diately, then deproteinized in the usual way (0.1 cc. plasma + 0.1 cc. water + 0.2 cc. 5% HPO_3) and 0.2 cc. deproteinized plasma titrated with 2,6-dichlorophenolindophenol (1 ≏ 0.02 mg. ascorbic acid) using our micropipette. A portion of each plasma was held for later titration at hourly intervals.

The remainder of each blood sample was allowed to stand at laboratory temperature and a portion of each centrifuged and deproteinized at intervals as shown in Table IV. We present data on 3 bloods of ascorbic acid contents frequently observed.

The above data are typical of similar studies conducted upon 17 different bloods.

Whole blood to which KCN is added becomes hemolysed after a short period of standing.

Bloods differ one from another considerably in the loss of reductive power upon standing.

Conclusion. For dependable ascorbic acid values, blood should be centrifuged, the plasma deproteinized, and the plasma-HPO_3 filtrate titrated in immediate sequence after the blood is drawn. Whole blood which stands in a closed small phial, with a minimum air space, may be depended upon to give results of clinical value for ½ hour. The higher values obtained with bloods to which KCN has been added represent an enhancement due to the action of KCN upon the 2,6-dichlorophenolindophenol, and in no wise a more accurate determination of their ascorbic acid content. This is particularly true with blood of low ascorbic acid value. KCN does not prevent the loss of ascorbic acid from blood.

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Vitamin B_1 Metabolism in Man. Excretion of B_1 in Urine and Feces.

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Goodhart and Jolliffe[1] have emphasized the need for a practical chemical method for studying vitamin B_1 metabolism. The fer-

[1] Goodhart, Robert, and Jolliffe, Norman, *J. Am. Med. Assn.*, 1938, **110**, 414.

mentation method recently published by our laboratories[2,3] is, we believe, admirably suited to this purpose.

After, and in conjunction with, considerable work on rat metabolism of vitamin B_1, we have applied our method to a group of human subjects. Five adults and 3 children, all 'normal' subjects with the possible exception of Subject E, who was under treatment for nervous breakdown, coöperated with us in this project. The main diet was in all cases normal, *i. e.*, no attempt was made to restrict these individuals to a standard diet. The average daily urinary output of the adults (average of 32 determinations) was 497.7 ± 47 gamma. For the children the urinary average was 333 ± 60 gamma.

Having established the normal urinary output on the average or normal diet, doses of 5 and 10 mg. (5000 and 10,000 gamma) were given once daily *per os* to the adult subjects. Table I gives the results obtained in this experiment.

TABLE I.
Daily Excretion of B_1 in the Urine.

B_1 dose per day	No. days on diet	Urinary B_1 of Subjects per Day				
		A	B	C	D	E*
Normal diet	8	Average for group 497 ± 47 gamma				
" + 5000 gamma	1	800	1000	1300	1320	600
" + " "	2	1000	1375	1800	1600	660
" + " "	3	1200	2000		1700	700
" + " "	4		1500			1068
" + " "	5-8 (aver.)					969
" + " "	19-21 "	1687	2383			
" + 10,000 "	Aver. maximum excreted		2414		2400	

*Not normal.

The first point to be noted is the non-uniform response to the 5 mg. dose. We feel that this variation in response may be related to either the level of body stores or individual rates of absorption.

After sufficient time has elapsed on the 5 mg. level, we find that urinary excretion of the vitamin takes place at a steady rate. This condition we designate as excretion equilibrium. When a subject in excretion equilibrium at 5 mg. per day is placed on the 10 mg. level, we do not obtain a significant rise in urinary output even after 15 days at this level. This phenomenon cannot be explained by body storage and the explanation must be sought elsewhere. Accordingly we made a series of parallel observations on the vitamin content of urine and feces. The results of this experiment are contained in Table II.

2 Schultz, A. S., Atkin, L., and Frey, C. N., *J. Am. Chem. Soc.*, 1937, **59**, 948.
3 Schultz, A. S., Atkin, L., and Frey, C. N., *J. Am. Chem. Soc.*, 1937, **59**, 2457.

TABLE II.
Daily Excretion of Vitamin B_1 in Urine and Feces.

	Aver. daily excretion after 6 to 19 days on supplement of 10,000 gamma Vit. B_1 per day		Aver. daily excretion on 2nd and 3rd days after reducing supplement from 10,000 to 5,000 gamma Vit. B_1 per day	
	Urine	Feces	Urine	Feces
Subject D	2350 gamma	7360 gamma	2600 gamma	2210 gamma
Subject F	2600 "	6900 "		

Primarily, Table II shows that doses in excess of 5 mg. result in incomplete absorption. Indeed, we find virtually all of the additional vitamin in the feces, *i. e.*, 5 mg. (5000 gamma). Thus the results clearly indicate that one must determine vitamin B_1 in feces as well as urine in order to obtain a true picture of vitamin metabolism.

It does not necessarily follow that incomplete absorption manifests itself only at doses of 5 mg. and above, for we find considerable fecal excretion of the vitamin at the 5 mg. level. Subject E. described in Table I as not normal, apparently failed to absorb vitamin B_1 in excess of 1-2 mg. per day. The indications are that if we had examined his feces when he had reached excretion equilibrium at the 5 mg. level, we would have found 2-3 mg. not being absorbed.

The oral administration of 5 to 10 mg. of the vitamin daily is considered, if we may judge from current medical literature, to be a moderate dosage. The data which we have presented would show that oral doses of this size are excessive or at least inefficient.

Schultz, Atkin and Frey[3] raised the question of the identity of the vitamin measured by the fermentation method. They showed that a fraction of the vitamin molecule 2-methyl-5-ethoxymethyl-6-aminopyrimidine will also give the fermentation response. However, irrespective of whether or not the urine and feces responses obtained represent true vitamin B_1, there is a close correlation between the determined values and the vitamin B_1 intake. The indications are that the method may be a valuable aid in the study of human vitamin metabolism.