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[9]. 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[10]. The fact that this gas has been observed to produce a high frequency of chromosome aberrations in several organisms including man[11], supports this conclusion. However, the reactivity of this strong oxidant towards nucleic acids and their derivatives, as well as proteins and amino acids[12], 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.

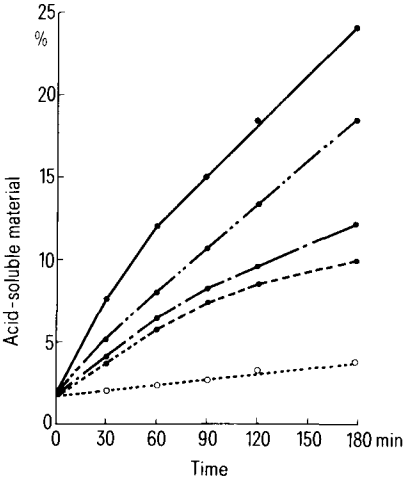

Fig. 2. Release of radioactivity into growth medium from the DNA of strain B251 as a function of ozone dose (30-min exposure) and of time of post-ozonation incubation with aeration at 37 °C. The experimental conditions and symbols are the same as in figure 1. The symbol (O · · · · · O) represents the 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, α-amylase, maltase, sucrase, lactase and lipase.

Information on the digestive physiology of dragon flies is poor[1-4] 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[5], for pepsin- and trypsin-like proteinases after Cole[6], for chymosin after Pavlovsky and Zarin[7], for aminopeptidase after Colowick and Kaplan[8] and for cellulase after Hawk et al.[9]. 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[10] resulting from the activity of carbohydrases and proteinases respectively. Lipase was tested strictly according to Hawk et al.[9].

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[11]. 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;

Digestive enzymes in the alimentary canal of *B. geminata*

Enzymes	Foregut (crop) Tissue	Foregut (crop) Contents	Midgut Tissue	Midgut Contents	Hindgut Tissue	Hindgut Contents
Pepsin-like protease	–	–	–	–	–	–
Trypsin-like protease	+	+ + +	+ +	+ + +	+	+ + +
Chymotrypsin	–	+ + +	+ +	+ + +	–	+ + +
Aminopeptidase	–	+ +	+ +	+ + +	–	+ +
α-Amylase	+	+ + +	+ +	+ + +	–	+ +
Cellulase	–	–	–	–	–	–
α-Glucosidase						
a) Maltase	–	+ + +	+ +	+ + +	–	+ +
b) Sucrase	+	+ + +	+ +	+ + +	+	+ + +
β-Galactosidase						
Lactase	–	–	+	+ +	–	+
Lipase	–	+ + +	+ +	+ + +	–	+ +

ileum and rectum, both 6.7–6.8. These values differ from those reported by Mehrotra and Sen[4] for 3 species of dragon-flies in which the pH of foregut contents ranged pH 8.4–8.9 and of midgut pH 5.4–5.9.

In *B. geminata*, digestive enzymes have been found to be present in both the tissues and contents of each region of the alimentary canal (table). It will be seen that since the midgut tissue secretes 8 enzymes, this region, is the main site of enzyme secretion. Enzymes detected in tissues of the foregut and hindgut are probably endoenzymes[12]. Whereas those present in the foregut contents presumably reach there by regurgitation, a well-known phenomenon[13], and those in hindgut arrive along with the digested food. An additional enzymic source can be the alimentary canal of the preyed insect. According to the array of enzymes, *B. geminata* is capable of metabolising carbohydrates, proteins and fats, unlike *Libellula luctuosa* in which the enzyme capable of digesting starch (amylase) is absent[2].

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Use of ^{31}P NMR to measure pH of membrane-enclosed solutions

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Summary. Phosphorus nuclear magnetic resonance was employed to measure the internal pH of unsonicated multilamellar liposomes. A steady transmembrane pH gradient could be obtained in the presence of sulfate and citrate anions. The effects of some uncouplers and ionophores on this controlled pH gradient were studied.

Available methods for estimating pH of membrane-enclosed solutions include measurements of the absorbance of indicator dyes[3], the equilibrium distribution of weak acids[4] or amines[5,6] in response to pH gradients, the fluorescence quenching of freely diffusible fluorescent amines[7]. The main limitation of these techniques, when applied to living organelles, lies in their great potential for interfering with biological processes, e.g. indicator dyes are likely to participate in oxidation-reduction reactions. Such methods appear rather laborious and can only provide indirect information about internal pH.

^{31}P and proton NMR techniques offer a very simple, direct method for measuring pH gradients across biomembranes[6,8,9]. ^{31}P NMR seems in this respect more promising than proton NMR, considering the much easier interpretation of ^{31}P NMR-spectra, the number of phosphorylated metabolites that can be monitored in living cells and the high resonance resolution of this method due to the large chemical shift range.

In the present study, the potentiality of this approach was tested in a series of experiments on the proton permeability of phospholipid liposomes which were loaded with inorganic phosphate to detect the internal pH.

Multilayer liposomes were chosen for such an approach as they have a large internal volume and are readily washed by centrifugation. Multilamellar liposomes were prepared by evaporating chloroform solutions containing 75 μmoles of egg phosphatidylcholine plus 5% egg phosphatidic acid, and shaking the lipids in 3 ml of a buffer containing either 0.3 M Na_2HPO_4 or 0.3 M K_2HPO_4 at pH 7. The milky