

Fig. 2. Transverse section of epidermis showing the green fluorescent nerve leaving a sense organ. The cuticle is autofluorescent. Bar = 50  $\mu$ m.

The sensory nerves of peripheral sense organs fluoresce with a blue-green colour similar to that of tracts in the nerve cords (figure 2). As they join segmental nerves, some of the fluorescent tracts in the nerve cords may be afferent. It has been established that the sensory nerves of many invertebrates contain catecholamines<sup>5</sup>.

Fine fluorescent fibres occur among the body wall muscle. They appear to be yellow in colour but their distribution has not yet been ascertained. Should they prove to be motor axons they could be either excitatory or inhibitory, the latter possibility being particularly interesting<sup>6</sup>.

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### Induction of deoxyribonucleic acid degradation in *Escherichia coli* by ozone<sup>1</sup>

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**Summary.** Cell survival and deoxyribonucleic acid (DNA) degradation were measured for wild-type *Escherichia coli* B251 cells after exposure to different concentrations of ozone. The results show that extensive breakdown of DNA occurs after ozonation and that the extent of ozone-induced DNA degradation generally correlates with the colony-forming ability of the cells.

Previous work on the effect of ozone on the survival of radiation-resistant and -sensitive strains of *Escherichia coli* suggested that lesions to deoxyribonucleic acid (DNA) might be responsible for killing of bacteria by ozone<sup>3</sup>. In agreement with this, ozone has been shown to modify markedly nucleic acids and bases<sup>4,5</sup> and to produce specific mutants in *E. coli*<sup>6</sup>. Therefore, it seemed interesting to determine the amount of DNA degraded in wild-type strain B251 of *E. coli* following exposure to different concentrations of ozone.

**Materials and methods.** Cells were grown overnight at 37 °C in glucose-salts medium (M9)<sup>7</sup> containing 10  $\mu$ Ci of thymidine-<sup>3</sup>H (New England Nuclear Corp.; 40–60 Ci/mM) and 250  $\mu$ g deoxyadenosine per ml. After several washings, cell suspensions were exposed in M9 medium to 5, 10, 25 and 50  $\mu$ l/l ozone for 30 min as described<sup>6</sup>. Ozone survival curves were done on nutrient agar plates (Difco) as previously described<sup>6</sup>. The procedure for measuring ozone-induced DNA degradation was that of Strike and Emerson<sup>8</sup>. Control experiments were carried out as above, except that clean air was used instead of ozone.

**Results and discussion.** The survival of strain B251 to different concentrations of ozone and for different intervals of treatment-time is represented in figure 1. According to these results, ozone showed no discernable effect on survival for exposure times ranging between 1 and 30 min at 5  $\mu$ l/l. However, the killing capacity of ozone which appeared first at 10  $\mu$ l/l (about 55% lethality after 30 min), was greatly increased at higher ozone concentrations (25 and 50  $\mu$ l/l), more than 3 decades of killing occurring within the 30-min treatment period.

The amount of DNA degraded in B251 cells following a 30-min exposure to ozone was examined (figure 2). Expressed

as a percentage of total radioactivity, the data show that more DNA is degraded and released into the medium by ozonated than by unozonated cells. Furthermore, the extent of this DNA breakdown increases both with ozone treatment and incubation time. This suggests that ozone produces lesions in the DNA which stimulate degradation; and

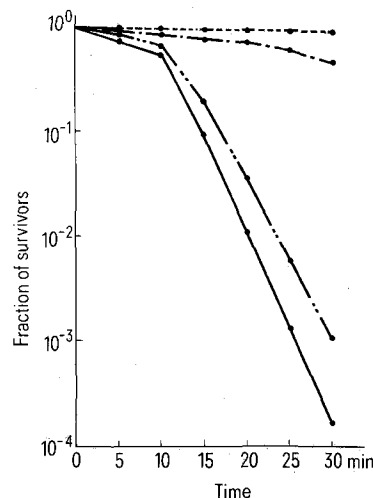


Fig. 1. Survival of wild-type strain B251 of *Escherichia coli* treated with different concentrations of ozone in complete growth medium: 5  $\mu$ l/l (●---●); 10  $\mu$ l/l (●---●); 25  $\mu$ l/l (●...●); 50  $\mu$ l/l (●—●). Average of 3 independent experiments.

that cell recovery and DNA degradation are interrelated under the present experimental conditions. The fact that, at low doses, ozone induces some DNA degradation with practically no decay in viability may reflect the high degree of repair capacity in wild-type cells.

It is well established that the DNA of *E. coli* is also degraded in the cells after exposure to radiation, and that base damage and/or single-strand breaks in the DNA are responsible for the degradation of this molecule<sup>9</sup>. Evidence obtained by comparing the relative sensitivity of selected mutants of *E. coli* to ozone indicates that an unrepaired single-strand break in the DNA might be the lesion which

initiates the extensive DNA degradation<sup>10</sup>. The fact that this gas has been observed to produce a high frequency of chromosome aberrations in several organisms including man<sup>11</sup>, supports this conclusion. However, the reactivity of this strong oxidant towards nucleic acids and their derivatives, as well as proteins and amino acids<sup>12</sup>, suggests that base damage and/or DNA-protein crosslinks may also be induced by ozone. Therefore, the possibility that the degradation results from nucleolytic breaks at the sites of ozone-produced DNA lesions is not excluded from the present data.

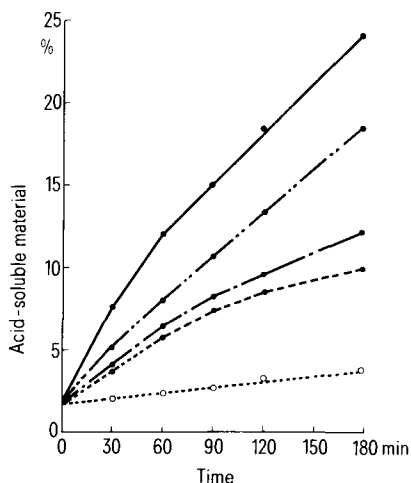


Fig. 2. Release of radioactivity into growth medium from the DNA of strain B251 as a function of ozone dose (30-min exposure) and of time of post-ozonation incubation with aeration at 37°C. The experimental conditions and symbols are the same as in figure 1. The symbol (○ ····· ○) represents the unozonated control. Average of 3 independent experiments.

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### Digestive physiology of *Bradynopyga geminata* (Odonata: Libellulidae)

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**Summary.** The pH of the gut of *B. geminata* ranges from 5.5 to 6.8. The midgut is the main source of the digestive enzymes, secreting trypsin-like protease, chymotrypsin, aminopeptidase,  $\alpha$ -amylase, maltase, sucrase, lactase and lipase.

Information on the digestive physiology of dragon flies is poor<sup>1-4</sup> as compared to that on other insects. Consequently a study of this problem in *Bradynopyga geminata* Rambur was undertaken.

**Material and methods.** The pH of different regions of the gut was determined by pH paper method and the digestive enzymes as follows. The isolated gut was divided into fore-, mid- and hind-gut. For each experiment, gut contents from 10 corresponding parts were collected in 1 ml distilled water and 10 gut tissues of corresponding parts homogenized in 0.5 ml distilled water. The contents and homogenate were centrifuged at 3000×g for 15 min and the supernatant raised to 1 ml with distilled water. The substrates for carbohydrases were prepared after Khan and Ford<sup>5</sup>, for pepsin- and trypsin-like proteinases after Cole<sup>6</sup>, for chymosin after Pavlovsky and Zarin<sup>7</sup>, for aminopeptidase after Colowick and Kaplan<sup>8</sup> and for cellulase after Hawk et al.<sup>9</sup>. The reaction mixture contained 0.2 ml of each

of the enzyme extract, 0.1 M phosphate buffer of appropriate pH and of the substrate solution, with 2-3 drops toluene serving as bacteriostat. It was incubated at 37°C for 24 h, but for 6 days for cellulase and, thereafter, subjected to unidimensional ascending paper chromatography on Whatman No.1 filter paper, using n-butanol-acetic acid-water as the solvent. The usual benzidine reagent was used for locating sugars and 0.2% ninhydrin solution for the amino acids<sup>10</sup> resulting from the activity of carbohydrases and proteinases respectively. Lipase was tested strictly according to Hawk et al.<sup>9</sup>.

**Results and discussion.** A microscopical examination of the crop contents of *B. geminata* revealed the presence of triturated insects which confirms the entomophagous nature of dragonflies<sup>11</sup>. The pH of the contents of different regions of the alimentary canal is as follows: crop, 5.5-5.8; midgut, anterior half 5.8-6.1 and posterior half 5.5-5.8;