CLI. THE CHEMICAL NATURE OF VITAMIN B₁ FROM EVIDENCE AFFORDED BY ITS ELECTRICAL TRANSFERENCE.

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SINCE the recognition of the dietary principle now known as vitamin B₁ a great number of attempts have been made to isolate it during the last thirty years. Though nearly all these attempts have admittedly failed in their main objective, the methods of fractionation generally employed suggest that the vitamin is probably of a basic nature. This conclusion is, however, complicated by the probability that adsorption plays a more important rôle in these fractionations than does true chemical reaction. Moreover, ample evidence has been brought forward by several investigators [Kinnersley and Peters, 1928; Guha and Drummond, 1929] to show that the behaviour of vitamin B₁ is to a large extent modified by the associated substances present. The adsorption of vitamin B₁ by fuller's earth and "acid clay," however, is apparently of the polar type and, therefore, strengthens the supposition that vitamin B₁ is a base. All this evidence, however, is indirect. The present paper records the results of experiments on the behaviour of various preparations of vitamin B₁, as also of crude yeast extracts, on being subjected to electro-dialysis at different hydrogen ion concentrations. This investigation had three objects in view:

(1) to obtain what might be regarded as direct and conclusive evidence as to the basic nature of vitamin B₁ and to attempt to find the approximate p_K value of its basic group;

(2) to examine the possibilities of concentration of vitamin B₁ by this method;

(3) to attempt a separation of vitamin B₁ from other members of the vitamin B complex.

After this work had been completed, our attention was drawn to a paper by Williams and Waterman [1929], in which they discuss the possibility of the separation of ampholytes by electrodialysis using a multiple compartment cell. They state that "the two factors which can be recognised most definitely by our present feeding technique migrate towards the alkaline region where they undergo fairly rapid decomposition. These factors are the antineuritic

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vitamin and the one designated as 'the third factor.' No details, however, are given. Professor Drummond has also recently informed us of the unpublished work of A. F. Watson in his laboratory in 1921, who observed with crude yeast extracts that "vitamin B" (its complex nature not being established at that time) migrated towards the cathode at p_H 3.8.

EXPERIMENTAL.

A three compartment cell was used for the electrolysis. It was made by cutting a wooden box, open at the top and of suitable dimensions, into three vertical sections of equal length. Each section was soaked in paraffin-wax to render it impermeable to water. Four rubber washers were cut to fit the ends of the sections, a parchment membrane was placed between each pair so as to separate the three sections, and the whole box was then clamped together by means of two iron clamps. The vitamin solution was placed in the middle compartment, the two end compartments each being filled with distilled water in which platinum electrodes were immersed. The cathode electrode acted both as stirrer and electrode. A cork pulley coupled to an electric motor was fitted to the upper end of a glass tube, the other end being fused on to the platinum and connection made by means of a copper wire dipping into mercury contained in the tube. This procedure was found necessary as in a previous experiment, using stationary carbon electrodes, a nearly total loss of activity was recorded. This was thought to be due either to adsorption on the carbon or to a local concentration of alkali near the electrode inactivating the vitamin. A cooling coil made of glass tubing was placed in each compartment. The coils were connected in series with a pump and a coil of lead tubing immersed in a freezing mixture, brine being continuously pumped round through this closed circuit to conduct away the heat generated by the passage of the current. During the course of the electrolysis sulphuric acid was added to the cathode compartment and baryta to the anode. The p_H of the middle compartment was similarly adjusted when necessary. In some of the experiments, as indicated below, certain substances were introduced into the end compartments, both to act as buffers and to reduce the resistance of the cell. The p_H was determined colorimetrically by the use of external indicators.

Exp. 1. Electrolysis of lead filtrate at p_H 7 and p_H 4.

50 cc. of a lead filtrate fraction prepared by extracting yeast with hot 50 % alcohol, precipitating with lead acetate and removing the lead with hydrogen sulphide were diluted to 150 cc., placed in the middle compartment and adjusted to p_H 7 with baryta. 150 cc. of distilled water were placed in both the anode and cathode chambers. The cathode liquid was kept at p_H 1.5-2, the anode liquid at p_H 2-5. Electrolysis was carried on for 4 hours with a current of 0.8 amp. (the temperature being 10-15°). At the end of this time 15 cc. or 1/10 of the total volume of the middle compartment and

the whole of the anode and cathode solutions were removed for feeding tests. The barium was precipitated by sulphuric acid, the precipitate filtered and washed, and the filtrates and washings were made up to definite volumes. The dosage was computed by finding the number of day-doses present after electrolysis on the basis of the original activity present in the middle compartment. The method of assay of the vitamin was essentially the same as that employed by Guha [1931]. Fig. 1 shows the activity of these solutions and also that

Fig. 1.

Fig. 2.

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Curve (1). Lead filtrate before electrolysis; $\uparrow$ 0.15 cc. (day-dose).

Curve (2). Fraction of middle chamber after electrolysis at p_H 7.0; $\uparrow$ dose $\equiv$ 3 times day-dose of original lead filtrate; $\downarrow$ dose $\equiv$ 1.5 times day-dose of original lead filtrate; $\downarrow$ dose $\equiv$ 1 original day-dose.

Curve (3). Cathode fraction; $\uparrow$ dose $\equiv$ 3 times original day-dose.

Curve (4). Anode fraction; $\uparrow$ dose $\equiv$ 6 times original day-dose.

Fig. 2. Electrolysis at p_H 4.4 (Exp. 1).

Curve (1). Middle fraction; $\uparrow$ dose $\equiv$ 1.5 times original day-dose; $\downarrow$ dose $\equiv$ 4.5 times original day-dose.

Curve (2). Cathode fraction; $\uparrow$ dose $\equiv$ 1.8 times original day-dose; $\downarrow$ dose $\equiv$ 1 original day-dose; $\downarrow$ dose $\equiv$ 1.5 times original day-dose; $\times$ dose $\equiv$ 2 times original day-dose.

Curve (3). Anode fraction; $\uparrow$ dose $\equiv$ 9 times original day-dose.

of the original lead filtrate. The greater part of the vitamin is seen still to be contained in the middle compartment, the cathode and anode fractions showing no activity. The fluid from the middle compartment was then adjusted to p_H 4.4 and further electrolysed for $4\frac{1}{2}$ hours the current being 0.3 amp. From Fig. 2 it is seen that over 50% of the activity has passed into the cathode compartment while the middle compartment contains the remainder of the activity, the anode fraction being totally inactive.

Exp. 2. Re-electrolysis of cathode solution at p_H 8.5.

80 cc. of the active cathode solution obtained at the previous stage were adjusted to p_H 8.5 by means of baryta and re-electrolysed for half an hour with a current of 0.3 amp. at 10–15°. Attempts were made to keep the anode and cathode liquids at approximately p_H 8.5 by buffering them with small amounts, about 0.3 g., of glycine and ammonium sulphate respectively. Owing to the rapid liberation of acid and alkali in these compartments great difficulty was experienced, but for the most part they were kept on the alkaline side of

neutrality. Fig. 3 shows that about 50 % of the vitamin has migrated to the cathode and none to the anode, which confirms the earlier finding.

Fig. 3.

Fig. 4.

Fig. 3. Electrolysis at p_H 8.5 (Exp. 2).

Curve (1). Middle fraction; $\uparrow$ dose $\equiv$ 3 times original day-dose; $\downarrow$ dose $\equiv$ 4.5 times original day-dose; $\circ$ dose $\equiv$ 6 times original day-dose.

Curve (2). Cathode fraction; $\uparrow$ dose $\equiv$ 8 times original day-dose; $\downarrow$ dose $\equiv$ 4 times original day-dose.

Curve (3). Anode fraction; $\uparrow$ dose $\equiv$ 12 times original day-dose.

Fig. 4. Electrolysis of a chemically purified fraction at p_H 8.5 (Exp. 3).

Curve (1). Middle fraction; $\uparrow$ dose $\equiv$ 2 times original day-dose; $\downarrow$ dose $\equiv$ 1.5 times original day-dose.

Curve (2). Cathode fraction; $\uparrow$ dose $\equiv$ 1.5 times original day-dose; $\downarrow$ dose $\equiv$ 1 original day-dose; $\circ$ dose $\equiv$ 1.25 times original day-dose; $\times$ dose $\equiv$ 1.5 times original day-dose; $\times$ dose $\equiv$ 2 times original day-dose.

Curve (3). Anode fraction; $\uparrow$ dose $\equiv$ 4 times original day-dose.

Exp. 3. Electrolysis of a chemically concentrated preparation of vitamin B₁ at p_H 8.5.

This moderately concentrated preparation was obtained from brewer's yeast approximately by the method previously described [Guha, 1931]. The solution was active in a day-dose of 0.25 cc. and contained 1.5 mg. solids (1.3 mg. organic) per day-dose. 21 cc. were taken, adjusted to p_H 8.5, diluted to 50 cc. and electrolysed for 40 mins. with an average current of 0.2 amp. at about 10°. The solutions in the side chambers were treated as in Exp. 2. Fig. 4 shows that nearly 40 % of the vitamin migrated to the cathode under these conditions, which is in agreement with the earlier experiment.

Exp. 4. Electrodialysis of crude yeast extracts at p_H 7 and 4.

A crude yeast extract was made by extracting 1055 g. of yeast with boiling water. The filtrate, 1600 cc., was electrolysed for 12 hours at p_H 7 (1.5 amp.). About 30 % of the vitamin B₁ activity passed to the cathode. The cathode solution contained no vitamin B₂. The solution from the middle compartment was still able to maintain growth of rats totally deficient in the "vitamin B-complex" (Fig. 5). On further electrolysis at p_H 4 there was a loss of vitamin B₂ activity from the middle compartment, with no corresponding increase in activity in either of the side compartments.

Table I summarises the results obtained.

Table I.

Exp.	Conditions	Result	Total organic solids (g.) per day-dose	
			In the middle chamber before electrolysis	In the chamber containing the active fraction after electrolysis
1	Lead filtrate electrolysed at p_H 7	Cathode fraction inactive	0.036	—
		Anode fraction inactive		—
		Middle fraction active		0.037
	Further electrolysis at p_H 4.4	Cathode fraction active Middle fraction slightly active Anode fraction inactive	0.037	0.018 —
2	Re-electrolysis at p_H 8.5 of the active cathode fraction obtained in Exp. 1	Anode fraction inactive	0.018	—
		Cathode fraction, 50 % activity		0.024
		Middle fraction, 50 % activity		0.060
3	Chemically concentrated fraction electrolysed at p_H 8.5	Anode fraction inactive	0.0013	—
		Cathode fraction, 50 % activity		0.0012
		Middle fraction, 40 % activity		0.003
4	Electrolysis of crude yeast extract at p_H 7	Anode fraction inactive	—	—
		Cathode fraction, 30 % activity		0.014
		Middle fraction, 60–70 % activity		0.018

Fig. 5. Electrolysis of crude yeast extract at p_H 7.

- Curve (1). Vitamin B₁-deficient rat; cathode fraction; ↑ dose ≡ 3 g. fresh yeast.
 Curve (2). Vitamin B₁-deficient rat; middle fraction; ↑ dose ≡ 0.75 g. fresh yeast.
 Curve (3). Vitamin B₂-deficient rat; middle fraction; ↑ dose ≡ 3 g. fresh yeast.
 Curve (4). Vitamin B complex-deficient rat; middle fraction; ↑ dose ≡ 2 g. fresh yeast; growth falls off later.

DISCUSSION.

From the results of Exps. 2 and 3 it is obvious that vitamin B₁ behaves like a base even at p_H 8.5, and, therefore, must be either a true base or an ampholyte whose isoelectric point is higher than p_H 8.5. Considering the latter case, such an ampholyte might be of the type carrying an excess basic group, or one whose basic dissociation constant is much greater than its acid dissociation constant.

Concerning Exp. 1 with lead filtrate, the fact that the vitamin did not

migrate at p_H 7 and did so at p_H 4.4 can probably be explained by taking into account the resistance of the cell at these p_H values. The much lower resistance at p_H 7 (noted by higher current passing) is no doubt due mainly to the fact that weak acids or ampholytes acting as acids at this p_H value exist as their ionised barium salts so that the barium cations may carry the major part of the current while at p_H 4 they are non-ionised and the barium is precipitated as barium sulphate. Hence the cations at p_H 4 are mostly bases other than barium, and so a much greater proportion of the current is carried by the vitamin. Owing to this variation in resistance of the cell at different p_H values due to the presence of large and varied amounts of ionised impurities, we did not succeed in getting even an approximate idea of the p_K value of the vitamin, since the experimental technique here adopted did not permit of a sufficiently adequate control of all the variables. From experiments with the crude yeast extract it is seen that the vitamin even in the crude state behaves like a base. The fact that the vitamin migrates in the crude state at p_H 7 appears to be in conflict with the result of Exp. 1 but the conditions of the two experiments are not comparable, as the total amount of current passed and the quantity and variety of ionised substances present are not the same. It was found possible to obtain vitamin B_1 in the cathode compartment practically free from vitamin B_2 , but it was not possible to obtain vitamin B_2 free from vitamin B_1 , as apparently there was a loss of activity of the former during the course of the electrolysis. This loss was probably due to the adsorption of vitamin B_2 on the precipitate of barium salts, proteins, *etc.*, which formed in the middle compartment. Very little of vitamin B_1 is apparently destroyed by electrolysis. Appreciable concentration may be obtained by this method at some of the earlier stages of fractionation.

SUMMARY.

1. Vitamin B_1 in both the crude and relatively purified state behaves as a base even at p_H 8.5.
2. By electrolysis of a crude yeast extract, vitamin B_1 can be concentrated in appreciable quantities free from vitamin B_2 at the cathode.

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