Nervous Control of Flight Orientation in a Beetle

In a recent publication about the nervous control of dipteran flight, Wilson and Wyman[1] confirmed and extended previous observations of the absence of phase relationship between the movements of the wings and the nervous input to the muscles of insects possessing a fibrillar flight-motor. Their records show, however, that an increase in wing-beat frequency is accompanied by an increase in the frequency of nervous stimulation to the power muscles. In a concluding sentence, Wilson and Wyman suggest that frequency control may operate in the regulation of parameters like direction of locomotion.

In an investigation of flight orientation in the rhinoceros beetle, *Oryctes boas*, I have found that change of direction about the yaw axis is accompanied by a unilateral increase in frequency of the nervous input to the fibrillar flight muscles. Extracellular recordings have been obtained from the left and right members of all six pairs of fibrillar flight muscles during straight flight and yawing rotations of tethered beetles. The traces in Fig. 1 show the marked increase in frequency of the nervous input to the left muscle of a pair during yaw to the right induced by rotation of a striped cylinder.

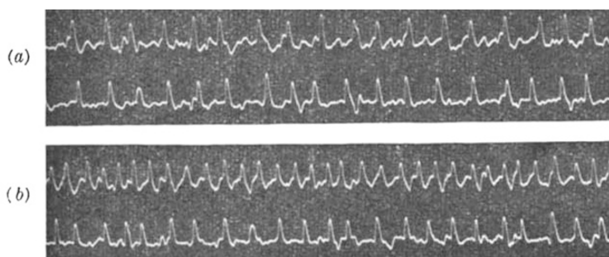

Fig. 1. Extracellular recordings from left (upper trace) and right (lower trace) dorsal oblique muscles of *Oryctes boas*. (*a*) Straight flight; (*b*) yaw to the right

Stroboscopic photographs of tethered beetles executing yawing rotations show that yaw is achieved chiefly by a unilateral increase in amplitude of the wing stroke. Machin and Pringle[2] observed that an increased frequency of electrical stimulation to the basalar muscle of *Oryctes rhinoceros* resulted in an increase in the amplitude of oscillation of the muscle.

It is concluded that, during yawing rotations, the observed increase in frequency of the nervous input to the flight muscles of the appropriate side brings about a greater amplitude of oscillation of these muscles and so leads to a unilateral increase in amplitude of the wing stroke.

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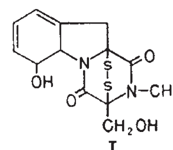
[1] Wilson, D. M., and Wyman, R. J., *J. Insect Physiol.*, **9**, 859 (1963).
[2] Machin, K. E., and Pringle, J. W. S., *Proc. Roy. Soc.* (B), **151**, 204 (1959).

VIROLOGY

Antiviral Activity of Gliotoxin and Gliotoxin Acetate

During a search for antiviral materials, we found several *Penicillium* culture filtrates that inhibited the cytopathic effect of type 2 poliovirus MEF_1 in monkey kidney primary cell cultures. The antipolio activity of these filtrates was found to be due to gliotoxin and gliotoxin acetate.

Gliotoxin (I) (ref. 1) was first isolated in 1936 by Weindling and Emerson[2] and afterwards by others from culture filtrates of species of *Gliocladium* (?), *Trichoderma*, *Aspergillus* and *Penicillium*[3]. Its inhibitory activity towards bacteria, fungi and transplantable neoplasms is well known[4]. There has been no report of antiviral activity for gliotoxin. It is highly toxic for rodents[4] and as a result has no practical value as a therapeutic agent. For this reason we have not investigated its antiviral effect or mode of action, but we present this communication: (1) to demonstrate that methods are available for detecting potentially useful antiviral materials; (2) to report that this long-known compound exhibits antiviral activity; and thereby (3) to provide another tool for virus and chemotherapy research.



One monoverticillate *Penicillium*, strain *F*1072, had been isolated from a soil sample from Bath, Maine, U.S.A., and fermented aerobically at 26° C for 4 days in a medium containing glucose, acid-hydrolysed casein, enzymatically hydrolysed yeast, and sodium chloride. Crystalline material was first obtained from such culture filtrates by *n*-butanol extraction followed by alumina chromatography and countercurrent distribution. By modifying fermentation media and conditions, more potent culture filtrates were prepared. These filtrates were extracted with ethylene dichloride; and, after partitioning the dried extract between methanol and heptane, the active material was crystallized from the methanol fraction. The identity of this material with gliotoxin was established by physical and chemical comparison with an authentic sample.

Another monoverticillate *Penicillium*, *F*642, isolated from soil collected in Anvers, Belgium, yielded both gliotoxin and its monoacetate, which were differentiated chromatographically and separated by fractional crystallization. Both were active against type 1 (Mahoney) poliovirus.

Fermentation and isolation were assisted by agar diffusion assays and bio-autography with poliovirus type 1 (Mahoney) in *H.Ep.* 2 cells, using methods described by Herrmann and by Siminoff[5]. It was found that assays with *Micrococcus lysodeikticus* reflect poliovirus-inhibitory activity and that similar R_F values are obtained with samples developed in the same solvent system. Hence, agar diffusion with *M. lysodeikticus* was used to help guide fermentation, fractionation, and isolation because of its greater precision, speed and convenience. Poliovirus assays and bio-autograms were limited to confirmation of key samples.

Table 1 gives the antiviral spectrum of gliotoxin when tested at half-log serial dilutions with *H.Ep.* 2 and *KB* cell suspensions in plastic panels and with monkey kidney primary cell and *KB* cell monolayers in tubes. The test procedures in plastic panels have been described[6]. Similar methods were used for tests in tubes except that virus and drug were added simultaneously to 3–5 day monolayer cultures containing 1·0 ml. of maintenance medium (synthetic medium 199 for monkey kidney, Eagle's plus 10 per cent horse serum for *KB* cells). Gliotoxin was active against poliovirus, herpes and Asian influenza; but the end-points were more definite with poliovirus. Gliotoxin was inactive against rabies when added to a CVS infected mouse brain homogenate, and this mixture inoculated intracerebrally into healthy adult mice.

Chemotherapy trials were limited because of the poor solubility of gliotoxin and its toxicity for animals. The maximum tolerated single dose for mice was found to be 7·5 mg/kg intravenously (*N,N*-dimethylacetamide solution) and <19 mg/kg intraperitoneally (sodium carboxymethyl cellulose emulsion). When gliotoxin was admini-

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