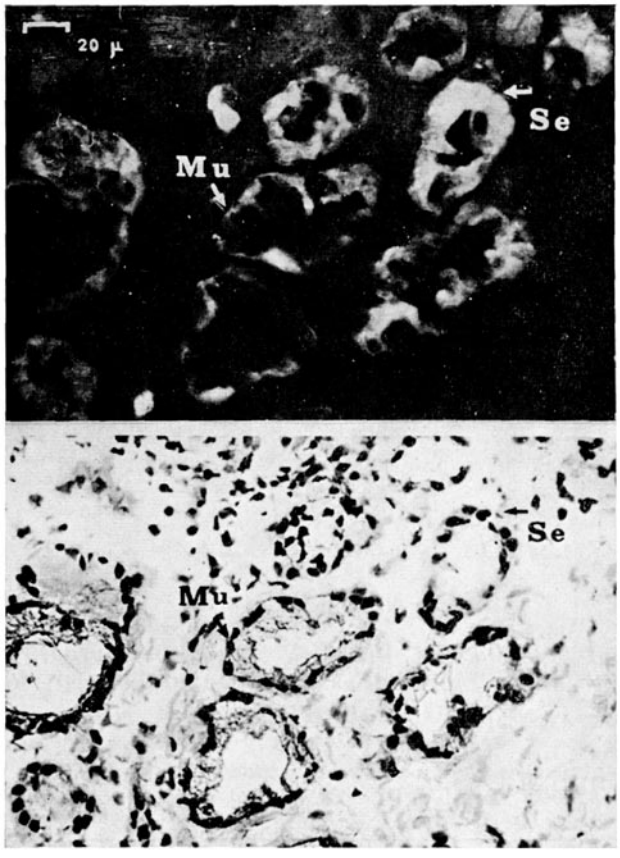

by the mucin-containing vacuoles, and which surrounded the nucleus. It should be stressed, however, that the contents of the secretory vacuoles of the goblet cells were usually lost during the processing of the tissues, so that no conclusion can as yet be drawn regarding the absence or presence of lactoferrin in these structures[9].

Résumé. Les techniques immunohistochimiques ont permis de localiser la lactoferrine dans les cellules séreuses des acini des glandes bronchiques.

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Laboratoire de Recherches de Pathologie interne des Cliniques universitaires Saint-Pierre, Louvain (Belgium), March 18, 1965.

[9] Acknowledgment: This investigation was supported by Grant No. 461 from the Fonds de la Recherche Scientifique Médicale, Bruxelles (Belgium).

Fig. 3. Comparative staining of serous and mucous acinar cells. Above: high-magnification view of bronchial biopsy section stained with fluorescein-labelled antilactoferrin. Below: same section after staining by the periodic acid-Schiff procedure. Se: serous cells. Mu: mucous cells.

Effect of Thiamine Analogs on the Electrical Activity of the Rabbit Vagus Nerve[1]

In an investigation of the role of thiamine in nervous tissue, Armett and Cooper[2] found that pyrithiamine, an antimetabolite of thiamine, when applied to the non-myelinated fibers of the rabbit vagus nerve produced an increase in the amplitude of the compound action potential that was irreversible over a 90 min period (Figure 1). These effects could be prevented by pretreatment of the nerve with thiamine. In subsequent biochemical investigations[3], no correlation could be obtained between the electrophysiological effect of pyrithiamine and an interference with the known enzymatic reactions that require thiamine pyrophosphate (TPP) as a coenzyme. In addition, no inhibition by pyrithiamine of the synthesis of TPP could be demonstrated. However, it could be shown that incubation of the vagus nerve with the antimetabolite caused a release of thiamine from the nerve.

In an effort to gain some information on the specificity of the electrical effect of pyrithiamine, a variety of other analogs of thiamine were tested. These experiments are the basis of this report.

The structures of the compounds that were examined are shown in Figure 2; these analogs were kindly supplied by Merck, Sharp and Dohme Research Laboratories. Pyrithiamine and thiamine were obtained from Calbiochem.

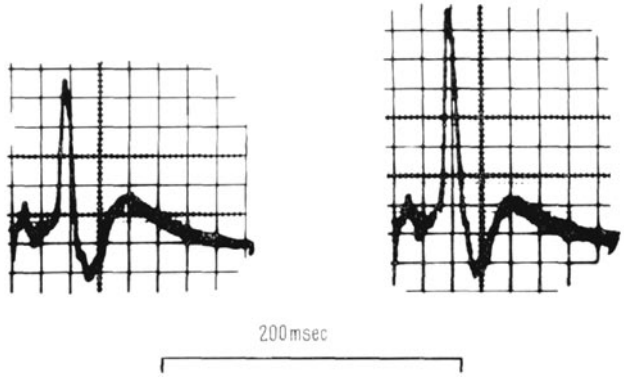


Fig. 1. The effect of pyrithiamine on the compound diphasic action potential of the non-myelinated nerve fibers of the rabbit vagus nerve. The left-hand record is a control action potential and the right-hand record is the action potential 10 min after the addition of pyrithiamine (5 m*M*) to the bathing solution.

[1] This work was supported by grants from the US Public Health Service, Merck, Sharp and Dohme, and The Burroughs Wellcome Company.

[2] C. J. Armett and J. R. Cooper, J. Pharmacol. exp. Therap. *148*, 137 (1965).

[3] J. H. Pincus and J. R. Cooper, in preparation.

Vagus nerves were dissected from rabbits killed by the injection of air into the marginal ear vein; they were then desheathed and mounted in a chamber similar to that designed by ECCLES[4]. The evoked compound action potential was recorded diphasically through a pair of external platinum electrodes. The main elevation of this action potential was a function of the non-myelinated (or C) fiber activity; the stimulus used to evoke the action potential was 0.5 msec in duration and about 30 V in amplitude; any change in the amplitude of the maximal action potential on a 10 min exposure of the nerve fibers to the test compounds was recorded[2].

The compounds were dissolved in Locke's solution of the following composition in mmoles/l: NaCl, 156; KCl, 5.6; $CaCl_2$, 2.2; dextrose, 10.0; sodium phosphate buffer pH 6.9, 2.0. When necessary, test solutions were readjusted to pH 6.9 by the careful addition of NaOH.

Results and conclusions. The results are shown in the Table. Amprolium (III) produced some increase in the amplitude of the action potential, but the results were variable; in two of the experiments the nerve was subsequently exposed to pyrithiamine (5.0 mmoles/l) and a further increase in amplitude was observed. Amprolium is a coccidostatic agent that is presumed to act by interfering with the absorption of thiamine[5].

When the vagus nerve was exposed to IV (L-593045-1-2) at a concentration of 0.5 to 5.0 mmoles/l its evoked action potential always increased. Unfortunately, a limited supply of this agent prevented further experiments to determine the lower limit of potency. Unquestionably this compound is much more potent than pyrithiamine since the latter was only occasionally effective at a concentration of 2.5 mmoles/l and rarely had an effect on the action potential at 1.0 mmole/l[2]. This finding with IV is of interest, since in experiments in vivo it has been found that IV is at least ten times more potent than pyrithiamine in producing polyneuritis in experimental animals[6].

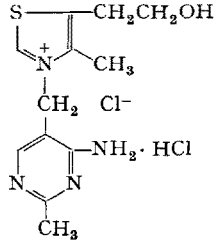
With V (L-58257-1-1) the action potential of the nerves also increased; no significant addition to the amplitude of the action potential occurred when the nerve was subsequently exposed to pyrithiamine.

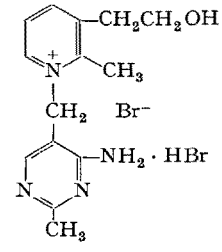
The effect of thiamine analogs on the action potential of the non-myelinated nerve fibers of the rabbit vagus nerve

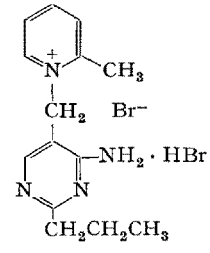
Test compound	Concentration (mmoles/l)	% increase in amplitude of action potential
I. Thiamine	5.0	0[a]
II. Pyrithiamine	5.0	47.5 ± 9.1 (S.E.)[b]
III. Amprolium	5.0	2.5
	5.0	33.0
	5.0	11.5
IV. L-593045-1-2	5.0	13.5
	5.0	36.5
	1.0	53.5
	0.5	26.5
	0.5	58.0
V. L-58257-1-1	5.0	40.0
	5.0	6.0
	5.0	18.0
	2.0	20.0
VI. L-522132-0-0	5.0	0
	5.0	0
VII. L-584424-0-1	5.0	– 7.5
	5.0	– 25.5
VIII. L-591515-0-4	5.0	8.5
	5.0	5.5

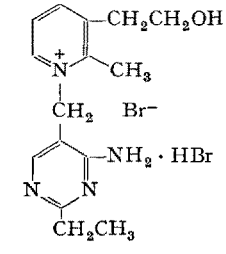
[a] Average of 10 experiments. [b] Average of 14 experiments.

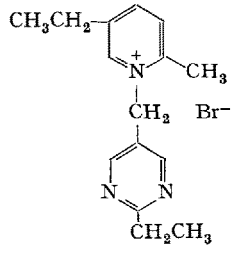
[4] R. M. ECCLES, J. Physiol. *117*, 181 (1952).
[5] D. POLIN, E. R. WYNASKY, and C. C. PORTER, Poultry Sci. *42*, 1057 (1963).
[6] E. F. ROGERS, personal communication.

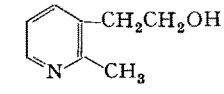

I Thiamine

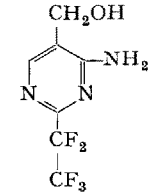

II Pyrithiamine


III Amprolium


IV L-593045-1-2


V L-58257-1-1


VI L-522132-0-1


VII L-584424-0-1

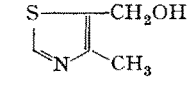

VIII L-591515-0-4

Fig. 2. Formulae of thiamine and analogs.

The addition of VI (L-522132-0-0) to the vagus nerve had no effect on the action potential. Similarly VIII (L-591515-0-4) had an insignificant effect. The addition of VII (L-584424-0-1) produced a slight decrease in the amplitude of the action potential.

Although limited in scope, these experiments suggest that both the pyridine and pyrimidine ring of pyrithiamine are required for its effect on the action potential of the non-myelinated nerve fibers of the rabbit vagus nerve. The greater sensitivity of the nerve to IV compared to II (pyrithiamine) may be a reflection of ease of transport since this agent with an ethyl group on the 1 position of the pyrimidine ring would be more lipid soluble than pyrithiamine with a methyl group on this position.

The high correlation between the effect of these analogs of thiamine on the electrical activity of the nerve fibers and their effectiveness in producing polyneuritis in animals[6] suggests that a further investigation with this in vitro system may be useful in elucidating the etiology of the neuropathy that is associated with a thiamine deficiency state.

Zusammenfassung. Der Einfluss einer Anzahl Thiaminanaloga auf die elektrische Aktivität des Vagus wurde untersucht. Das Äthylanalogon des Pyrithiamins hatte mindestens die zehnfache Wirkung des Pyrithiamins auf die Erhöhung der Amplitude des Aktionspotentials. Die Resultate stimmen gut überein mit der Fähigkeit dieser Antimetabolite, in vivo Polyneuritis hervorzurufen.

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Potentiation of the Cerebral Vascular Action of Bradykinin by the 'Bradykinin Potentiating Factor' (BPF) in the Dog

The vasodilating effect of bradykinin upon the cerebral vessels has already been described[1–3]. It has also been indicated that bradykinin may play a role in the physiopathological control of the cerebral circulation[1,2]. On the other hand, the 'bradykinin potentiating factor' (BPF), a purified extract from the venom of *B. jararaca*, has been shown to act in vitro as well as in vivo[4].

Eight dogs, weighing 18 to 22 kg, were employed in the experiments. The animals were anaesthetized with morphine (2 mg/kg, s.c.) and chloralose (90 mg/kg, i.v.) and the intra-cranial pressure recorded by means of a catheter introduced in a cephalad direction through the external jugular vein[5]. The tracings of the intra-cranial blood pressure together with the femoral arterial blood pressure and the respiration were simultaneously recorded in a Grass, 6-channel Polygraph. Synthetic bradykinin (BRS 640 Sandoz), kallidin (KL 695 Sandoz), eledoisin (ELD 950 Sandoz) and histamine were administered through a small catheter, placed into the lingual artery and directed towards the carotid artery. Drugs were diluted in saline and 0.2 ml of this solution followed by 0.2 ml saline were injected each time. The same route was used for the administration of BPF (10 mg in 0.3 ml of saline). Though by slow injection no effects could be observed, when the factor was injected quickly, a moderate fall in arterial blood pressure together with mild acceleration in respiratory movements and increase of 3 to 5 cm H_2O in the intracranial venous pressure occurred. Consequently, in all experiments presented in continuation, the factor was injected slowly, in about 30 sec (0.3 ml).

The intra-carotideal injection of bradykinin in doses varying from 0.01 to 0.1 μg, according to the sensitivity of the preparation, produced a clear-cut rise in the intracranial blood pressure, ranging from about 1 to 6 cm H_2O, without any change in the systemic blood pressure and respiration (Figure 1). Similar effects were obtained with the other three agonists, kallidin being in all cases more potent than bradykinin, and histamine being always less effective than either agent; eledoisin was more potent in some experiments and less in others (Table). Neat dose-

Effect of BPF upon the cerebral vascular action of bradykinin, kallidin, eledoisin, and histamine

Experiment number		Increase in intra-cranial venous pressure (cm H_2O)[a]		Potentiation ratio			
		before	after	BK	KL	ELD	H
I	Bradykinin (0.01 μg)	1.25	7.20	5.7	–	–	–
	Histamine (0.4 μg)	5.30	3.12	–	–	–	0.6
II	Bradykinin (0.01 μg)	1.25	2.50	2.0	–	–	–
	Histamine (0.03 μg)	1.25	1.88	–	–	–	1.5
III	Bradykinin (0.1 μg)	5.95	9.68	1.6	–	–	–
	Histamine (1 μg)	5.30	5.30	–	–	–	1.0
IV	Bradykinin (0.02 μg)	5.30	14.20	2.7	–	–	–
	Eledoisin (0.04 μg)	4.68	10.30	–	–	2.2	–
	Kallidin (0.02 μg)	8.10	14.28	–	1.8	–	–
V	Bradykinin (0.01 μg)	5.61	18.80	3.3	–	–	–
	Eledoisin (0.05 μg)	5.30	3.75	–	–	0.7	–
VI	Bradykinin (0.1 μg)	4.68	9.05	1.9	–	–	–
	Eledoisin (0.1 μg)	7.20	8.41	–	–	1.2	–
	Kallidin (0.1 μg)	12.45	10.30	–	0.8	–	–
VII	Eledoisin (0.1 μg)	12.20	9.69	–	–	0.8	–
	Kallidin (0.005 μg)	5.61	9.35	–	1.7	–	–
VIII	Eledoisin (0.2 μg)	7.80	6.75	–	–	0.9	–
	Kallidin (0.005 μg)	3.12	12.45	–	4.0	–	–
Mean potentiation ratio				2.9	2.1	1.2	1.0

[a] The values presented refer to the increase in intra-cranial venous pressure due to the drugs injected, respectively, during the 10 min time interval preceding (before) and the 10–20 min interval following (after) the intra-carotideal administration of BPF (10 mg).

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[4] S. H. Ferreira, Brit. J. Pharmacol. *24*, 163 (1965).
[5] D. Bovet, M. Virno, G. L. Gatti, and A. Carpi, Arch. int. Pharmacodyn. *110*, 380 (1957).