1946. While attending these meetings he received official notification that his services had been recognized by the award of the C.M.G.

Cameron was a Fellow of the Royal Society of Canada, a Fellow of the Royal Institute of Chemistry, a Past President of the Canadian Institute of Chemistry (now the Chemical Institute of Canada), and a member of various chemical, biochemical, and medical societies. One of his life-long interests was the Scientific Club of Winnipeg, of which he was one of the early members, for twelve years its Secretary, and to whose Scientific Proceedings he made 46 contributions.

Although of a very reserved nature, Cameron made friends in all parts of the Dominion, and they, together with many others to whom he was only known by name, mourn the passing of a scientist and scholar.

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A Note on the Estimation of Vitamin B_1 in Urine

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The accurate measurement of the vitamin B_1 content of urine by the thiochrome method is made difficult by the interference of non-specific fluorescent substances. Adsorption on the zeolite Decalso (The Permutit Co. Ltd.) as described by Hennessy & Cerecedo (1939) removes some of these, but not nicotinamide methochloride which occurs in the urine of man and some other animals including the rat. Treatment with alkali, ferricyanide and *iso*-butanol in the thiochrome procedure converts nicotinamide methochloride to a violet-fluorescing compound (F_2) (Coulson, 1944), which is indistinguishable fluorimetrically from thiochrome. When ferricyanide is omitted, another substance (F_2) with a bluish-white fluorescence is formed from nicotinamide methochloride (Najjar & Holt, 1941). This makes the so-called 'NaOH blank' unsatisfactory. Several ways of overcoming this difficulty have been proposed (Mason & Williams, 1942; Najjar & Ketron, 1944; Coulson, 1944; Mickelsen, Condiff & Keys, 1945), but for reasons which will be given subsequently we believe that none of them is entirely satisfactory.

During work on the effect of sulphonamides on the excretion of vitamin B_1 by rats, we developed a procedure for avoiding interference by nicotinamide methochloride which, in our opinion, is an improvement on those at present in use. It depends on the fact that the blue fluorescence of thiochrome disappears on addition of acid to the *iso*butanol extract, whereas the fluorescence of F_3 is unaffected. By measuring the fluorescence before and after the addition of acid, a measure of the fluorescence due to thiochrome is obtained.

METHOD

A sample of urine (2–20 ml.), containing if possible 2–3 μg. vitamin B_1, is adjusted to pH 4·5 with glacial acetic acid, and diluted to 50 ml. with glass-distilled water. One 25 ml. portion is poured on to a Decalso column 5 cm. long and 0·6 cm. in diameter; 1·3 μg. vitamin B_1 is added to the other 25 ml. portion which is poured on to another column. The shape and size of the adsorption tube and the activation of the zeolite are as described by Hennessy (1941). The columns are washed three times with 10 ml. water, and the vitamin B_1 is then eluted with successive portions of 3, 3 and 5 ml. 25% (w/v) KCl in 0·1 N-HCl. The volume of the eluate is adjusted to 11·0 ml.

For oxidation to thiochrome, 40% NaOH (1 ml.), freshly prepared 1% $K_3Fe(CN)_6$ (0·1 ml.) and redistilled *iso*butanol (15 ml.) are added to a 5 ml. portion of the eluate in a glass-stoppered 2 oz. bottle. The contents of the bottle are well mixed after each addition and allowed to stand for 1 hr. If measurement of the fluorescence of F_2 is desired, a second bottle may be prepared in the same way except that ferricyanide is omitted, but this is not part of our routine procedure. Otherwise, the second 5 ml. portion of the eluate may be used for a duplicate determination of vitamin B_1.

Measurement of the fluorescence in a Cohen-type instrument (Henry, Houston, Kon & Osborne, 1939) is made with a 10 ml. portion of the *iso*butanol layer. While this is still in the test tube of the fluorimeter, seven drops of a mixture of three parts methanol and four parts N-HCl are added, the contents mixed, and the fluorescence measured again. The difference between the two readings is a measure of the fluorescence of thiochrome. This procedure makes the 'NaOH blank' unnecessary for the measurement of vitamin B_1, but useful as an indication of the amount of nicotinamide methochloride present.

It should be mentioned that this technique cannot be used with extracts containing methanol, which markedly increases the solubility of alkali in the *iso*butanol phase, as addition of sufficient HCl to neutralize this alkali leads to turbidity.

RESULTS

The results of an experiment, in which pure substances were used (Table 1), show that the fluorescence of thiochrome, as observed visually and as measured fluorimetrically, disappears after treatment with acid whereas that of F_3 does not. The galvanometer deflexion due to thiochrome, obtained by difference, is almost the same for vitamin B_1 alone as in the presence of nicotinamide methochloride. Use of the 'NaOH blank' (F_2) would give a negative result for vitamin B_1 in the presence of nicotinamide methochloride. In our fluorimeter the fluorescence of F_2 appears greenish instead of bluish white.

Typical results obtained with normal human urine, and with urine of normal and vitamin B_1-deficient rats, are given in Table 2. Two assumptions are unavoidable in the application of our method, as of many other methods for the estimation of vitamin B_1 in urine. One is that vitamin B_1 is adsorbed and eluted to the same extent from the zeolite columns, through which the unknown with and without added vitamin B_1 had been passed; the other is that thiochrome is extracted to the same degree from eluates with and without added vitamin B_1. On the other hand, the measurement of the blank with the same *iso*butanol extract used for measurement of the unknown obviates two other possible sources of error: (*a*) that due to differences in the extent to which nicotinamide methochloride is adsorbed on, and eluted from, different zeolite columns, and (*b*) that due to differences in the extent to which F_3 and other fluorescent substances (not thiochrome) are ex-

Table 1. *The effect of* HCl *treatment on the fluorescence of thiochrome and of* F_3

	Substance and quantity taken for zeolite adsorption					
	Vitamin B_1 (2·57 μg.)		Nicotinamide methochloride (250 μg.)		Vitamin B_1 (2·57 μg.) + nicotinamide methochloride (250 μg.)	
Treatment	Galvanometer deflexion*	Colour of fluorescence	Galvanometer deflexion*	Colour of fluorescence	Galvanometer deflexion*	Colour of fluorescence
(1) NaOH, then *iso*butanol extraction	24	Not visible	228	Greenish	218	Greenish
(2) NaOH ferricyanide, then *iso*butanol extraction	138	Blue	102	Violet	207	Bluish violet
(3) *iso*Butanol extract (2) treated with HCl	35	Not visible	103	Violet	102	Violet
Deflexion due to thiochrome ((2) – (3))	103	—	–1	—	105	—

* Linear scale.

Table 2. *The determination of vitamin* B_1 *in rat and human urine by the thiochrome method involving the use of* HCl

(Values are given as galvanometer deflexions, linear scale.)

	Rat urine				Human urine	
	Normal diet		Vitamin B_1 deficient diet			
Treatment	Without added vitamin B_1*	With 1·3 μg. added vitamin B_1*	Without added vitamin B_1†	With 1·3 μg. added vitamin B_1†	Without added vitamin B_1‡	With 1·3 μg. added vitamin B_1‡
(1) NaOH, then *iso*butanol extraction	142	140	320	360	364	374
(2) NaOH and ferricyanide, then *iso*butanol extraction	236	300	79	158	161	233
(3) *iso*Butanol extract (2) treated with HCl	58	56	80	89	76	77
Deflexion due to thiochrome ((2) – (3))	178	244	–1	69	85	156
Calculated vitamin B_1 content of urine	2·7 μg./ml.		0·0 μg./ml.		0·16 μg./ml.	

* 1 ml. urine taken. † 4 ml. urine taken. ‡ 10 ml. urine taken.

Table 3. *Urinary excretion of vitamin B_1 by rats with different intakes of vitamin B_1*

Treatment	No. of observations	Urinary excretion (μg./rat/day) Mean	Range
Diet deficient in vitamin B_1	7	0	0–0
Diet deficient in vitamin B_1 + 20 μg. vitamin B_1 daily	11	0·36	0·1–0·6
Diet deficient in vitamin B_1 + 40 μg. vitamin B_1 daily	3	1·89	1·3–3·0

Table 4. *A comparison of the determination of vitamin B_1 in pure solutions in the presence and absence of nicotinamide methochloride by the method of Mickelsen et al.* (1945) *and by our method*

(Values are given as galvanometer deflexions, linear scale.)

Treatment	Substance and quantity taken for zeolite adsorption: Vitamin B_1 (1·3 μg.)	Nicotinamide methochloride (250 μg.)	Nicotinamide methochloride (250 μg.) + vitamin B_1 (1·3 μg.)
(1) NaOH, then *iso*butanol extraction at pH 12	34	270	293
(1*a*) NaOH, then *iso*butanol extraction at pH 8–9·5	40	37	39
(2) NaOH and ferricyanide, then *iso*butanol extraction at pH 12	98	103	180
(2*a*) NaOH and ferricyanide, then *iso*butanol extraction at pH 8–9·5	97	108	175
(3) *iso*Butanol extract (2) treated with HCl	37	103	116
Deflexion attributed to vitamin B_1:			
Method of Mickelsen *et al.*: ((2*a*) – (1*a*))	57	71	136
Our method: ((2) – (3))	61	0	64

Table 5. *A comparison of measurement of vitamin B_1 in rat and human urine, by the method of Mickelsen et al.* (1945) *and by our method*

(Values given as galvanometer deflexions, linear scale.)

Treatment	Urine* of rats on vitamin B_1 deficient diet: Without added vitamin B_1	With added vitamin B_1 (1·3 μg.)	Normal human urine†: Without added vitamin B_1	With added vitamin B_1 (2·6 μg.)
(1) NaOH, then *iso*butanol extraction at pH 12	400	400	142	150
(1*a*) NaOH, then *iso*butanol extraction at pH 8·0–9·5	39	40	27	28
(2) NaOH and ferricyanide, then *iso*butanol extraction at pH 12	148	225	68	158
(2*a*) NaOH and ferricyanide, then *iso*butanol extraction at pH 8·0–9·5	148	226	63	147
(3) *iso*Butanol extract (2) treated with HCl	148	150	47	50
Method of Mickelsen *et al.* ((2*a*) – (1*a*)):				
Deflexion attributed to vitamin B_1	109	185	36	119
Calculated vitamin B_1 content of urine	0·45 μg./ml.		0·11 μg./ml.	
Our method ((2) – (3)):				
Deflexion attributed to vitamin B_1	0	75	21	108
Calculated vitamin B_1 content of urine	0·0 μg./ml.		0·06 μg./ml.	

* 4 ml. urine taken.

† 10 ml. urine taken.

tracted by *iso*butanol in separate samples of the extract. We have frequently observed that the blank obtained for urine with added vitamin B_1 is slightly higher than that for urine alone, which may be due to a greater elution of nicotinamide methochloride from the zeolite column in the presence of additional vitamin B_1.

Results similar to those in Table 2 have been obtained with human urine on many occasions, but our experience has been primarily with rat urine. Typical results (Table 3) show that the excretion of vitamin B_1 by rats, as determined by our method, is closely related to the vitamin B_1 content of the diet.

DISCUSSION

We believe that our procedure is an improvement on those of other workers for the following reasons. We are in agreement with Najjar & Ketron (1944) that nicotinamide methochloride is partly destroyed by sodium sulphite and that the procedure suggested by Mason & Williams (1942) gives too high results. Najjar & Ketron (1944) and Coulson (1944) allow for the interference of nicotinamide methochloride by assuming that the galvanometer deflexion due to F_2 in the NaOH blank bears a fixed relation to that due to F_3 in the unknown. No such assumption is necessary in our method as we are able to measure F_3 directly.

In the method of Mickelsen *et al.* (1945) the *iso*butanol extraction is carried out at a pH 8·0–9·5, at which F_2 in the NaOH blank does not fluoresce, but the fluorescence of thiochrome in the unknown is unimpaired. Table 4 confirms this but shows that this treatment does not affect F_3, so that in the presence of nicotinamide methochloride both thiochrome and F_3 contribute to the fluorescence. Table 5 shows that for this reason the method of Mickelsen *et al.* (1945) gives too high results when applied to rat and human urine; e.g. the urine of vitamin B_1-deficient rats was found by this method to contain 0·45 μg. vitamin B_1/100 ml., whereas none was found by our method. For the sample of human urine the discrepancy was less since less nicotinamide was present.

SUMMARY

A modification of the thiochrome method for the fluorimetric determination of vitamin B_1 in urine in the presence of nicotinamide methochloride is described. Since treatment of the *iso*butanol extract with HCl removes the thiochrome fluorescence, the difference between readings taken before and after this treatment is a measure of the vitamin B_1 content of the urine.

We wish to thank Dr A. C. Bottomley for preparing the nicotinamide methochloride.

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Aggregate Formation in Soil

1. INFLUENCE OF SOME BACTERIAL POLYSACCHARIDES ON THE BINDING OF SOIL PARTICLES

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For maximum crop production it is essential that the soil should have a good crumb structure and that the crumbs should be stable and resistant to the dispersing effects of rain, frost, etc. Aggregate formation facilitates cultivation, drainage and aeration, increases the moisture-holding capacity of soil and reduces erosion. It also maintains sufficient cohesion in the soil to give anchorage to plants, and yet sufficient incoherence to facilitate root penetration and emergence of seedlings. Large additions of organic matter and the growth of grassland vegetation are credited with having an ameliorative effect on the physical state of the soil, but there is considerable diversity of opinion as to the exact processes involved in the formation of water-stable aggregates.

Stable structure formation under natural conditions is possibly a gradual process influenced by many factors. It is reasonable to expect that physical, chemical and biological agencies are involved. The biological agencies include plants, animals and micro-organisms; and of these the micro-organisms are of great importance in pro-