TABLE 1

HYDROLYSIS OF ACETYLCHOLINE, MECHOLYL AND BENZOYLCHOLINE

Gland	Species	Number of animals	Sex	Average weight of tissue mg	Q_{ChE}				
					Q_{ACh}	Q_{Mech}	Per cent of ACh*	Q_{Benz}	Per cent of ACh*
Liver	Cat	4		19.4	4.40 ± 0.513	0.26 ± 0.039	*6 ± 0.9*	1.81 ± 0.122	*42 ± 3.4*
	Rat	3	male	21.0	0.54 ± 0.021	0.09 ± 0.024	*17 ± 4.0*	0.32 ± 0.004	*59 ± 2.3*
	Guinea pig	4	2 male 2 female	14.0	0.31 ± 0.031	0.23 ± 0.031	*74 ± 6.0*	2.50 ± 0.507	*810 ± 125*
	Rabbit	4	female	21.2	1.58 ± 0.241	1.29 ± 0.191	*82 ± 5.1*	0.92 ± 0.135	*62 ± 13.7*
Pancreas	Guinea pig	2	female	12.6	7.52 ± 0.145	0.50 ± 0.007	*6 ± 0.7*	2.00 ± 0.125	*26 ± 1.6*
Salivary gland	Guinea pig	1	male	6.7	15.3	1.47	*10*	4.72	*31*

* The hydrolysis of mecholyl and benzoylcholine have been compared with ACh hydrolysis, assuming the latter to be 100 per cent. The standard errors have been calculated according to the formula S. E. = $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

same order as that of the liver, hydrolyze ACh in amounts large enough to make the enzyme present in these tissues fulfill the expectations of pseudo-ChE. True ChE, while active, is of secondary importance in the total ACh hydrolysis. In other guinea pig tissues and organs assayed, including skeletal muscle, peripheral nerve, sympathetic ganglia, anterior hypophysis, adrenal medulla and spleen, the hydrolysis of ACh, as in pancreas and salivary gland, is invariably greater than that of benzoylcholine.

Presumably the low ACh hydrolysis by the guinea pig liver might be due to the presence of inhibitors. If this were true, then an extract of guinea pig liver should also inhibit ACh hydrolysis by pseudo-ChE elsewhere. To test this possibility a sample of rat serum (largely pseudo-ChE) was found to have an ACh hydrolyzing capacity, in arbitrary units of 2.98. Guinea pig liver extract had an activity, in the same units, of 0.16. A mixture of the rat serum and the guinea pig liver extract together showed an activity of 313, clearly demonstrating that the two activities are additive (theoretical, 3.14) and that the low ACh hydrolysis by guinea pig liver is not due to the presence of inhibitors.

There is therefore an esterase present in strong concentrations in the guinea pig liver, and to a lesser extent in the rabbit liver, which splits benzoylcholine but is not concerned with ACh hydrolysis. Nachmansohn and Rothenberg[3] have recently shown that the guinea pig kidney also hydrolyzes benzoylcholine faster than ACh. It may perhaps be of significance that this enzyme, which will be temporarily designated benzoylcholine-esterase, is located in those tissues in the body, liver and kidney, in which it has been demonstrated that the enzymic detoxication of benzoic acid chiefly occurs.[4]

Although the distribution of benzoylcholine-esterase appears to be limited, the fact that it occurs at all sounds a note of caution to the acceptance of benzoylcholine hydrolysis as an absolute measure of pseudo-ChE activity. Pseudo-ChE assays should be made, as appears to have been done,[1] only in conjunction with assays both of ACh and mecholyl hydrolysis.[5]

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[3] D. Nachmansohn and M. A. Rothenberg, SCIENCE, 100: 454, 1944.

[4] H. Waelsch and A. Busztin, *Jour. Physiol. Chem.*, 249: 135, 1937.

A CATALYTIC EFFECT OF THIAMINE AT THE DROPPING MERCURY ELECTRODE[1]

A WAVE occurring near −1.9 volts in the polarograms of extracts of stimulated cholinergic nerves,[2] of solutions used for bathing such nerves[3] and of heart fluids of frogs following vagal stimulation[4] has been attributed by v. Muralt to the liberation of thiamine or a related compound. The concentration of material set free, however, was found to be far lower than the lowest concentration of pure thiamine which could be detected polarographically. v. Muralt therefore ascribes the wave to a catalytic effect of the liberated thiamine or thiamine derivative at the dropping mercury electrode.

The present communication describes a polarographic effect of chemically pure thiamine[5] which in sensitivity approximates those of the nerve action-substance and what v. Muralt calls the "second vagus-substance."

When highly dilute solutions of thiamine are electrolyzed in ammonium chloride, boric acid—KCl mixtures, or phosphate buffer as supporting medium, the resulting current-voltage records show a prominent wave with a maximum at −1.7 volts with respect to the saturated calomel electrode (Fig. 1). The wave does not appear in the absence of thiamine. Air need not be removed from the solution, as the preceding

[5] The author is indebted to Drs. F. Bernheim, E. J. Boell, J. E. Markee and W. A. Perlzweig for reading the manuscript, and to Hoffmann-La Roche, Inc., for supplying the benzoylcholine chloride.

[1] Aided by a grant to Dr. George Wald from the Williams-Waterman Fund of the Research Corporation. The author is deeply grateful to Professor Wald, at whose suggestion and in whose laboratory this study was conducted, and also wishes to thank Dr. James J. Lingane for valuable advice.

[2] A. v. Muralt, *Pflügers Arch.*, 245: 604, 1942.

[3] *Idem, Helv. Physiol. Acta*, 1: C20, 1943.

[4] *Idem, Nature*, 154: 767, 1944.

[5] Obtained, together with several derivatives used in this study, through the courtesy of Dr. D. F. Robertson, Merck & Co., Inc., Rahway, N. J.

reduction of oxygen not only does not interfere with, but even increases the thiamine current. Since the reduction of thiamine at the dropping mercury elec-

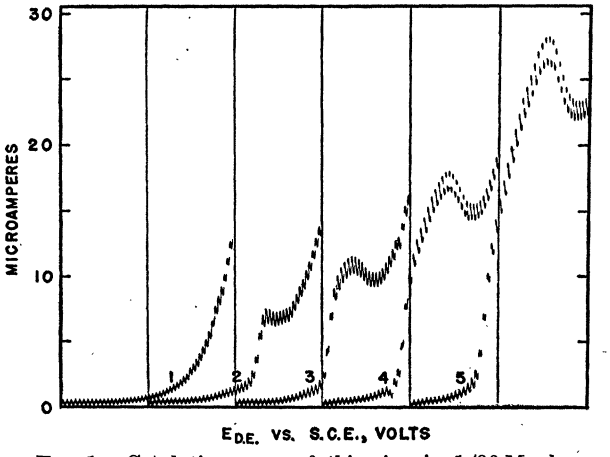

FIG. 1. Catalytic waves of thiamine in 1/30 M phosphate buffer of pH 5.89 equilibrated with air. Temp. 25° C. All waves run from −1.4 to −1.8 v. Molar concentrations of thiamine were: (1) zero; (2) 2.5×10^{-7}; (3) 5×10^{-7}; (4) 10^{-6}; (5) 2×10^{-6}.

trode takes place at about −1.3 volts,[6] the current must be due to reduced thiamine.

The best results were obtained using prosphate buffer. The strength of the current was found to be roughly proportional to the logarithm of the hydrogen-ion activity. The variation of the current with the concentration of thiamine at pH 5.89 is illustrated in Table 1.

TABLE 1

DEPENDENCE OF THE CATALYTIC CURRENT ON THE THIAMINE CONCENTRATION (1/30 M PHOSPHATE BUFFER, PH 5.89; AIR ATMOSPHERE; TEMPERATURE 25° C.)

Thiamine concentration, moles $\times 10^7$/liter	Current,* microamperes
0	0
1.0	1.8
2.5	4.5
5.0	8.4
7.5	11.6
10.0	14.4
20.0	24.4
40.0	38.6
60.0	49.1
80.0	54.8
100.0	60.5

* Corrected for non-catalytic hydrogen current of buffer.

In order to obtain a current of 4.7 microamperes by the simple reduction of thiamine in unbuffered 0.5 N potassium chloride under otherwise identical conditions, a concentration of 10^{-3} M is required. That is, the current obtained in phosphate buffer of pH 5.89 is about 4,000 times as sensitive to thiamine concentration as the diffusion current. It is obvious that one is dealing here with a powerful catalytic action of thiamine. According to the prevailing concept regarding catalyzed electrode reactions,[7] catalytic currents obtained with the dropping mercury cathode are hydrogen currents, caused by an increase in the rate of discharge of hydrogen ions under the influence of substances that decrease the overvoltage of hydrogen on mercury. It can be concluded that thiamine is such a substance.

Table 1 indicates that the relation between the concentration of thiamine and the magnitude of the current is not linear. The current tends to increase less and less in proportion to the rise in concentration. At a concentration of 3×10^{-5} M a limiting value of 90 microamperes is reached which is not exceeded on further addition of the vitamin. Like the concentration-current relations of other catalytically active compounds[7] the dependence of the catalytic current on the thiamine concentration can be expressed by a Langmuir adsorption isotherm.

The inference that adsorption of thiamine at the electrode surface is a determining factor in the catalytic process is supported by the fact that the vitamin is a capillary-active compound. It is able to suppress and eliminate the oxygen maximum[8] and, according to our observations, has the same action on the maxima of cobalt and other metallic ions. Incidentally, v. Muralt[2] reports that the oxygen maximum in oxygenated Ringer solution is suppressed by extracts from stimulated and particularly from unstimulated nerves. We have obtained further proof for the surface-activity of thiamine by studying its effect on the electrocapillary curve of mercury. The interfacial tension in a capillary-inactive electrolyte solution is significantly decreased over a wide potential range by addition of small quantities of thiamine.

The pyrimidine moiety of thiamine and the ternary as well as the quaternary thiazole portion[9] do not yield the catalytic wave. Addition of sulfite to thiamine solutions completely abolishes it. On the other hand, both thiochrome and the product of prolonged action of strong alkali on thiamine are characterized by waves similar to that of thiamine itself. The pyrophosphate of thiamine produces only a faint current; thiamine disulfide[10] yields a wave comparable to that of thiamine in twice its molar concentration.

The occurrence of the catalytic thiamine wave has escaped the attention of earlier investigators[6] who have worked with thiamine in phosphate buffer, pos-

[6] J. J. Lingane and O. L. Davis, *Jour. Biol. Chem.*, 137: 567, 1941.

[7] R. Brdička, *Coll. Czech. Chem. Commun.*, 11: 614, 1939.

[8] V. Zambotti and A. Ferrante, *Boll. Soc. Ital. Biol. Sper.*, 14: 689, 1939.

[9] 2,5-dimethyl-6-aminopyridine, 4-methyl-5-β-hydroxyethylthiazole, and 3,4-dimethyl-5-β-hydroxyethylthiazolium iodide.

[10] O. Zima and R. R. Williams, *Ber. Deutsch. Chem. Ges.*, 73: 941, 1940.

sibly because it is superimposed upon the non-catalyzed hydrogen wave of the buffer acid (*cf.* Fig. 1), and is of such magnitude that the maximum goes unnoticed unless a low galvanometer sensitivity is employed. The values of current shown in Table 1 are corrected for the non-catalytic buffer current.

It should be pointed out that the polarographic effect of thiamine described here is entirely distinct from the thiamine wave which Bergh *et al.*[11] discovered to be partly responsible for the polarographic cancer serum reaction. The latter wave occurs in ammoniacal cobalt solution at a more positive potential than the present wave, and is much less sensitive to the presence of thiamine. It closely resembles Brdička's[12] catalytic sulfhydryl waves, and in the opinion of the writer is due to the sulfhydryl group of the thiol into which thiamine rearranges on treatment with alkali.[13]

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THE NUTRITIVE VALUE OF FUSARIA[1]

EXTENSIVE studies have been made on the enzymatic performances of members of the *Fusarium* family.[2] We were particularly interested to find that some Fusaria were capable of synthesizing thiamin.[3] With a view to ascertaining whether these organisms could synthesize also the other members of the vitamin B complex, a study of the food value of Fusaria has been carried out.

It has been well established that brewer's yeast at a 10 per cent. level in a synthetic diet supplies sufficient amounts of the B complex, so that normal growth, reproduction and lactation in mice are obtained.[4] The first experiment which, therefore, suggested itself was to determine whether Fusaria could adequately replace brewer's yeast in such a purified ration.

In these experiments two strains of the genus Fusarium were used, *viz.*, *Fusarium lini* Bolley (FlB) and *Fusarium graminearum* Schwabe (*Gibberella Saubinettii*) (Fgra). The Fusaria were grown on an artificial stock culture medium[5] containing glucose. After a growth period of three weeks in a sterilincubator, the mats were removed, washed with water, air dried and ground up into a powder. This powder was incorporated into the diets. The percentage composition of the experimental diets used is given in Table 1.

TABLE 1

	Diet V–3	Diet V–4
Purified casein (Smaco)	25	25
Sucrose	45	45
Salts (Osborn and Mendel)	5	5
FlB	10	..
Fgra	..	10
Crisco	7	7
Lard	5	5
Cod liver oil	3	3

The mice used in these experiments were of two strains, *viz.*, the Rockland black and an albino strain raised in these laboratories. They were placed on the experimental diets at weaning. Eleven mice were placed on the V-3 diet, and seven animals received the V-4 diet.

The V-4 diet containing Fgra was totally inadequate for growth in both strains. The animals ate very little of the diet and died within three weeks. The V-3 diet containing FlB, on the other hand, proved to be excellent for growth in both strains of mice over a period of 30 to 35 days. The growth during this period was superior to that obtained with diets containing 10 per cent. brewer's yeast. The food intake averaged 3–3.5 grams daily. After the first month on this diet, however, growth fell off and a loss in weight occurred. The daily food intake dropped to 1–1.5 grams. Since this loss in appetite suggested a possible thiamin deficiency, a supplement of this vitamin (10 micrograms daily) was either injected or fed. An immediate resumption of growth occurred, and the food intake was tripled over night. An effect comparable to the injection of Vitamin B_1 was obtained when the amount of FlB in the diet was doubled. FlB contained[3] about 20 micrograms of this vitamin per gram of dried material so that approximately 2 micrograms were present in one gram of the diet.

It should be noted that Fgra, which was totally inadequate as a source of the B-complex vitamins, was found to contain only 5 micrograms of vitamin B_1 per gram of dried material, thus supplying 0.5 micrograms per gram of diet. However, thiamin is not the only deficient vitamin that is responsible for the insufficiency of the Fgra diet, since a supplement of

[11] F. Bergh, O. M. Henriques and C. G. Wolffbrandt, *Nature*, 142: 212, 1938.
[12] R. Brdička, *Coll. Czech. Chem. Commun.*, 5: 148: 1933.
[13] R. R. Williams and A. E. Ruehle, *Jour. Am. Chem. Soc.*, 57: 1856, 1935.
[1] This investigation was supported by grants from the Rockefeller Foundation and the John and Mary R. Markle Foundation. The authors are under obligation to Dr. L. J. Sciarini for his cooperation in the production of large amounts of Fusaria. The brewer's yeast was obtained through the courtesy of Anheuser-Busch, Inc., St. Louis, Mo.
[2] F. F. Nord, *Ergebn. Enzymforsch.*, 8: 149, 1939; R. P. Mull and F. F. Nord, *Arch. Biochem.*, 5: 283, 1944.
[3] L. J. Sciarini and F. F. Nord, *Arch. Biochem.*, 3: 261, 1943.
[4] L. J. Vinson, Dissert. Fordham Univ., 1944; L. R. Cerecedo and L. J. Vinson, *Federat. Proceedings*, 3, 55: 1944.
[5] F. F. Nord *et al.*, *Proc. Nat. Acad. Sci.*, U. S., 29: 121, 1943.