THE COURSE OF THIAMIN METABOLISM IN MAN AS INDICATED BY THE USE OF RADIOACTIVE SULFUR*,†

By Henry Borsook, Edwin R. Buchman, John B. Hatcher, Don M. Yost and Edwin McMillan

California Institute of Technology, Pasadena, Calif., and Radiation Laboratory, University of California, Berkeley, Calif.

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When supplementary thiamin (Vitamin B_1) is ingested or injected, the amount which appears in the urine during the succeeding twenty-four hours usually increases, but the total additional excretion always falls short of the supplement, even when the vitamin is injected and there can be no question of incomplete absorption. We have sought information on this unaccounted-for moiety by synthesizing thiamin from sulfur which contains the radioactive isotope S^{35} (designated as B_1*) and by following, after injection of the B_1*, the excretion of the radiosulfur (S*) in the urine and feces and the excretion of total free B_1 in the urine.

Radiosulfur was prepared by bombarding elementary sulfur with 7.5 MEV. deuterons, and thiamin bromide hydrobromide containing S^{35} was synthesized from the bombarded sulfur.[1]

The half-life of radiosulfur is about 88 days and it emits negative electrons with a maximum energy of 0.107 MEV.[2] This long half-life makes extended observations possible; some samples showed measurable activity 18 months after the radiosulfur had been prepared.

The radioactive vitamin was injected intramuscularly in two series of experiments; in the first series the subject, a young man in good health, was on a vitamin B_1-free diet for 36 days prior to the first injections and for the 15 days of the experimental period; in the second series the same subject was on a normal diet. Essentially the same results were obtained in both series.

Free (unphosphorylated) vitamin B_1 was determined in the urine by a modification of the thiochrome method. The urinary sulfur compounds were fractionated into the inorganic sulfate, ethereal sulfate and neutral sulfur components, the sulfur of each component was converted into elementary sulfur,[3] and the radioactivity of the sulfur was then measured quantitatively by comparison with a standard sample of the same radiosulfur. In the feces only the total radiosulfur was determined.

The radioactivity measurements were made with open, coincidence Geiger counters. The sulfide-coated copper counter tubes and the samples were enclosed in a large partially evacuated bell jar, provided with an externally operated arrangement for moving the samples successively to a definite position in front of the counters. The standard Rossi coincidence circuit was used in the amplifiers; these had time constants of 3×10^{-4} seconds. It was possible to measure samples as weak as 3% of the background by making very long runs.

Separate experiments showed that B_1^* added to urine does not exchange its radiosulfur with the inorganic or ethereal sulfur compounds; the S* was quantitatively recovered from the neutral sulfur fraction.

Figure 1 shows the daily excretion of radiosulfur (all forms) in the urine and feces after a daily intramuscular injection of 16 mg. of B_1^* for four

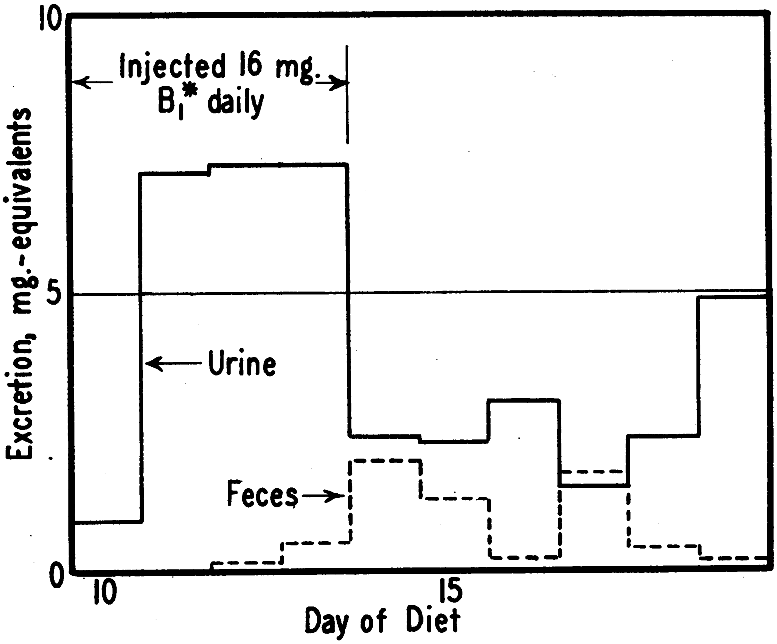

FIGURE 1

The daily recovery of radiosulfur (calculated as mg. of vitamin B_1) after intramuscular injection of B_1^*; normal diet.

days, while the subject was on the normal diet. Six days after the last injection a total of 61% of the injected radiosulfur had been recovered from the urine and 11% from the feces; 28% of the injected material remained unaccounted for. It is clear from the low and diminishing excretion in the feces that the urine is the major excretory medium of parenterally administered B_1 and its decomposition products.

Figure 2 shows the daily excretion of free thiamin in mg. and of the radiosulfur present in the neutral sulfur fraction expressed in terms of mg. of B_1^*. If only pure B_1^* is present in urine these two should be the same;

consequently the excess of thiamin over radiosulfur shown in the figure indicates excretion of thiamin already present in the body. Since the total amount of B_1 in the blood in this case could hardly have exceeded 1 mg.[4] the excess of 7.5 mg. of non-radioactive vitamin in the urine on the first day of the injections must have come from the tissues. This indicates that the injected vitamin interacts very rapidly with that preëxisting in the tissues.

Although the whole of the neutral radiosulfur in the urine during the injection period shown in the figures may have been vitamin B_1, this is improbable in view of the findings on the later days. The difference between the total free B_1 and the total neutral radiosulfur is certainly the minimum amount of preëxisting vitamin which was displaced from the tissues.

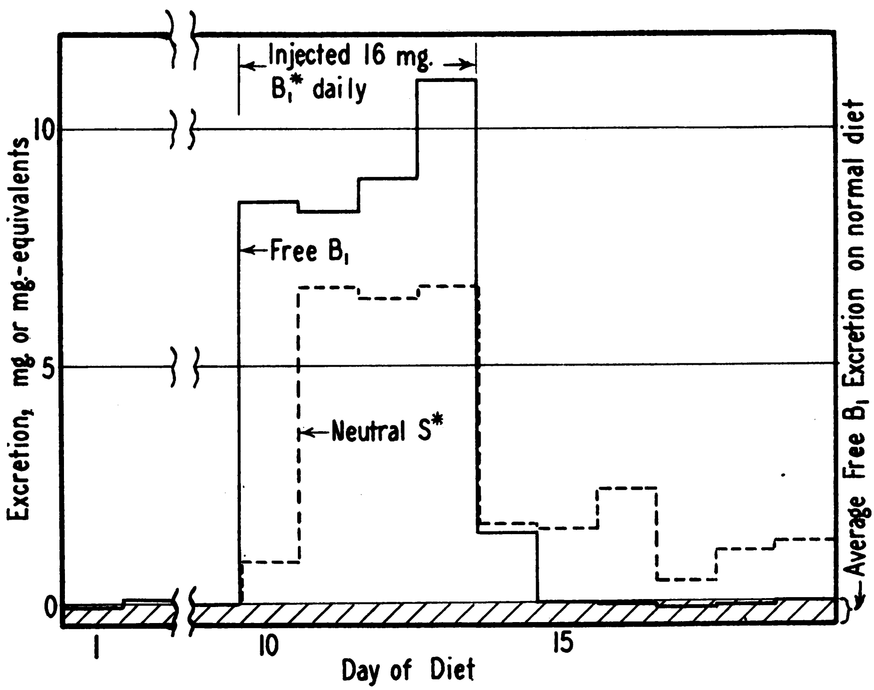

FIGURE 2

The daily excretion of neutral radiosulfur and free thiamin in the urine after intramuscular injection of $B_1{}^*$; normal diet. The radiosulfur has been calculated in terms of mg. of vitamin B_1.

During the six days following the termination of the radiothiamin injections the neutral radiosulfur in the urine exceeded the thiamin excretion. The difference between the neutral radiosulfur and the free B_1 sulfur is the minimum amount of neutral sulfur-containing decomposition products of the thiamin which was excreted. The continued excretion of radiosulfur is evidence that tissue thiamin is continuously undergoing destruction at a rapid rate.

These features were shown even more clearly in the series of experiments when the subject was on the B_1-free diet and 2.7 mg. of B_1* were injected daily from the 37th to 46th days, inclusive, of the B_1-free diet (Fig. 3). Thiamin excretion in the urine increased immediately, but no radiosulfur was detected in the urine for the first two days (less than 20 γ). The entire increment in the urine came from preëxisting thiamin in the tissues which was displaced by the injected material, again indicating that injected B_1

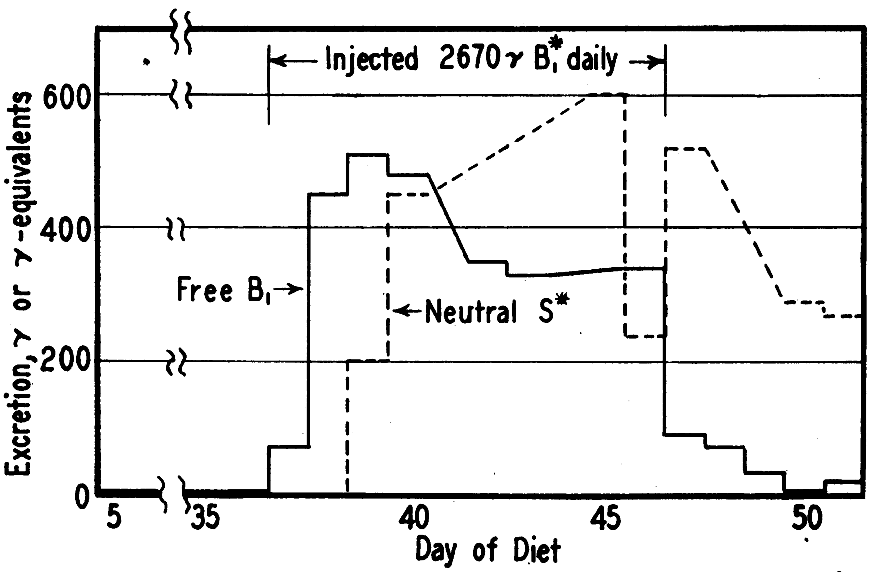

FIGURE 3

The daily excretion of neutral radiosulfur and free thiamin in the urine after intramuscular injection of B_1*; B_1-free diet. The radiosulfur has been calculated in terms of mg. of vitamin B_1.

rapidly enters the tissues from the blood. Since no thiamin was detectable in the urine for 30 days prior to the injections, the total quantity in the blood must have been less than 0.2 mg. For a short time at least after the injection of 2.7 mg., the ratio of radiothiamin to normal thiamin in the blood was probably greater than 1. If the excreted vitamin is an "overflow" moiety excreted before it enters the tissues, it should have been accompanied by a corresponding radiosulfur activity, but this was not found to be the case. The injected thiamin, therefore, must have entered the tissues very rapidly and interacted with the considerable quantities of thiamin already present.

Table 1 is a summary of some of the data illustrating this feature of thiamin metabolism. It is interesting that any free thiamin was displaced from the tissues of a subject who had been for 36 days on a B_1-free diet.

TABLE 1

THE DISPLACEMENT OF PREËXISTING THIAMIN FROM THE TISSUES BY ADDITIONAL THIAMIN INJECTED INTRAMUSCULARLY

DIET	INJECTION AND EXCRETION PERIOD (DAYS)	THIAMIN INJECTED (MG.)	MINIMUM AMOUNT OF PREËXISTING THIAMIN EXCRETED (MG.)
B_1-Free	3	8	0.8
Normal	1	16	7.5
Normal	4	64	16.0

The neutral sulfur and thiamin data in the two experiments are summarized in figure 4 which shows the difference between the thiamin and the neutral radiosulfur excretion. During the injection period the deficit of neutral radiosulfur indicates the minimum amount of preëxisting vitamin displaced from the tissues; the excess of neutral radiosulfur found in the urine after stopping the injections represents destroyed vitamin.

Figure 5 summarizes the urinary excretion of radiosulfur as inorganic sulfate; the radioactive sulfur appeared in this form in small quantities on the second day of the injections, and increased to considerable amounts by the end of the experiment, which is proof of the destruction of the injected vitamin and oxidation of the thiazole ring. The SO_4^* from this oxidation mixes with the large amount of SO_4 in the body;[5] the excretion of the radiosulfur is therefore delayed. The oxidation of the thiazole ring was therefore probably much more extensive than is indicated by the amount of SO_4^* in the urine in the first few days after beginning the injections.

The amounts of radiosulfur found as ethereal sulfate were in most cases very close to the experimental error of the radioactivity measurements; only a very small fraction of the radiosulfur appears in this form, and it may have arisen from exchange with the inorganic sulfate.

The extensive destruction of B_1 in the body is indicated in table 2.

TABLE 2

DESTRUCTION OF INTRAMUSCULARLY INJECTED THIAMIN

	TOTAL RECOVERY OF S* FROM B_1* (% OF TOTAL INJECTED)		PER CENT OF RECOVERED S* IN THE URINE WHICH REPRESENTS DESTROYED VITAMIN		
	FECES	URINE	AS NEUTRAL S COMPOUNDS	AS INORGANIC SULFATE	TOTAL
B_1-Free	..	26	21	21	42
Normal	11	61	18	25	43

Summary.—1. There is a rapid interaction of injected B_1 with that present in the blood and tissues.

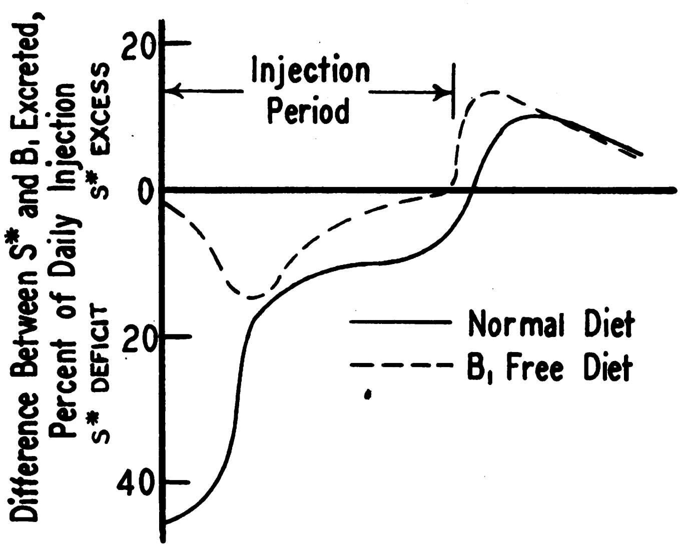

FIGURE 4
The difference between neutral radiosulfur and thiamin excretion after the injection of B_1*.

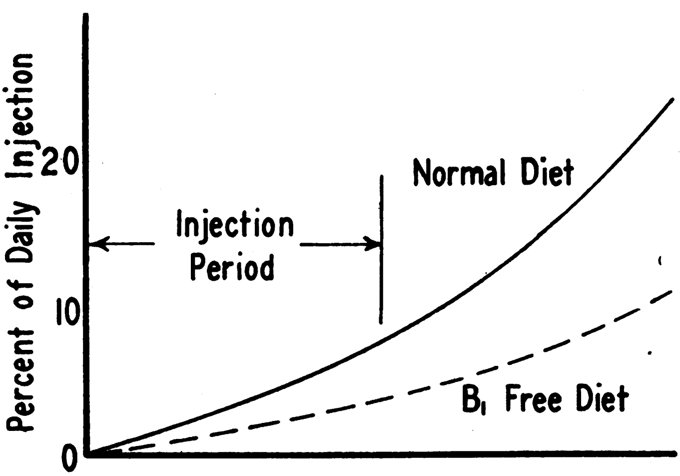

FIGURE 5
The urinary excretion of radiosulfur as inorganic sulfate after the injection of B_1*.

2. The displacement of preëxisting thiamin by injected thiamin demonstrates that a significant amount of the vitamin remains in the tissues even after 36 days of a B_1-free diet; larger quantities are present under normal nutritional conditions. This does not imply that the amount retained after a prolonged B_1-free diet is an adequate protective amount of this vitamin.

3. The metabolism (interchange and destruction) of vitamin B_1 is rapid and thus resembles that of the main metabolites—protein, fat and carbohydrate.

4. The rapid destruction of thiamin yields in the urine neutral sulfur compounds and inorganic sulfate.

5. The losses incurred by excretion and destruction are inevitable in the maintenance of a physiologically adequate concentration of thiamin and cocarboxylase in the blood and tissues.

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[1] Cf. E. R. Buchman, *Jour. Am. Chem. Soc.*, **58**, 1803 (1936); J. K. Cline, R. R. Williams and J. Finkelstein, *Ibid.*, **59**, 1052 (1937).

[2] W. F. Libby and D. D. Lee, *Phys. Rev.*, **55**, 245 (1939).

[3] R. A. Cooley, Don M. Yost and Edwin McMillan, *Jour. Am. Chem. Soc.*, **61**, 2970 (1939).

[4] Robert Goodhart and H. M. Sinclair, *Jour. Biol. Chem.*, **132**, 11 (1940).

[5] Henry Borsook, Geoffrey Keighley, Don M. Yost and Edwin McMillan, *Science*, **86**, 525 (1937).

RADIOACTIVE CARBON IN THE STUDY OF RESPIRATION IN HETEROTROPHIC SYSTEMS

BY S. RUBEN AND M. D. KAMEN

DEPARTMENT OF CHEMISTRY AND THE RADIATION LABORATORY, UNIVERSITY OF CALIFORNIA

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It is now well established[1] that the presence of small amounts of CO_2 are indispensable for the growth of many types of heterotrophic organisms. Also it has been reported[2] that the reduction of methylene blue by certain bacteria is dependent upon traces of CO_2. It appears that some of the experiments we have been doing with radioactive carbon have direct bearing on these interesting results.