The dose of HEH was less effective in inhibiting the brain enzyme but more effective in inhibiting the liver enzyme than the dose of 3-amino-2-oxazolidinone. One possible explanation, based on the premise that 3-amino-2-oxazolidinone acts only after it is converted to HEH, is as follows. In the first pass through the liver after i.p. injection, HEH enters as an active inhibitor whereas 3-amino-2-oxazolidinone enters as an inactive precursor to an inhibitor. Thus greater inhibition of liver MAO occurs with HEH. By the time these agents reach the brain, much of the HEH has been inactivated by metabolism whereas the 3-amino-2-oxazolidinone has been activated by metabolism, so the latter compound has a greater effect on brain MAO.

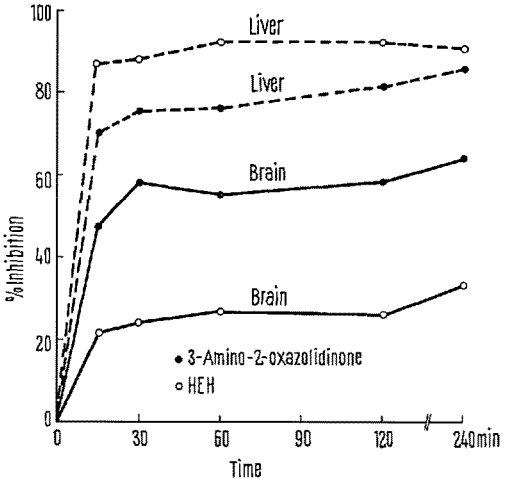

Fig. 1. Time course of in vivo inhibition of MAO. 3-amino-2-oxazolidinone (0.01 mmole/kg) or 2-hydroxy-ethylhydrazine (0.05 mmole/kg) was injected i.p. into mice. MAO activity was determined, and results are expressed as percent inhibition compared to activity in untreated mice. Mean values for 3 mice per group are shown. Activity in untreated mice was 42.5 ± 1.3 nanomoles of substrate oxidized/min/g brain and 577 ± 20 nanomoles/min/g liver.

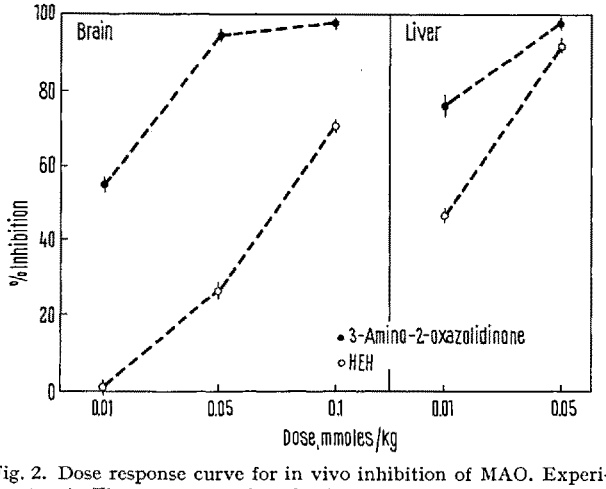

Fig. 2. Dose response curve for in vivo inhibition of MAO. Experiment as in Figure 1 except that the dose was varied and drugs were injected 60 min before the mice were killed. Means and standard errors for 3 mice per group are shown. Activity in untreated mice was 45.1 ± 5.1 nanomoles of substrate oxidized/min/g brain and 615 ± 42 nanomoles/min/g liver.

Our interest in the lipid content of musculature in cases of various forms of Myotonia congenita has been aroused by 2 findings: first a myotonic syndrome could be produced by the supply of azacholesterol[1,2]. Second Kuhn et al.[2] found a change of the fatty acid composition of the lipids in an experimentally induced myotonic syndrome. They discussed the possibility of a membrane lesion as the effect of the enzymatic basic defect of this disease. A changed conductivity of the plasma membrane proved to be the cause of the myotonic syndrome also from electrophysiological view[3].

We had the opportunity to take a larger bioptic sample from a 9-year-old boy suffering from Paramyotonia congenita (P.c.). The patient shows the typical clinical

A comparison of the potency of these compounds in inhibiting brain and liver MAO is shown in Figure 2. Comparing equimolar doses, 3-amino-2-oxazolidinone was more effective than was HEH in inhibiting both brain and liver MAO.

Since 3-amino-2-oxazolidinone does not inhibit MAO in vitro, its in vivo effects presumably occur after it is converted to some active metabolite. Previously that active metabolite was suggested to be HEH[1]. If that were so, then 3 findings that we report here would not have been expected: 1. there was no lag of in vivo MAO inhibition by 3-amino-2-oxazolidinone behind that of HEH, 2. 3-amino-2-oxazolidinone was actually more potent than HEH in causing in vivo inhibition, and 3. the relative liver/brain effects were different with the 2 agents. If 3-amino-2-oxazolidinone does act through conversion to HEH, then it must be an efficient vehicle for delivering HEH to tissues, especially the brain. Alternatively, 3-amino-2-oxazolidinone may act in vivo by conversion to some metabolite other than HEH, that metabolite being a more powerful inhibitor than HEH and having less selective affinity for liver MAO relative to brain MAO.

Zusammenfassung. Die MAO-hemmende Aktivität von 3-Amino-2-Oxazolidinon und von 2-Hydroxy-Äthylhydrazin wird beschrieben.

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The Lipid Content of Musculature and of Tissue Preparations with Enriched Plasma Membranes in Paramyotonia congenita

Neutral lipids and phospholipids in bioptic samples of human musculature with congenital Paramyotonia and in intact controls (A) and in tissue preparations with enriched plasma membrane content in P.c. and normal controls (B)

	A			B		
	Controls		P.c.	Controls		P.c.
	$\bar{x}$	s		$\bar{x}$	s	
Lysophosphatidylcholine	60	25	15.2	145	65	232
Sphingomyeline	77	32	9.2	149	46	147
Phosphatidylcholine	380	86	109	758	247	1005
Phosphatidylethanolamine	126	25	49	349	98	582
Phosphatidylserine	26	10	6	94	29	103
Phosphatidic acid	35	14	3	81	20	82
Cholesterol	1.94	0.54	0.43	7.3	2.1	8.3
Cholesterol ester	0.196	0.098	0.23	0.09	0.005	0.1
Triglycerides	31.2	14.2	2.1	47	15	10
Free fatty acids	2.6	1.52	0.09	3.8	1.5	8.9

$n = 12$. Phospholipids: µgP/100 mg collagenfree N. Neutral lipids: mg/100 mg collagenfree N.

symptoms and electromyographically signs; in the histological examination there was no change at the myofibrils, 4 members of the family had the same disease. The tissue piece, covering about 5 g, enabled us to engage in making a plasma membrane preparation. In the present paper we report on the lipid content in the total musculature and in the tissue preparation enriched with plasma membranes in the normal muscle and P.c. by way of comparison.

The bioptic sample originates from the M. quadriceps fem. The musculature of the control group belonged to the M. pect. major and had been taken from 46 to 73-year-old women at mammary gland operations. We could not make the membrane preparation according to KONO[4], as the tissue quantity was too small. Judging from our experience, the method according to MCCOLLESTER[5] does not yield any pure preparations, but requires less initial material[6]. As the taking of bioptic samples of tissue material is limited, on the one hand, and as, on the other hand, fresh tissue samples can hardly be obtained by autopsy, we decided to attempt a preparation in spite of the methodic difficulties involved. The phase contrast optic control yielded only some single, scattered myofibrils, while we found by electronoptical control undestroyed muscular tissue beside areas full of membraneous rags and membraneous debris. Lipid analysis also indicates the fact that there is a preparation with enrichment of membraneous rags. In our pure membraneous preparations, the phospholipids recorded are about 3 times as high. Corresponding to the processing with P.c., the comparative material was prepared by the same method. The analysis of the neutral lipids and phospholipids was made by thinlayer chromatography according to methods already published[7]. For the choice of the reference parameter, we refer to a previous publication[8]. In the total muscle we found in P.c. an uniform decrease of all phospholipids, which was particularly marked with sphingomyelin.

For the neutral lipids we also recorded a decrease concerning especially fatty acids and cholesterols. As the interstitial lipid share is covered by this determination, a binding statement on genuine, intracellular shiftings of content is impossible. Contrary to this, the membraneous preparations permit – considering the methodic restrictions stated above – conclusions as to specific lipid changes which would concern, above all, cellular membraneous structures. There results a conformity in the phospholipids of normal and myopathic muscle. In the neutral lipid content there is also a conformity in the fractions triglycerides, cholesterol and cholesterol ester. The content of fatty acids is, however, strikingly different. While a decrease of free fatty acids has been recorded in the overall musculature, the content of fatty acids in the membraneous preparations is more than double as high with P.c.

The differences recorded in the lipid content of the total musculature are probably differences in interstitial fattening which is more marked in advanced age (i.e. with normal samples) than in adolescence. This difference is removed by the preparation. Both in the total muscle and in the preparation, the behaviour of the fatty acids is conspicuous. The diametrically opposed behaviour, in the overall muscle and in the preparation, can be interpreted only as follows: Along with a shortage of fatty acids of the overall muscle there occurs an enrichment in the muscle plasma membrane. In view of the complex mechanism of the transfer of fatty acids through cellular membranes[9] an interpretation from this individual finding is impossible. This finding will, however, have to be duly considered at future interpretations of the myotonic syndrome.

Zusammenfassung. In einer Biopsieprobe menschlicher Muskulatur bei Paramyotonia congenita wurden Neutrallipide und Phosphatide im Gesamtmuskel und in Präparationen mit angereicherten Plasmamembranen bestimmt und mit den entsprechenden Werten in normaler Muskulatur verglichen. Dabei findet sich eine Erhöhung der freien Fettsäuren in den Plasmamembranen bei Paramyotonie.

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