eggs will be laid in the following spring. A spacing reaction may tend to disperse widely the wintering populations and to increase the proportion of larval hosts that they discover in the following spring.

Another field trial at Warren, New South Wales, suggested that two massive releases of sterile flies at one point in the center of town at the beginning of winter (May 1963) may have driven the wild population to the periphery of town and thus changed the pattern of infestation of the loquat crop in the following spring (September-October) (*6*).

Nadel and Peleg (*7*) have reported a different but related response in the Mediterranean fruit fly, *Ceratitis capitata* Wied. They found that females were more strongly attracted to traps baited with "tri-med" lure (a methylcyclohexane derivative, *7*) when food was short than when food was abundant. Food shortage might be accentuated by release of sterile flies, which might even be used to increase the number of wild flies entering traps or settling on baits.

The efficacy of population flushing is yet to be established, but the procedure is worth developing, both as an ecologic tool and as a means of controlling harmful populations.

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Marking Single Neurons by Staining with Intracellular Recording Microelectrodes

Abstract. *Glass microelectrodes filled with a saturated solution of methyl blue or fast green in 1.0M potassium acetate can be used to mark penetrated neurons from which intracellular recordings have been made. Many cells can be marked in one experiment and can easily be located in subsequent histological sections.*

When making electrophysiological recordings from the mammalian central nervous system, it is desirable to know the position of the electrode tip and, hence, the site of recorded activity. A number of techniques (*1, 2*) are available for use with extracellular recording electrodes, but these do not permit the exact identification of the neuron from which recordings were made. However, no simple or reliable methods are available for use with intracellular recording electrodes, which allow the study of single nerve cells in effective electrophysiological isolation. In particular, there is no method for marking a series of neurons over a period of hours in one experiment. We have now developed such a method for routinely marking neurons penetrated by microelectrodes, so that the marked cells may be identified easily in subsequent histological sections.

We have described a method for making extracellular marks by passing current through glass electrodes filled with 2*M* sodium chloride saturated with fast green FCF (*2*). It seemed possible that this technique could be adapted for intracellular marking. For this purpose we chose 1.0*M* potassium acetate as the basic solution for the electrodes; this salt does not cause reversal of inhibitory postsynaptic potentials (*3*), and we have found that electrodes filled with it have excellent recording properties.

Saturated solutions of a number of water-soluble dyes in 1*M* potassium acetate were made by shaking an excess of the dye with acetate solution and filtering. Glass microelectrodes, previously filled with distilled water, were individually filled with the dye solutions by means of a fine Pasteur pipette. Alternatively, a number of water-filled electrodes were placed in a large volume of dye solution. The electrodes were then left overnight so that the dye could diffuse to the tips and, in initial trials, were tested to see whether appreciable quantities of the dye could be ejected electrophoretically into agar. Electrodes filled with dyes passing this test were selected for resistances of 5 to 15 megohms and used to penetrate antidromically activated motoneurons in the lumbosacral cord of decapitate cats. When a stable penetration was obtained we attempted to stain the cell by passing a current of a few microamperes through the electrode. The electrode was then withdrawn from the track, so that it did not further penetrate the marked cell. After several test marks were made, segments of the cord containing marks were fixed overnight in 10 percent formalin. In some cases the animal was perfused with saline and formol-saline to remove blood from the cord; this procedure did not seem to affect the marks in any way. Segments of the cord containing test marks were sectioned at 100 μ on a freezing microtome, and the sections were mounted serially on glass slides coated with albumin. The unstained sections were then examined under a dissecting microscope to locate the marked cells. Sections containing marked cells were dried at 50°C for 1 hour before they were stained in thionin and mounted in DPX in the usual manner. In some cases sections were mounted in DPX without staining so that the marked cell could be examined in isolation.

Of the 16 dyes tested, only two have reliably made easily detectable marks—fast green FCF (color index No. 42053) and methyl blue (color index No. 42780), with the latter giving the better results. In the case of both dyes, current was passed with the electrode negative. Electrodes containing these dyes in 1.0*M* potassium acetate have proved excellent for intracellular recording, and the presence of the dye did not affect the impaled cell in any obvious way. Cells impaled for periods of over 30 minutes did not show any deterioration, and one electrode could be used to make several marks in the same experiment.

The appearance of the marked cell varied with the size of the current and the time that elapsed before the animal was killed. The cell shown in Fig. 1 was marked with a methyl blue electrode 9 hours before the animal was killed. The color in the unstained

section (Fig. 1*A*) was quite intense, but the cell did appear damaged. In the thionin-stained section (Fig. 1*B*) the marked cell was surrounded by many glial cells, but it was still easy to distinguish. Cells marked with methyl blue less than 4 to 5 hours before the animal's death were not only relatively undamaged, but often showed a spectacular array of stained dendrites. Figure 2 shows a cell marked 2 hours before death, and dendrites in the unstained section (Fig. 2*A*) can be seen over 500 μ from the cell body. Figure 2*B* shows the same cell after being stained with thionin. Some of the dendritic stain has been lost, this loss being minimized if dioxan rather than alcohols was used for dehydration. The marked cell is of a quite different color from that of the other neurons and can easily be identified in the section. Electrodes containing fast green do not produce quite such intense staining, and we have not found them as satisfactory as electrodes filled with methyl blue; but, in cases where marks of two different colors are desired, fast green is quite acceptable.

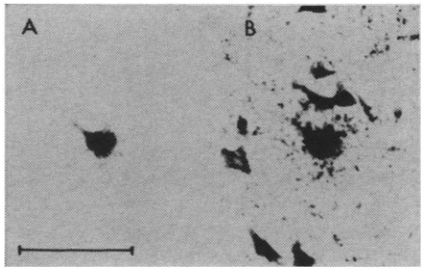

Fig. 1. Motoneuron marked 9 hours before the end of the experiment by passing 2.4 μa for 3 minutes through an electrode containing methyl blue. (*A*) The cell photographed in the unstained section. (*B*) After thionin staining, at the same magnification. Scale, 200 μ.

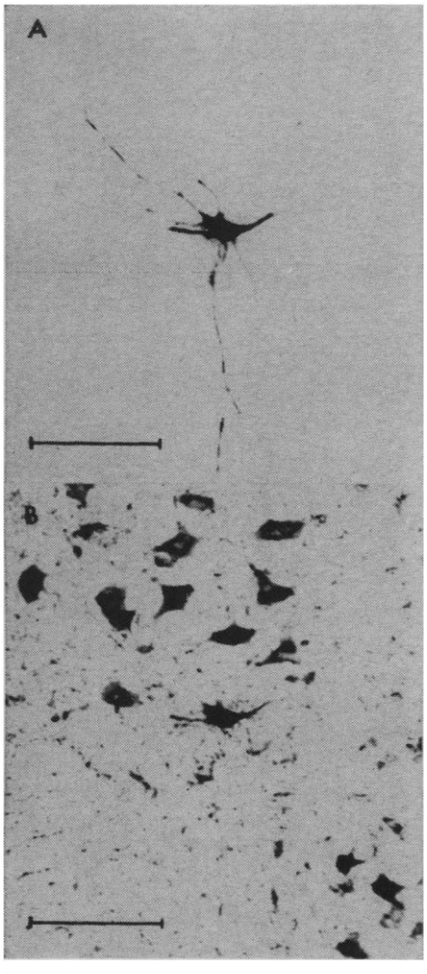

Fig. 2. Motoneuron marked 2 hours before the end of the experiment by passing 1.8 μa for 1 minute through a methyl blue electrode. (*A*) Cell in unstained section; the staining of the dendrites is extensive. (*B*) The same after thionin staining, at the same magnification. Some of the dendritic stain has been lost. Scale, 200 μ.

Currents tested were from 6 to 0.5 μa. However, currents in excess of about 3 μa often caused the dye to spread to neurons near the one impaled. On the other hand, marks made with less than 1 μa have been lost when made more than 2 hours before death. Thus for best results about 2 μa for 3 minutes should be used up to 3 hours before death; less current may be used nearer the end of the experiment. With such currents, we have never found more than one stained cell in the area. We have usually passed the current for 3 minutes, but this does not seem to be necessary in the case of methyl blue, except for marks made many hours before the animal was killed. Using currents in these ranges we have marked over 26 motoneurons with methyl blue and have been able to find all but two of them, probably lost because of bad penetrations. With electrodes filled with fast green we have made 19 marks, with currents ranging from 1.7 to 3 μa for 3 minutes, and, while many of the marks were rather pale, we were able to locate all but one.

Thus the above technique, while not sacrificing the quality of electrodes for intracellular recording, permits the routine marking of single penetrated neurons. The marked cells can easily be located in subsequent histological sections and, in particular with methyl blue, can show some details of the dendritic spread.

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Aflatoxin B_1: Binding to DNA in vitro and Alteration of RNA Metabolism in vivo

Abstract. *Aflatoxin B_1 binds to both native and denatured DNA, as shown by spectroscopy and equilibrium dialysis. It also strongly inhibits incorporation of cytidine into rat liver nuclear RNA and lowers the RNA content of the nucleus. The extreme toxicity and carcinogenicity of aflatoxin B_1 may be direct results of the affinity of this agent for DNA.*

Aflatoxins, metabolites produced by some strains of the mold *Aspergillus flavus*, are among the most potent carcinogenic agents for rat liver, if judged by the minimum dose required to induce liver tumors (*1*, *2*). The structure of aflatoxin B_1 (Fig. 1), the most toxic of the four known aflatoxins produced by *A. flavus*, has recently been elucidated (*3*). We report here that aflatoxin B_1 binds to DNA in vitro, and that in vivo aflatoxin B_1 rapidly inhibits incorporation of cytidine-H^3 into rat liver nuclear RNA and lowers the ratio of nuclear RNA to DNA. These results suggest that the binding of aflatoxin B_1 to DNA may be of prime importance in the mechanism of action of this compound.

The binding of aflatoxin B_1 to DNA in vitro was demonstrated both by the shift in the absorption spectrum which occurs when aflatoxin B_1 is bound to DNA and by equilibrium dialysis techniques (*4*). Upon binding, there is a shift in the absorption maximum