tween hexenolactone and cysteine. The presence of beta-alanine or the age of the hexenolactone solution did not alter the nature or significantly influence the kinetics of this process, as given in Fig. 1. Alanine,

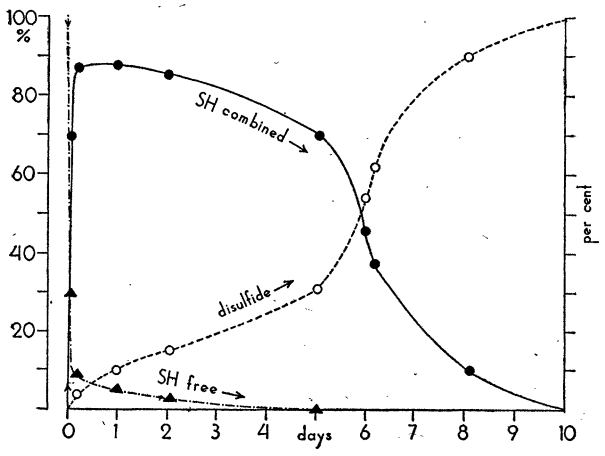

FIG. 1. Interaction between hexenolactone and cysteine (under relatively anaerobic conditions).

therefore, appears to counteract the biological effect of hexenolactone through a mechanism distinct from inactivation by SH, as borne out also by the concentration optimum of beta-alanine (Table 1).

The hexenolactone-cysteine addition compound is gradually decomposed again, yielding disulfide in sufficient quantity to account for all the cysteine. Decomposition under relatively anaerobic conditions is five to seven times slower than in the presence of O_2.

The ultraviolet absorption peak, characteristic for the conjugated double bond in the hexenolactone molecule (I), is markedly lowered during the height of the reaction with cysteine, but 9 days later has approximately returned to pre-reaction shape and magnitude. At no time does the spectrum resemble the typical absorption curve for sorbic acid (II). The spectral changes are consistent with the colorimetric data and suggest regeneration of hexenolactone. Furthermore, the complete inactivation of the hexenolactone (5×10^{-5} M) effect on Dugesia by cysteine (25×10^{-5} M), shown in Table 1, lasts for only 20 to 24 hours. After this period triclad lesions gradually appear in the test animals. However, the molecule which emerges from the reaction with cysteine is only about one third to one half as active as a hexenolactone control. This raises the problem of a structural change, now under investigation.

Hammett[9] has demonstrated the rôle of the sulfhydryl group in cell proliferation. The activity of the many enzymes with SH groups in the protein portions of their molecules appears dependent on the maintenance of SH in the reduced state which, according to Barron and Singer,[10] is insured by the presence of glutathione. Inactivation by SH of the antibiotic properties of penicillin and several widely different bacteriostatic substances was recently reported by Cavallito and Bailey.[11] In view of our similar findings for hexenolactone it is suggested that some of the most effective antibiotics interfere with cellular proliferation mainly through their reactivity with SH groups essential to enzyme function.

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HYDROLYSIS OF CHOLINE ESTERS BY LIVER

IN a recent paper Mendel, Mundell and Rudney[1] have shown that acetyl-beta-methylcholine (Merck's mecholyl) is a specific substrate for true cholinesterase (ChE) and benzoylcholine, a specific substrate for pseudo-ChE. Both of these enzymes hydrolyze acetylcholine (ACh) more efficiently than their specific substrates, and when a mixture of the enzymes occurs, both take part in the total ACh hydrolysis.

While investigating the true ChE and pseudo-ChE content of various organs by a microchemical titrimetric method[2] it was noted that the above conditions did not always hold in the case of rodent livers. The results (Table 1) are expressed in terms of Q_{ChE} (mgms ACh hydrolyzed by 100 mgms tissue per hour) and for the sake of comparing enzyme activities on a molecular basis, the data for mecholyl and benzoylcholine have been calculated in terms equivalent to that of ACh.

In the cat and rat livers ACh hydrolysis appears to be dependent on the enzymes hydrolyzing mecholyl and benzoylcholine, principally pseudo-ChE. But in the rabbit and especially in the guinea pig livers, ACh hydrolysis seems to be purely a function of true ChE; the relatively high means and low standard errors in the appropriate "percentage of ACh" column testify to this relationship. Benzoylcholine hydrolysis by rabbit and guinea pig livers, however, show no constant ratio to ACh hydrolysis. In fact, the guinea pig liver splits benzoylcholine 5 to 11 times as rapidly as ACh.

The guinea pig pancreas and submaxillary salivary gland, whose ability to split benzoylcholine is of the

[9] Hammett and Hammett, *Protoplasma*, 15: 59, 1932.

[10] Barron and Singer, SCIENCE, 97: 356, 1943.

[11] Cavallito and Bailey, SCIENCE, 100: 390, 1944.

[1] B. Mendel, D. B. Mundell and H. Rudney, *Biochem. Jour.*, 37: 473, 1943.

[2] C. H. Sawyer, *Jour. Exp. Zool.*, 92: 1, 1943.

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

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[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.