FTG-culture containing an inoculum of 6×10^5 microorganisms/ml could be detected, was regarded as the MLC.

3. In-vivo test. Female NMRI-mice weighing 10–15 g were treated orally 2, 18 and 24 h after intraperitoneal infection with 8×10^5 trichomonads of a 24 hour culture. The results were evaluated 5 days after the last treatment. The efficacy of the compounds was determined by the presence or absence of trichomonads in the abdominal cavity, as tested by re-isolation.

Results. The comparative analyses of the efficacy of different 5-nitro-imidazole-derivatives against the *T. foetus*-strains KV 1 and KV 1/M 100 in vivo and in vitro are summarized in Tables I and II. It can be seen (Table I) that the strain KV 1/M 100 is both resistant to metronidazole and also shows different decrease of sensitivity to all 5-nitroimidazole-derivatives. Differences are revealed by comparison of their resistance factors. As far as metronidazole and nitrimidazine are concerned, these factors represent, however, only a minimal value, as we have not determined the DC_{50} owing of the high dosages necessary.

Similar results were obtained from the experiments carried out in vitro (Table II). Here also the strain KV 1/M 100, proved less sensitive to metronidazole, showed to be less sensitive to all 5-nitroimidazole-derivatives in comparison with the strain KV 1.

Discussion. A comparison of the efficacy of several 5-nitroimidazole-derivatives against the metronidazole resistant strain *T. foetus* KV 1/M 100 shows that an extremely high metronidazole resistance is accompanied by a clearly marked cross-resistance to all 5-nitroimidazole-derivatives studied in the experiments. This corresponds to the findings of McLOUGHLIN[9], who not only observed a cross-resistance of a dimetronidazole resistant *T. foetus* strain to metronidazole, but also to aminitrazole, a nitrothiazole derivative. It confirms also the findings of BENAZET and GUILLAUME[5], who, by using both a metronidazole-resistant and a nitrimidazine-resistant strain of *T. vaginalis*, described a complete cross-resistance between these two 5-nitroimidazole-derivatives. On the other hand, CARNERI[10] found only a slight cross-resistance between these two drugs on testing nitrimidazine, using different metronidazole-resistant strains of *T. vaginalis*. This phenomenon might be related to the degree of metronidazole resistance of the strains used. Both our experiments and those of BENAZET and GUILLAUME[5] have been carried out with highly metronidazole-resistant strains with factors of > 68 and > 48 respectively, while the resistance factors of the strains used by CARNERI[10] were in the range of 1.5 to 12.1.

Under in-vitro conditions, the strain KV 1/M 100 was also found to be cross resistant to different degrees to all 5 nitroimidazole-derivatives tested. Here, as under in-vivo conditions, the strain KV 1/M 100 demonstrated the highest resistance factor to metronidazole. However, we did not find an exact correlation between the factors of resistance observed in vitro and those found in vivo. Such a correlation could hardly be expected, as the results of the experiments in vivo are influenced qualitatively and quantitatively by the specific pharmacokinetics and biotransformation of the drugs in animals.

[9] D. K. McLOUGHLIN, J. Parasit. *53*, 646 (1967).
[10] I. DE CARNERI, Trans. R. Soc. trop. Med. Hyg. *65*, 268 (1971).

The Problem of Detecting a Free Glutamate Decrease in the Dorsal Sensory Neuron Following Dorsal Root Crush[1]

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Summary. Dorsal root crush results in a highly significant decrease in the free glutamate/total free amino acid concentration ratio over an extended time period. Perhaps this is a good measurement to use in glutamate crush studies.

Since glutamate is a potent spinal cord excitant[2], and free glutamate levels are higher in the dorsal root than in the ventral root[3–5] or distal sensory root[4,5], this amino acid is presently a transmitter candidate at the dorsal root terminals[3,6]. In analogy with the cholinergic transmitter system where nerve section or crush has led to definite changes in the regulatory enzymes[7,8] or in the acetylcholine level[9], it has been tested whether this would be true for the glutamate content of the dorsal root after such injury[10–12]. Although there is a decrease in free glutamate per g tissue in the root following injury, neither proximally[12] nor distally[10,11] is this decrease significant. This can be interpreted to support any one of three alternative possibilities: 1. glutamate is not a transmitter candidate here, 2. the glutamate transmitter system is different from the cholinergic system in this respect, or 3. the usual means of expressing such amino acid data has not been able to uncover any significant free glutamate change. The purpose of this study is to analyze free glutamate changes and total free amino acid changes proximal and distal to a dorsal root crush in order to discern if there is any way to detect a significant free glutamate change as a result of the crush. Such a study has not been carried out using several time periods over the time when maximal crush effects should be seen[8].

Adult cats (3.5–4.2 kg) were anesthetized with sodium pentobarbital (Nembutal, 35 mg/kg) and the lumbosacral spinal cord and roots were exposed. The dura was carefully cut rostrocaudally in order to expose the dorsal roots of L7 and S1. The rootlet bundles of L7 and S1 on one side were crushed with a fine forceps 13 mm from the cord. The dura was then stitched and the skin was closed with wound clips after stitching the underlying fascia. Skin-Hesive liquid skin cement (United Surgical) was applied to the wound area to facilitate complete closure. 300,000 units of procaine Penicillin-G were given i.m. to each animal. After times ranging from 2 to 25 days after crushing, the cat was sacrificed and the root and ganglionic tissue of L7 and S1 were removed rapidly and frozen in isopentane in dry ice. The 13 mm segment of

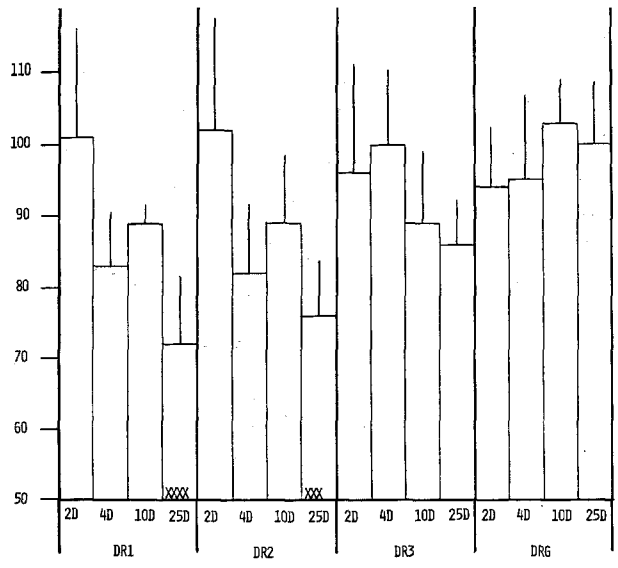

Fig. 1. The free glutamate is shown on the Y-axis as the percentage of the control level. The control level of glutamate was 4.49 ± 0.50 μmoles/gT in the dorsal root and 4.52 ± 0.42 μmoles/gT in the dorsal root ganglion ($N = 38$). On the X-axis, the time period after crushing is shown in days (D) for each tissue segment. The dorsal root segments are numbered from the cord towards the dorsal root ganglion (DR1, DR2 and DR3). Each segment of root is 12–14 mm long, with DR1 being distal to the root crush and DR3 being located nearest the dorsal root ganglion (DRG). The bar for each time period is the percentage of free glutamate remaining in that tissue after crushing and the line above each bar shows the standard deviation. Using the Student's t-test, the number of X's at the base of each bar shows the significance of the difference between control (100%) and crush (X = 5%, XX = 2.5%, XXX = 1% and XXXX = 0.5% level of significance).

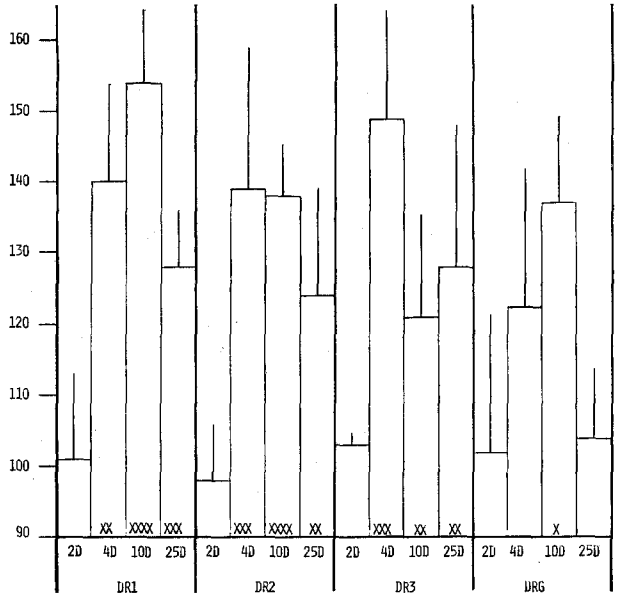

Fig. 2. The total free amino acids are shown on the Y-axis as the percentage of the control level. The control levels of total free amino acids were 22.9 ± 5.6 μmoles/gT in the dorsal root and 32.8 ± 4.2 μmoles/gT in the dorsal root ganglion ($N = 38$). On the X-axis, the time period after crushing is shown in days (D) for each segment of tissue labeled as indicated for Figure 1. The bar for each time period is the percentage of total free amino acids in that tissue compared to the control, and the line above each bar shows the standard deviation. The number of X's at the base of each bar shows the level of significance for the difference from the control value (100%) as indicated in the legend to Figure 1.

dorsal root distal to the crush was labeled DR1. The proximal root tissue between the crush and the ganglion was divided into 2 segments of equal length each averaging 12–14 mm, and labeled DR2 (segment just proximal to the crush) and DR3 (segment nearest the ganglion). The dorsal root ganglion was also taken for analysis. The contra-lateral control tissue was treated in a similar manner. Glutamic acid was determined by the method of GRAHAM and APRISON[13], and total free amino acids were determined by the method of MCCAMAN and ROBBINS[14] as modified by GRAHAM et al.[15].

Figure 1 shows the temporal effect of the root crush on free glutamate, and Figure 2 shows the concomitant change in total free amino acids when expressed per g tissue. At 2 days after crush no effect was evident on free glutamate or total free amino acids. By 4–10 days there was a significant elevation of total free amino acids in all root tissues but an insignificant decrease in free glutamate. At 25 days after crushing there was a significant decrease in free glutamate in DR1 and DR2 only, with the total free amino acids showing a lower but still significant elevation. The damming of free amino acids immediately proximal to the crush was seen within 8 h after injury[16] which was much too early for the free amino acid increase along the extensive distance of the dorsal root observed in this study (Figures 1 and 2). Thus, the total free amino acid increase seen here may not be a damming phenomenon at all. The dorsal root ganglion shows a significant free amino acid increase only in the 10 day period.

Next, it was determined if this insignificant free glutamate decrease, when expressed on a tissue basis, could be significant when expressed as the glutamate/total free amino acid concentration ratio (Figure 3). The first significant decrease in this ratio occurred in DR1 and DR2 in the 4-day period. Since this change is evidently in process during the 4-day period, the variability in the time-span of this response from animal to animal would make highly significant changes here unlikely. In both 10 and 25-day periods there was a very highly significant decrease in this ratio in all root segments. Since the ganglion did not show any singificant changes here it is not included in Figure 3.

Thus, there is a significant decrease in free glutamate of the dorsal root for an extended period of time after

[1] This work was supported by Research Grant No. USPH-5-RO1-NS-10508 from the National Institutes of Health.

[2] D. R. CURTIS, J. W. PHILLIS and J. C. WATKINS, J. Physiol., Lond. *150*, 656 (1960).
[3] M. H. APRISON, L. T. GRAHAM, JR., D. R. LIVENGOOD and R. WERMAN, Fedn. Proc. *24*, 462 (1965).
[4] A. W. DUGGAN and G. A. R. JOHNSTON, J. Neurochem. *17*, 1205 (1970).
[5] J. L. JOHNSON and M. H. APRISON, Brain Res. *24*, 285 (1970).
[6] J. L. JOHNSON, Brain Res. *37*, 1 (1972).
[7] C. O. HEBB and G. M. H. WAITES, J. Physiol., Lond. *132*, 667 (1956).
[8] J. L. JOHNSON, Brain Res. *18*, 427 (1970).
[9] C. A. N. EVANS and N. R. SAUNDERS, J. Physiol., Lond. *192*, 79 (1967).
[10] I. M. JONES, C. C. JORDAN, I. K. M. MORTON, C. J. STAGG and R. A. WEBSTER, J. Neurochem. *23*, 1239 (1974).
[11] P. J. ROBERTS and P. KEEN, Brain Res. *74*, 333 (1974).
[12] T. TAKAHASHI and M. O. OTSUKA, Brain Res. *87*, 1 (1975).
[13] L. T. GRAHAM, JR. and M. H. APRISON, Analyt. Chem. *15*, 487 (1966).
[14] R. E. MCCAMAN and E. ROBBINS, J. Neurochem. *5*, 18 (1959).
[15] L. T. GRAHAM, JR., R. P. SHANK, R. WERMAN and M. H. APRISON, J. Neurochem. *14*, 465 (1967).
[16] P. J. ROBERTS, P. KEEN and J. F. MITCHELL, J. Neurochem. *21*, 199 (1973).

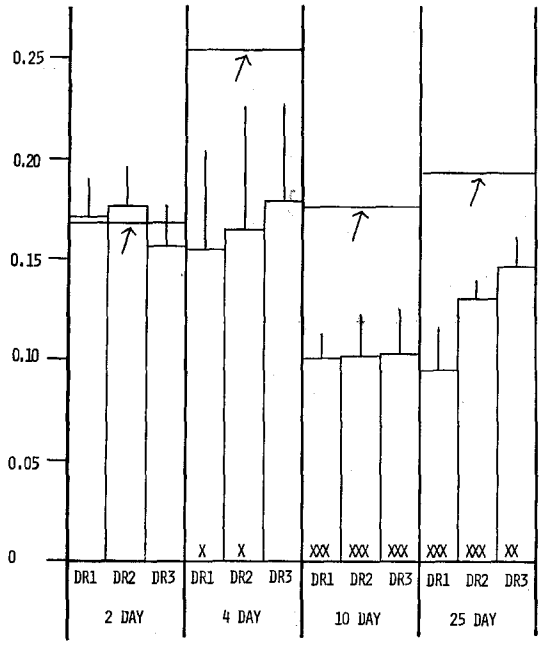

Fig. 3. The free glutamate/total free amino acid concentration ratio is shown on the Y-axis. Each tissue is shown at 2, 4, 10 and 25-day periods following crushing (between DR1 and DR2 as in Figure 1) on the X-axis. The arrow points to the control value glutamate/free amino acid concentration ratio obtained in each particular time period. The bar for each tissue on the X-axis represents the glutamate/free amino acid concentration ratio, and the line above this is the standard deviation. The significance of the difference between control and crush tissue was tested by the Student's *t*-test. The number of X's at the base of each bar represents the level of significance of this difference; X = 5%, XX = 2.5% and XXX = 0.5% level of significance.

crushing, not on absolute grounds (per g tissue), but on a relative basis (expressed as the ratio of free glutamate/total free amino acid concentrations). Perhaps, however, a significant free glutamate concentration decrease could be detected at times earlier than 25 days if a very large sample size was used with data expressed per g tissue only. Although this may suggest that there is a decrease in free glutamate in the dorsal sensory neuron following injury, some additional difficulties must be considered before this conclusion is reliable. Distal to the injury there is complete degeneration of the separated axons[17] with phagocytic satellite cells breaking down axonal debris[18]. The proteolytic activity of the satellite cells may cause free glutamate increases which cancel any decreased axonal glutamate when measured on a tissue basis. Proximal to the injury, the intact axons regrow in an attempt to reinnervate the end organ[17]. It is not yet known whether the total free amino acid increase observed proximally here is neuronal or extraneuronal in nature, although free glutamate is decreasing relative to total free amino acids at a great distance proximal to the crush. Therefore, the results of this study[1], and the fact that glutamate is such an active substance of widespread importance[6], indicate that one must ask the following three questions concerning such data. 1. How important are such observations in showing whether or not glutamate is a synaptic transmitter? 2. How specific are such crush effects on free glutamate levels where one is reasonably sure it is a synaptic transmitter as opposed to where it is not a transmitter? 3. What amino acid changes following axonal crush or section are associated with the axons and which are associated with satellite cells?

[17] J. Z. YOUNG, Adv. Surg. *1*, 165 (1949).
[18] M. SINGER and M. C. STEINBERG, Am. J. Anat. *133*, 51 (1972).

Long-Chain (Z)-9-Alkenes are 'Psychedelics' to Houseflies with Regard to Visually Stimulated Sex Attraction and Aggregation

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Summary. Three different tests on houseflies (*Musca domestica* L.) revealed that both pheromone-free pseudoflies and male partner flies exhibit in the presence of mixtures of long-chain (Z)-9-alkenes or pure (Z)-9-tricosene enhanced releasing effects for two optical cues, which stimulate male houseflies to mating strikes and houseflies of both sexes to aggregation.

Behavioral responses of long-chain (Z)-9-alkenes in the male housefly, *Musca domestica* L. are believed to be sex attraction[1–3], sex stimulation[3], excitement, mating and orientation[4]. Furthermore, aggregation of both sexes of houseflies is attributed to (Z)-9-tricosene[5,6]. Several homologous compounds show pheromone activity[3,4], especially a 7:3 mixture of (Z)-9-tricosene and (Z)-9-heneicosene[4]. Although it is well known that visual stimuli elicit strong sex attraction[7] and aggregation[8], the mutual interaction between visual and olfactory stimuli in the housefly has not been established[9].

In the present study, combinations of several (Z)-9-alkenes were tested to elucidate their effect on the behavior of houseflies in conjunction with visual stimuli. 3 types of tests were carried out, each in the absence of pheromones ('standards') and in the presence of pheromones: 1. The well established pseudofly test in Petri dishes[2,3,10], in which *one* pseudofly is exposed to 2 male houseflies; 2. a modified pseudofly test in Petri dishes, in which *two* pseudoflies are exposed to 1 male housefly; and 3. a large-scale test using pseudofly-flypaper strips. In 2. as well as in 3. pseudoflies containing no pheromones were employed as 'controls' in the presence of pseudoflies impregnated with pheromones.

Material and methods. The test materials (I–VI), as shown in Table I, contained various levels of biologically active hydrocarbons, such as (Z)-9-heneicosene[3,4], (Z)-9-docosene[3,4], (Z)-9-tricosene[1–6], (Z)-9-tetracosene[3,4], and (Z)-9-pentacosene[4].

1. The short range action of long-chain (Z)-9-alkenes on the sex attraction of male houseflies was studied using the pseudofly test in Petri dishes[2,3,10]. Covered Petri dishes, 9 cm in diameter, each with 2 male houseflies, 5 to 10 d old, from laboratory culture and 1 'pseudo-