CV. POTENTIOMETRIC TITRATIONS OF VITAMIN B₁ AND OF THIOCHROME.

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INTRODUCTION.

THIS work is a continuation of that of Moggridge and Ogston [1935]. It embodies titration curves of vitamin B₁ in more concentrated solution and of thiochrome [Kuhn and Vetter, 1935; Barger *et al.*, 1935], spectrographic measurements and catatorulin tests. Thiochrome is of interest since it is an oxidation product of the vitamin differing by only 2 or possibly 3 H atoms.

RESULTS AND DISCUSSION.

Vitamin B₁. The vitamin combines with only two equivalents of acid. The formula proposed by Williams [1935] demands that one of these shall be combined with a quaternary nitrogen of unusual type forming part of a thiazole group. The structure of the latter appears to have been completely settled by the synthesis of Clarke and Gurin [1935]. The peculiar N atom determines the character of a pseudo-acidic or pseudo-basic group which can be titrated in the neighbourhood of p_H 9.0 [Birch and Harris, 1935; Moggridge and Ogston,² 1935; Williams and Ruehle, 1935]. This group it is agreed requires two equivalents of alkali. All these workers agree that a further group is present which can be titrated near p_H 4.8, but Moggridge and Ogston described also a basic group of p_K 3.4. But this is inconsistent with the remainder of the evidence.

The present results show clearly that the titration curve of vitamin B₁ below p_H 7.0 is satisfactorily accounted for by a single group of p_K 4.8. The finding of Moggridge and Ogston was due therefore to the fact that too dilute solutions were employed; either there was some decomposition of the vitamin or possibly an error in the large water correction. Hence all workers now agree that vitamin B₁ has p_{K_1} 4.8 corresponding to a basic group and p_{K_2} about 9.0 corresponding to a pseudo-acidic or pseudo-basic group, the latter group requiring two equivalents.

The present results upon vitamin B₁ were obtained with the hydrogen electrode. They are consistent with the previous findings regarding p_K 4.8 obtained with the glass electrode; spectroscopic observations (Fig. 1, Curve 1) indicate that the vitamin structure is essentially intact after titration [*cf.* the curves of Holiday, 1935; Peters and Philpot, 1933]. On the other hand, exposure of a vitamin solution to the hydrogen electrode under the conditions of titration at p_H 7.0 for 12 hours in one experiment (5) reduced its catatorulin activity by 80 % without

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² These authors thought that this group must be regarded as pseudo-acidic because of the effect of alcohol upon the p_K . The group however is so unusual that the alcohol effect may well be anomalous.

causing any material change in the absorption spectrum. It is possible therefore that even in the shorter period of titration (30 min.) the vitamin may be slightly

Fig. 1. Ultraviolet absorption spectra. Curve I. Vitamin at p_H 3 after having been in contact with H_2/Pt in the titration vessel. Curve II. Thiochrome at p_H 8. Curve III. Thiochrome at p_H 3. Curve IV. Thiochrome at p_H 3 after titration with the glass electrode.

Fig. 2. Corrected titration curves of vitamin B₁ and thiochrome. $\times$ Experimental points for vitamin B₁ below p_H 7. Full line is theoretical for 1 equiv. of p_K 4.8. $\circ$ Experimental points for thiochrome; full line theoretical for 1 equiv. p_K 5.6. $\square$ Equilibrium points for thiochrome.

altered. It has always been found that the colour reaction is affected [cf. Moggridge and Ogston, 1935]. Kinnersley *et al.* [1935, 2] suggested that the group which can be titrated near p_H 4.8 contains quaternary nitrogen. The

elimination of the group of p_K 3.7 now means that we must return to the original view of Moggridge and Ogston that the group of p_K 4.8 is the amino-group attached to the pyrimidine ring. Since this group remains intact to the titration while the colour reaction disappears, it seems that the amino-group cannot be responsible for the diazo-reaction as has been suggested by Kinnersley *et al.* and Barger *et al.*

Thiochrome. Proper determination of the titration curve of thiochrome with the hydrogen electrode proved impossible, the curves being of indefinite form and changing with successive titrations of the same sample; this was associated with a change in absorption, diminution of the long-wave band and the appearance of a new band at about 2700 Å. This is a confirmation of the band given by Kuhn and Vetter [1935] for reduced thiochrome. Using a glass electrode however excellent titration curves (Fig. 2) were obtained, very similar to those of the vitamin; the ultra-violet absorption curve was not changed during titration (Fig. 1); there is a single group with p_K 5.6 the acid binding corresponding to one equivalent per mol. thiochrome (assumed to be $C_{12}H_{14}ON_4S=264$). Its titration seemed to correspond closely with the change from yellow to colourless of the solution. Between p_H 7.0 and 9.0 (the limit of the glass electrode) the solution behaved somewhat similarly to the vitamin; the p_H values drifted to equilibrium indicating the presence of a pseudo-acidic or pseudo-basic group titrating between p_H 8 and 10.

From determination of the fluorescence changes Kuhn and Vetter [1935] found a p_K of 6.3 for a basic group of thiochrome. Our data give a rather more acid value of 5.6.

Let us consider the interpretation of these results in relation to the recent formula proposed for thiochrome [Barger *et al.*, 1935; Kuhn and Vetter, 1935; Windaus *et al.*, 1934] with formation of a central glyoxaline ring (I). From the

titration data it seems that the p_K 5.6 of thiochrome belongs to the same N atom at 8 attached to the pyrimidine ring; the change from 4.8 to 5.6 might accompany the ring closure. The value of 5.6 is consistent with the p_K values of known glyoxaline basic groups [Levy, 1935]. It seems probable that the drift at alkaline reaction has the same cause both with vitamin and thiochrome. Formula I does not account satisfactorily for these points; we suggest formula II with a quaternary N atom. As Barger *et al.* point out there are objections to the assumption of a quaternary N atom in thiochrome; but without assuming this grouping we

cannot account for the alkali drift, since according to Clarke and Gurin the opening of the ring and alkali drift are associated with the quaternary attachment of the thiazole nucleus. Both Kinnersley *et al.* and Barger *et al.* consider that the acquisition of blue fluorescence (quinochrome and thiochrome) involves loss of sulphur lability.¹ No formula seems completely satisfactory. Thiochrome has been found to combine with 2 mols. of HCl, one of these being held less closely than that of vitamin B₁. This would suggest a weaker base for the second HCl whereas actually the titration data indicate a stronger base of p_K 5.6.

The changed absorption spectrum found after titration with the hydrogen electrode is sufficiently similar to that of vitamin B₁ to suggest the application of the catatorulin test, though Barger *et al.* state that hydrogenated thiochrome is not vitamin B₁. The short-wave maximum 2700 Å. is not very near that of B₁ in acid solution at 2470 Å. A few catatorulin tests have been made upon thiochrome itself and specimens after reduction by contact with hydrogen and platinum for 4 hours (see Fig. 3). The thiochrome both from Germany and Edinburgh showed

Fig. 3. Ultra-violet absorption spectra. Curve I. Thiochrome at p_H 3 after 4 hours' contact with H_2/Pt at p_H 7. Curve II. Thiochrome at p_H 3 after 4 hours' contact with H_2/Pt at p_H 5.5.

no catatorulin activity (Exps. 1 and 2) in confirmation of animal tests from these laboratories. Some specimens of reduced thiochrome (Exps. 3, 4, 5) showed a trace of apparent activity almost within limits of experimental error. This matter is being further investigated.

EXPERIMENTAL.

Hydrogen electrode titrations were performed in a small rocking half-cell (Fig. 4) working on the principles of that described by Clark [1920]. Its capacity was 0.15 ml. Hydrogen electrodes were small platinised plates [Ogston and Brown, 1935]. Hydrogen flowed over the surface of the liquid and escaped through a trap; contact of the liquid with hydrogen and the electrode and mixture of the liquid were assured by keeping the cell mechanically rocked through an

¹ On the other hand, Kuhn and Vetter state that after long boiling with 2N NaOH thiochrome liberates S as —SH.

angle of 20° about three times per sec. A 3.5 *N* calomel electrode was the reference electrode; the whole cell was in an air thermostat at $25 \pm 0.2^\circ$.

Glass electrodes were of the type described by MacInnes and Dole [1933] having very thin membranes of about 2 mm. diameter; they were calibrated against buffers standardised with the hydrogen electrode. Electrical measure-

Fig. 4. Hydrogen electrode vessel.

ments were made with a Tinsley slide wire potentiometer using a Tinsley valve electrometer with glass electrodes. Potentials were measured to the nearest millivolt. Titrant solutions were approx. *M*/150; 0.1 *N* titrant solutions were added from a capillary microburette which allowed reading to $\pm 2 \times 10^{-5}$ ml.

Ultra-violet absorption spectra were obtained with a Hilger E 316 Quartz spectrograph with a Spekker photometer. All spectra were measured in solutions of about p_H 3 except that of thiochrome which was measured at p_H 8.

The amount of thiochrome used for these experiments was under 1.0 mg.

The vitamin was prepared in the Department of Biochemistry, Oxford University [Kinnersley *et al.*, 1935, 1]; the thiochrome was available to us by permission and through the courtesy of Prof. Kuhn and Prof. Barger. All observations upon thiochrome with the exception of the catatorulin tests, Exps. 1 and 2, were made with Prof. Kuhn's specimen.

Catatorulin tests. Medium: Ringer phosphate of p_H 7.3, no pyrophosphate; substrate: pyruvate instead of lactate. Figures represent average O_2 uptake $\mu\text{l./g./hr.}$ for a period of 1–2 hours.

		Additions	$\mu\text{l./g./hr.}$
Exp. 1		0	861
	$\mu\text{l. B}_1$	1 γ	1345
	Thiochrome Kuhn	5 γ	848
	Thiochrome Barger	5 γ	869
Exp. 2		0	556
	Vitamin B_1	1 γ	995
	Thiochrome Kuhn	5 γ	550
	Thiochrome Barger	5 γ	572

The results show that less than 1% vitamin B_1 activity (catatorulin) is present in the thiochrome preparations of Kuhn and Barger.

	Additions	$\mu\text{l./g./hr.}$	
Exp. 3	0	565	
Vitamin B ₁	0.5 γ	973	
Vitamin B ₁	1.0 γ	1016	
Reduced thiochrome p _H 3-9 during titration	16 γ	679	Not more than 0.15 γ vitamin B ₁ present or 1%
Exp. 4	0	476	
Vitamin B ₁	0.25 γ	675	
Vitamin B ₁	0.5 γ	757	
Reduced thiochrome p _H 7.5 (4 hours)	{ A 1/5 B 1/5	489 Nil	
		508	Not more than 0.05 γ present
Exp. 5	0	618	
Vitamin B ₁	0.25 γ	924	
	0.5 γ	1010	
	2 γ original B ₁ reduced p _H 7.0 (12 hours)	912	Not more than 0.25 γ or not more than 12% present
	12 γ reduced thiochrome p _H 5.5 (4 hours)	772	0.12 γ or 1% present
Exp. 6	0	602	
Vitamin B ₁	2 γ	1219	
	0 + 22 γ reduced thiochrome p _H 2.5 (12 hours)	639	Not more than 0.05 γ or 1 in 500 probably
Vitamin B ₁	2 + 22 γ thiochrome	1228	

All reductions at 25° or room temperature with H₂ and Pt black.

SUMMARY.

1. The titration curve of vitamin B₁ on the acid side is completely described by one basic group having p_K 4.8. All workers are now in agreement.
2. Thiochrome (Kuhn) shows a basic p_K of 5.6 and like vitamin B₁ a tendency for a drifting titration value to the alkaline side of the neutral point. It has no vitamin B₁ activity by catatorulin test.

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