Results. The administration of amantadine alone in a dose of 100 mg/kg induced consequently parkinsonian symptoms, such as tremor and humpback followed by an increased motor activity. When injected simultaneously with reserpine, amantadine aggravated all manifestations of the model of reserpine parkinsonism in rats. When administered with a therapeutic purpose on the background of parkinsonian symptomatology previously induced by chronic reserpinization, amantadine delayed the persistence of the parkinsonian symptoms in the animals.

The Table shows that amantadine in doses of 50 and 100 mg/kg increased K-Na-ATPase activity in the stem. The ATP content and ATPase activity in the stem of chronically reserpinized animals were significantly decreased as compared with the controls. The animals treated simultaneously with reserpine and amantadine exhibited a significantly decreased ATP content and an increased ATPase activity. As can be seen from the Table, the amantadine therapy of chronically reserpinized animals led to normalization of ATPase activity and to a more difficult restoration of ATP content in the brain stem. The amantadine increases, while reserpine decreases the blood ATP level.

Discussion. The results of our investigations on rats treated with amantadine point to an increased K-Na-ATPase activity. A still higher activity is found in the brain stem of animals given simultaneous chronic treatment with reserpine and amantadine. This gives us reason to consider that the mechanism of amantadine consists in an action on the transfer mechanisms in which the ouabain sensitive K-Na-ATPase takes an active part. We found a decreased ATPase activity and ATP content in the brain stem of chronically reserpinized rats. The storage of CA in the amine granules is shown by means of a mechanism, which is dependent on ATP and ATP-ase[18–21]. The decreased ATP content and increased ATP-ase activity in the brain stem of the animals treated with reserpine and amantadine, explain also to a great extent the pharmacological potentiation of the reserpine effect by amantadine. The reduced content of the binding agent – ATP, and the increased membrane permeability (raised K-Na-ATPase), lead to an enhanced membrane transfer and intense release of CA[25].

Amantadine increases the total blood ATP level in these animals. This might also be evidence in support of our view for the mechanism of action of amantadine upon cell permeability and the storage of CA in the blood cells.

It seems likely that the good therapeutic effect of amantadine alone in parkinsonian patients is due to its property to exert an influence on the transfer mechanisms, which are disturbed in parkinsonism[25]. The results of our investigations make us believe that the amantadine effect consists in an increase of cell membrane penetration.

Zusammenfassung. Nachweis, dass Amantadin-HCl die Aktivität der K-Na-ATPase im Hirnstamm von Ratten erhöht. Für diese Wirkung bei extrapyramidalen Syndromen wird die Veränderung der Membranpermeabilität verantwortlich gemacht.

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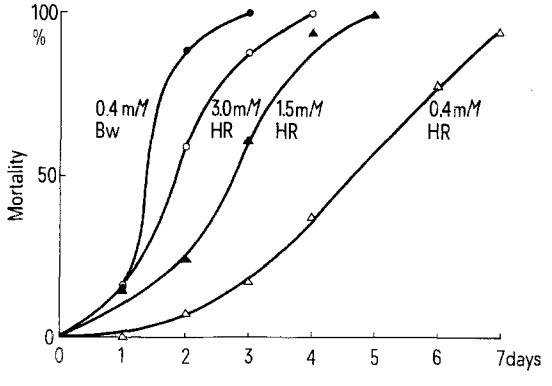
[25] V. VELKOV, Acta morph., Brno *21*, 345 (1973).

Mutagenic Activity of the Insecticide Oxydemetonmethyl in a Resistant Strain of *Drosophila melanogaster*

Oxydemetonmethyl (0,0-dimethyl-S-2-(ethylsulfinyl)-ethyl phosphorothioate) is widely used as a systemic and contact insecticide for the control of insects and mites It has a range of action similar to that of demetonmethyl of which it is a metabolic product[1]. It has been suggested that this insecticide, which has been proved to be a monofunctional alkylating agent, causes forward mutations in *Escherichia coli*[2] and produces trp-conversions in *Saccharomyces cerevisiae*[3]. Therefore, we were interested in whether this compound might also be mutagenic in a higher organism like *Drosophila*.

The strains used were Berlin wild and Hikone R (HR); HR shows resistance to a series of insecticides such as DDT, chlordane, parathion, and dinitrocresol (DNOC)[4,5]. It is, however, highly susceptible to phenylthiourea[6]. Both mortality and the induction of X-linked recessive lethals as a function of dose were compared by treating 1-2-day-old adult males of both strains using the adult feeding technique described in detail elsewhere[7].

Oxydemetonmethyl was dissolved in 5% sucrose solution and fed to the adult males for either 17 h or for several days. 1-2-day-old treated (control) males were mated individually (2♀♀:1♂) to virgin sc^{S1} In S B w^a sc^8 (for genetic symbols see ref.[8]). Recessive lethals were tested for in the the F_2. A breeding schema consisting of 3 broods of 3 days duration each was set up, so that treated mature sperm (brood I), spermatids (and sperm) in



Dosage-mortality relationships to oxydemetonmethyl for males of the Hikone R strain (HR) and the Berlin wild strain (Bw).

[1] H. MARTIN, *Pesticide Manual* (British Crop Protection Council, 1968), p. 495.
[2] G. MOHN, Mutation Res., *20*, 7 (1973).
[3] R. FAHRIG, Naturwissenschaften *60*, 50 (1973).
[4] H. KIKKAWA, Jap J. Genet. *28*, 171 (1953).
[5] M. OGAKI and M. TSUKAMOTO, Botyu-Kagaku *23*, 100 (1953).
[6] Z. OGITA, Botyo-Kagaku *23*, 108 (1958).
[7] E. VOGEL, Mutation Res. *11*, 397 (1971).
[8] D. L. LINDSLEY and E. H. GRELL, Carnegie Inst. of Washington Publ. No. 627 (1968).

The incidence of recessive lethals in male germ cells of *Drosophila* by oxydemetonmethyl

Experiment	Strain	Treatment	Brood I		II		III		I–III	
		(conc./m*M*)	lethals/chromos. (%)		lethals/chromos. (%)		lethals/chromos. (%)		lethals/chromos. (%)	
1	Hikone-R	0.43 m*M* fed for 2 days	4/998	0.40						
		Control	0/595	—						
2	Hikone-R	3.0 m*M* fed for 26 h	3/819	0.37	1/609	0.16	1/616	0.16	5/2044	0.24
		Control	2/611	0.33	0/614	—	1/620	0.16	3/1845	0.16
3	Hikone-R	1.5 m*M* fed for 3 days	4/607	0.66	2/618	0.32	2/539	0.37	8/1764	0.45
		Control	1/617	0.16	1/616	0.16	1/612	0.16	3/1845	0.16
4	Berlin wild	0.043 m*M* fed for 3 days	1/826	0.12	1/813	0.12	2/820	0.24	4/2459	0.16

brood II, and spermatocytes (and spermatids) in brood III were analyzed.

If the toxic effects of oxydemetonmethyl are analyzed first, both strains exhibit pronounced differences in response to the chemical (Figure). The Berlin wild strain is clearly much more sensitive to the insecticide chemical than the Hikone R strain.

In the sex-linked lethal experiments, a slightly higher incidence of recessive lethal mutations was determined after treatment of Hikone males with concentrations ranging from 0.043 m*M* to 3.0 m*M* (Table). The exposure time had to be shortened at higher doses due to the toxic property of the compound. Most of the lethals induced by oxydemetonmethyl were found in brood I, indicating sensitivity of mature sperm to the chemical.

When the data from all oxydemetonmethyl experiments with HR males are pooled and compared with the pooled data from the control sample, the X^2 test shows that the difference between these 2 samples is just significant ($0.05 > p > 0.01$). However, since a weak rise resulted in all experiments with the insecticide, the test substance may be considered a weak mutagen in Hikone R males. In contrast, no mutagenic effect could be found with Berlin wild males (expt. 4). Failure to recover mutations in Berlin wild males is obviously due to killing effect of the compound in this strain. Only 1/40 of the dose that can be given to Hikone R males for 3 days can be fed to Berlin wild males. Thus, this compound may be classified as non-mutagenic or a weak mutagen in *Drosophila*, depending on the genetic constitution of the test strain.

We plan to continue analyzing variations in response to treatment with chemical mutagens. Such differences promise to provide clues to the mechanism of action and of resistance to mutagens.

Zusammenfassung. Oxydemetonmethyl induziert rezessive Letalmutation im Insektizid-resistenten Stamm Hikone R von *Drosophila melanogaster*. Am Wildstamm Berlin ist, bedingt durch die hohe Toxizität des Insektizids, eine Testung mit höheren Dosen als 0.04 m*M* nicht möglich.

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Protein Half-Lives in Neonatal Mice After a Toxic Dose of Cyclophosphamide

Development represents a period of time during which immature cells and tissues of an organism acquire adult structure and function. This orderly transition of form and function ultimately depends on developmentally directed changes in tissue proteins. The protein composition of a tissue differs both quantitatively and qualitatively during development and between various tissue types in the adult. Agents which alter either protein acquisition or composition of a tissue may produce abnormal development.

Cyclophosphamide, a clinically useful alkylating agent, has toxic properties during both embryonic[1,2] and postnatal development[3,4]. One day-old neonatal mice treated with 80 mg/kg cyclophosphamide grew at reduced rate with increased mortality and were morphologically abnormal at maturity[4]. Toxicity, during postnatal development, was correlated with the presence of alkylating cyclophosphamide metabolites[5]. Postnatal toxicity was associated with an inhibition of DNA synthesis in the liver, brain, and carcass and RNA synthesis in the liver and brain during a 5 day observation period after cyclophosphamide[6]. Protein synthesis, however, was not affected in a manner which indicated that drug treatment altered this process of differentiation. Since the protein

[1] J. Wilson, J. Pharmac. exp. Ther. *144*, 429 (1964).
[2] J. Gibson and B. Becker, Cancer Res. *28*, 475 (1968).
[3] H. Nordlinder, Experientia *25*, 1296 (1969).
[4] R. Short and J. Gibson, Experientia *27*, 805 (1971).
[5] J. Bus, R. Short and J. Gibson, J. Pharmac. exp. Ther. *184*, 749 (1973).
[6] R. Short and J. Gibson, Biochem. Pharmac., *22*, 3181 (1973).