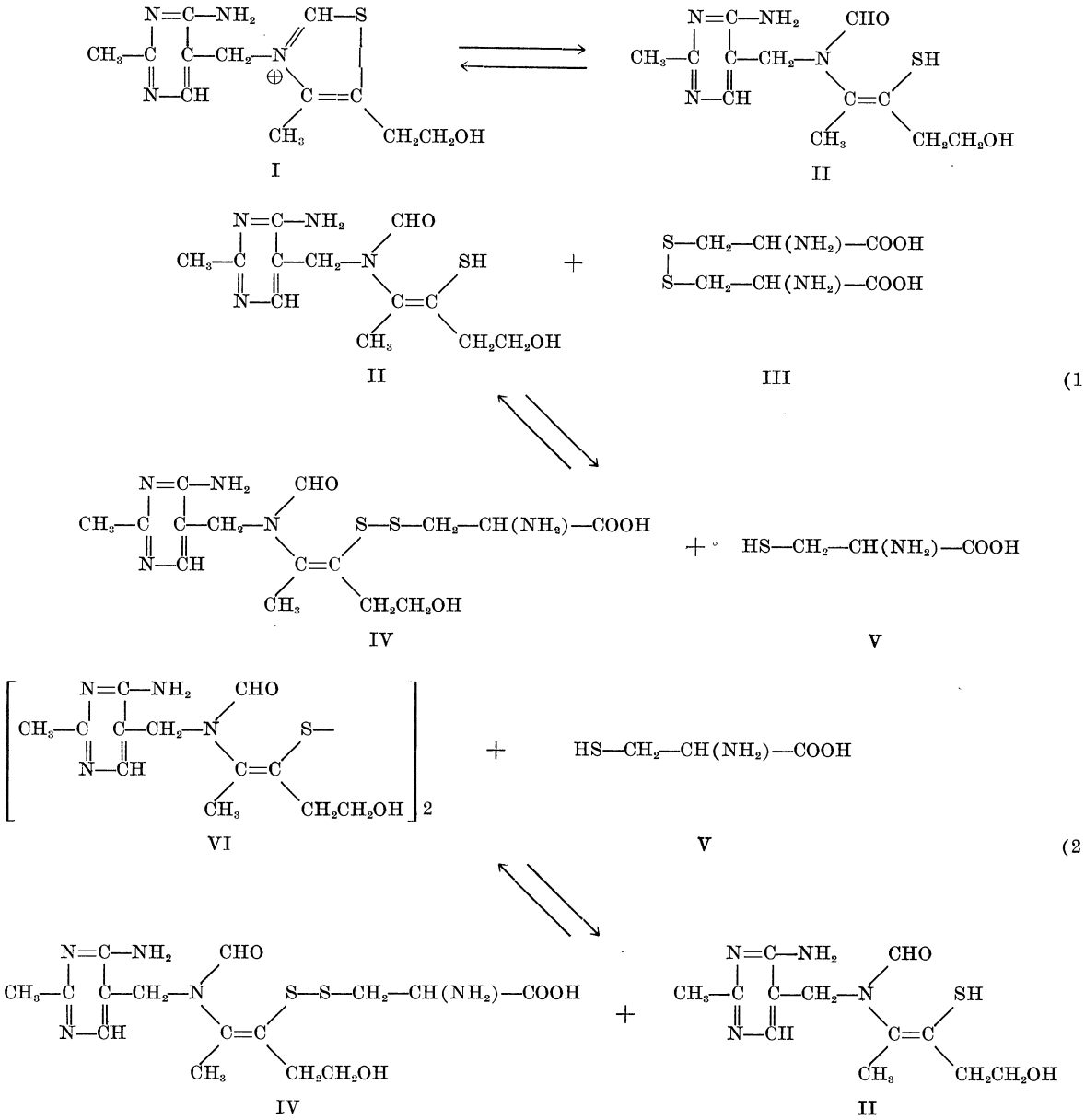
I = 0.05 *N* aqueous solution of iodine containing 1.5% of sodium azide. The reagent used for the detection of sulfur-containing amino acids (*3*) (thiamine and some related compounds can be detected by this reagent).

B = this reagent is prepared by adding 10% solution of sodium cyanide to the water saturated with bromine until the color of the bromine has disappeared (this is used instead of potassium ferricyanide to oxidize thiamine to thiochrome).

C = 0.4% solution of cysteine hydrochloride in water.

A = 5% solution of sodium hydroxide in the water saturated with butanol.

The mark (—) implies weak detection.



(2) Thiamine disulfide and equivalent cysteine were dissolved in a phosphate buffer solution (pH 7.5) and the solution was allowed to stand at 37° C for 20 min and then subjected to paper partition chromatography under the same conditions as in (1). The result was quite the same as that shown in Table 1.

From the above results the following mechanism might be suggested. Thiamine in a solution exists in a state of equilibrium between its ammonium (I) and thiol form (II) and the latter form takes part in the above reaction (1). In this reaction the thiol form (II) reacts with cystine (III) to produce thiamine cysteine (IV) liberating cysteine (V). The reaction will be an equilibrium one. In parallel with this reaction a secondary reaction (2) is imaginable in which (IV) reacts with the co-existing thiol form (II) to yield thiamine disulfide (VI) which in return reacts with cysteine (V) to form (IV), since the result of the reaction (2) is the same as that of the reaction (1). The secondary reaction will also be an equilibrium one.

These are, however, the results of the experiments carried out *in vitro* and with equimolecular substances. In the living body there is a large amount of cystine, cysteine, or their homologues, compared with that of thiamine; therefore, though the reaction (1) may be possible in the living body, the secondary reaction (2) seems impossible. In other words, the formation of thiamine disulfide (VI) seems unlikely in the living body.

From the results mentioned above it seems likely that the catalytic action played by thiamine in the metabolism in the living body is due to the following mechanism rather than to a redox system between the thiol form of thiamine and thiamine disulfide, proposed by Zima *et al.* Namely, an equilibrium reaction like (1) seems to be taking place in the living body, and the catalytic action of thiamine will be due to the dehydrogenating or oxidative action on certain substrata which are affected when thiamine cysteine (IV) or its homologues produced by the reaction revert to their original components.

References

1. ZIMA, O., and WILLIAMS, R. R. *Ber.*, **73**, 941 (1940).
2. ZAFFARONI, A., BURTON, R. B., and KEUTMANN, E. H. *J. Biol. Chem.*, **177**, 109 (1949).
3. CHARGAFF, E., LEVINE, C., and GREEN, C. *Ibid.*, **175**, 67 (1948).

Manuscript received January 12, 1953.

The Effect of Whole Blood Transfusion on Dogs Receiving a Maximal Sublethal (LD_0) Exposure to Ionizing Radiation[1]

R. W. Miller, W. K. Cotton, and S. N. Swisher

Departments of Radiation Biology and Medicine, University of Rochester, School of Medicine and Dentistry, Rochester, New York

Allen and his associates (*1*) have reported the effects of whole blood transfusion in the treatment of the acute illness in dogs produced by whole-body x-irradiation. Their data show that 4 of 10 transfused animals died consequent to a 175-r exposure that was not lethal for the control animals. This suggests that in the lower dose levels of irradiation, transfusions may kill animals not fatally injured by irradiation.

A similar study is reported here to help define the hazard, if one exists, of compatible whole blood transfusion to the dog suffering acute radiation sickness following a maximal LD_0 total body exposure.

Twenty unanesthetized mongrel dogs were paired as closely as possible with respect to weight, sex, age, and blood type. Each animal was subjected to 300 r of whole-body x-irradiation from a 1-Mev machine. One of each pair, following irradiation, served as a control while the other received typed, crossmatched, compatible whole blood on a predetermined basis of three transfusions a week beginning on the 4th day postirradiation and continuing for 4 weeks. The blood was drawn from donor animals into standard ACD solution 48–72 hr prior to its administration, and stored at 4° C. Blood was transfused into a leg vein in an amount of 5 cc/kg of body weight at each transfusion. Sufficient blood was withdrawn from each animal 3 times weekly in order to determine its hematocrit, RBC, WBC, and platelet counts.

[1] This paper is based on work performed under contract with the United States Atomic Energy Commission and the Armed Forces Special Weapons Project at the University of Rochester Atomic Energy Project, Rochester, New York.

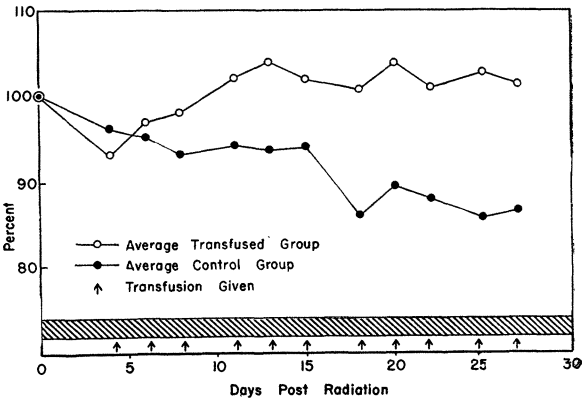


FIG. 1. Percentage preradiation hematocrit.

A fourteen-dog donor colony was maintained. Each donor and recipient animal was typed for the presence or absence of the canine A factor. A+ blood was given to A+ recipients and A− blood to A− recipients. The blood was crossmatched for major and minor incompatibility just prior to transfusion.

The diet of the donor colony was supplemented with daily feedings of horse meat and iron which maintained the donor hematocrits at approximately 95% of their control levels.

Results. Mortality: None of the animals in either group succumbed during the 28-day observation period.

Hematology: Figure 1 shows that the average hematocrit for the transfused dogs was restored to the preradiation value by the blood transfusions, whereas the control group's average hematocrit fell gradually in a linear fashion. The average platelet count (Fig. 2) and white blood cell count (Fig. 3) for the control and the treated groups were essentially the same.

Transfusion reactions: No clinical hemolytic or anaphylactoid reactions occurred.

Under the conditions of this experiment, the findings of Allen *et al.* could not be confirmed. Allen's x-ray factors differed from ours, but in both experiments the lethality of the exposure appeared to be

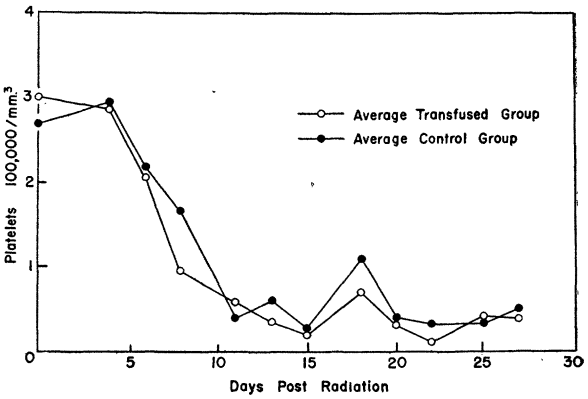


FIG. 2. Average platelet counts.