of the mice receiving the various immunosuppressive treatments but no AB-PC (table 1, figures in parentheses). However, in the mice that received combined treatments of AB-PC, X-ray irradiation and dexamethasone or trypan blue, *Candida* became detectable in the liver and kidneys at 4 and 6 days after oral administration (tables 1 and 2). The isolates from the liver and kidneys were identified with *C. albicans* subtype A. This suggested that the yeast detected in these organs was not a contaminant or endogenous organism but had spread to the organs from the GIT.

These findings support the idea of penetrative growth of *Candida* rather than persorption through the intestinal wall which would have occurred within a few hours of oral *Candida* administration. They also stress the importance of both *Candida* growth in the GIT and the suppressed state of the host defence mechanism.

Macrophages are known to be rather resistant to sublethal irradiation doses of X-rays which induce severe damage to T cells, B cells and polymorphonuclear leukocytes of mice[6]. However, the suppression of macrophages can be induced by dexamethasone[7] or trypan blue treatments[8]. The fact that the spread into the organs did not follow after single sublethal irradiation but followed after the combination of X-ray irradiation and dexamethasone or trypan blue, suggests an important role of macrophages in the defence against systemic *Candida* infection. Using *Candida* administered orally, several authors have already obtained colonization of the GIT of germ free, specific pathogen free, or antibiotic treated conventional mice, but the colonizing *Candida* did not spread to the inner organs through the intestinal wall[9,10]. The present study shows that *Candida* colonizing the GIT of mice can be the source of systemic *Candida* infection under immunosuppressive conditions brought about by X-ray irradiation combined with dexamethasone or trypan blue administration.

Table 2. Number of viable *Candida* in liver and kidneys of mice that received immunosuppressive treatments after oral *Candida* administration

Treatment			Liver		Kidneys	
			4[a]	6	4	6
AB-PC	X-ray	Dexa	3.4[b] ± 1.2	3.8 ± 0.6	3.7 ± 1.1	4.3 ± 0.8
AB-PC	X-ray	Tryp	-[c]	3.3 ± 0.2	3.5 ± 0.9	4.5 ± 0.5

[a]Days after oral *Candida* administration. [b]Number of viable *Candida* ($\log_{10}$ per organ). [c]Less than 10 *Candida* cells per organ.

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High levels of transition metals in dinoflagellate chromosomes

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Summary. X-ray microanalysis of fixed, sectioned chromosomes of the dinoflagellates *Glenodinium foliaceum, Prorocentrum micans* and *Amphidinium carterae* has revealed high levels of iron, nickel, copper and zinc. We report high levels of these transition metals in association with chromosomes in intact eukaryote cells.

Since Dodge[2] proposed, over a decade ago, that dinoflagellates were evolutionary intermediates between prokaryotes and eukaryotes, considerable attention has been focussed on the fine structure and composition of their nuclei[3-7]. As reported here, our use of X-ray probe microanalysis to investigate the elemental composition of dinoflagellate nuclei has revealed new information about the chemical composition of the nuclei, and in particular the chromosomes. High levels of the transition metals iron, nickel, copper and zinc were found to be associated with the chromosomes of 3 species of marine dinoflagellates. Although transition metals have been reported previously in small amounts in extracted mammalian DNA[8] and deoxyribonucleoprotein (DNP)[9], this is the 1st report of high levels of a wide range of transition metals in association with chromosomes in intact cells.

Materials and methods. 3 marine dinoflagellate species were examined (table). The cells were cultured in Cambridge medium A.E. 50 and prepared for X-ray microanalysis by fixing in 1% glutaraldehyde (buffered by 0.1 M sodium cacodylate, pH 7.2) for 1.5 h, followed by rinsing in buffer, dehydration in an ethanol series, and embedding in Spurr resin[10]. Ultrathin sections (~ 200 nm) were cut, collected on nylon grids and lightly coated with carbon. The sectioned cells were examined completely unstained in an aluminium grid holder using a Corinth analytical electron microscope (Cora-AEI Kratos Ltd, UK). Cora was operated at 60 kV, counting for 500 sec with a spot size of 0.75 µm. Examination of the processing solutions used in the preparation of the cells, by atomic absorption spectrophotometry, showed no detectable contamination by transition metals.

Results and discussion. For each species, 5 cells were analysed in detail, and for each cell X-ray emission spectra were obtained for chromosomes, nucleoplasm, cytoplasm and regions of adjacent resin (control). The elements found in the analyses, and their occurrence in the areas examined in each species, are shown in the table. A typical series of spectra, from 1 cell, showing the 3 regions studied and an adjacent resin control is illustrated in the figure.

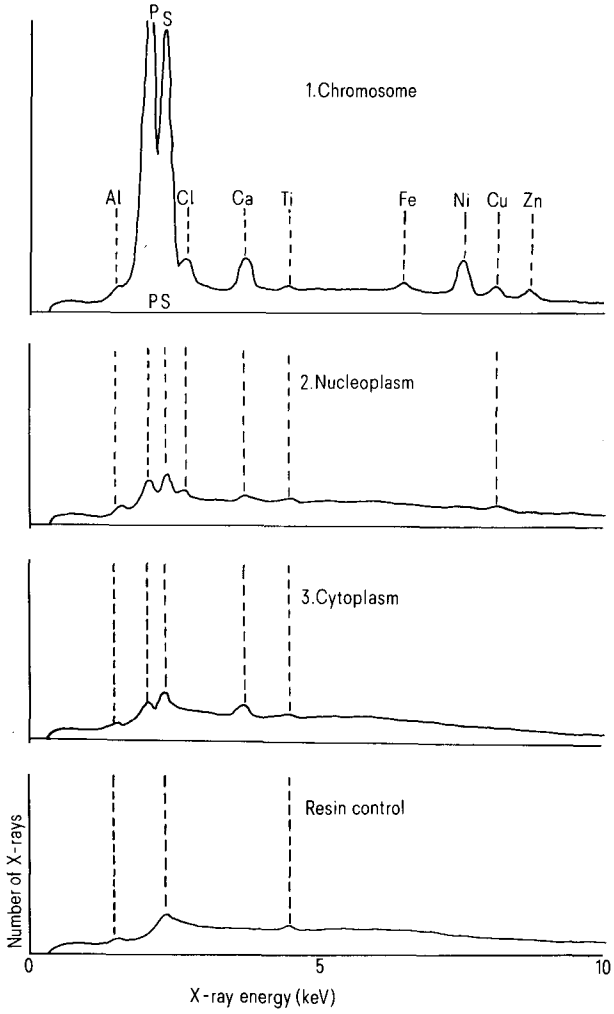
The techniques used in preparing the cells (fixation, dehydration and embedding) would result in the majority of weakly bound elements being lost from the cells[11]. Detectable elements retained by these cells were probably closely associated with, if not an integral part of, structural components of the cell.

The chromosomes were remarkable for the high levels (figure) and wide range (table) of elements detected. The transition metals were of particular interest since there are

Occurrence of elements in 3 species of dinoflagellates

Cell	Region	n	P	S	Cl	K	Ca	Fe	Ni	Cu	Zn
Glenodinium foliaceum	Chromosome	25	++	++	+	+	++	++	++	++	+
	Nucleoplasm	15	++	++	+	±	++	++	−	+	+
	Cytoplasm	15	++	++	+	−	+	−	−	−	−
	Resin control	5	−	+	+	−	−	−	−	−	−
Prorocentrum micans	Chromosome	25	++	++	±	+	++	++	++	++	++
	Nucleoplasm	15	++	++	++	±	++	+	−	++	+
	Cytoplasm	15	++	++	+	−	++	±	−	±	−
	Resin control	5	−	++	−	−	−	−	−	−	−
Amphidinium carterae	Chromosome	25	++	++	++	−	++	++	++	++	++
	Nucleoplasm	15	++	++	++	−	++	−	−	−	−
	Cytoplasm	15	+	++	++	−	++	±	−	−	−
	Resin control	5	−	++	+	−	−	−	−	−	−

n, Number of analyses; ++, element present in more than 90% of analyses; +, element present in between 20–90% of analyses; ±, element present in less than 20% of analyses; −, element not detected. NB. This table shows presence or absence of elements only and gives no indication of elemental concentrations.



Emission spectra from 3 areas of a single cell of *Prorocentrum micans* (glutaraldehyde fixed, resin embedded) and an adjacent resin control. The spectra are drawn to the same scale, the continuum ('white') radiation counts having been adjusted to the same value in all 4 spectra. With this adjustment the peak height of each element gives a direct indication of relative mass fractions[22]. The Al peak was produced by the gridholder, the Ti peak by titanium contaminants in the nylon grids, and the sulphur containing constituents of Spurr resin produced a small S count (see resin control).

apparently no previous reports of high levels of iron, nickel, copper and zinc in either chromatin or chromosomes. Biochemical analysis of extracted DNA from a variety of sources has demonstrated small amounts of iron, zinc and copper even after prolonged dialysis[7]. Similar observations, with the additional detection of nickel, have been made on calf thymus DNP[9]. The concentrations of transition metals found in extracted DNA and DNP were very low, not exceeding tens of ppm[8,9].

The sensitivity of X-ray microanalysis, at the present time, is such that the minimum detectable levels of transition metals would be in the order of 5 times[12] the maximum concentrations reported in extracted mammalian DNA and DNP. X-ray microanalytical studies on sectioned material have generally failed to detect a wide range of chromatin-associated metal elements. The highly condensed chromatin of the bryophyte *Phaeoceros laevis*[13] showed detectable calcium, but no other metals. In mammalian nuclei iron was shown to be localized to the nucleolus[14], and zinc to the nucleolus and chromatin[14,15]. Iron has been cited as an essential element in nucleotide synthetic pathways, and zinc as a component of DNA polymerase[14]. Whether the high levels of iron and zinc associated with dinoflagellate chromosomes have similar functions to those proposed for mammalian nuclei remains a matter for further work.

Metals have been found localized within nuclei of cells subjected to pollutant or potentially toxic external concentrations of metals[16,17]. This seems unlikely to be occurring in this case, since a) cell growth was vigorous, as measured by population increase and absence of dead cells; and b) no ultrastructural evidence of pollutant or toxic effects was observed. Nuclear precipitation complexes resulting from high levels of metal pollutants[16,17] have not been observed in any of the dinoflagellates discussed here.

Recent work has shown low concentrations of ionic copper are toxic to dinoflagellates[18] and that high levels of free ionic zinc limit plankton growth[19]. In view of this work, the high levels of transition metals reported here imply they have a low free ionic activity and therefore are organically complexed[19].

In addition to occurring invariably at high levels in the chromosomes, iron, zinc and copper were also detected in other regions of the cells (table). They were most frequently found in the nucleoplasm and always at consistently lower levels than in the chromosomes (figure). Nickel was exceptional in being the only transition metal strictly localized to the chromosomes in all the cells examined.

The chromosomes, as expected, showed the distinct

phosphorus and sulphur peaks associated with chromatin, as reported by other authors[13,14]. Calcium produced prominent peaks in the chromosomes (figure), indicating its presence in large concentrations. This is of particular interest in view of the permanently condensed nature of dinoflagellate chromosomes, as previous work[20,21] has suggested that high calcium levels are associated with the condensation of chromatin in mammalian nuclei.

The major conclusion to be drawn from this work is that high levels of the transition metals, iron, nickel, copper and zinc are present in the chromosomes (probably in an organic complex) of the dinoflagellates *A. carterae, G. foliaceum* and *P. micans*. It will be of considerable interest to determine if a similar situation exists in other dinoflagellates, or even in other members of the Protozoa.

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Registration of feeding behaviour in rats by recording food approach behaviour

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Summary. A method is described in which food approach behaviour of rats is recorded to study feeding behaviour. Between rats, differences in food approach behaviour were observed. For each rat, food approach behaviour was constant over a long period of time. This allows conversion of approach behaviour data into quantified feeding behaviour. Examples of long-term feeding behaviour and of reproducibility of food intake are given.

The investigation of 24 h eating rhythmicity in rats requires reliable but uncomplicated apparatus for monitoring food intake. For our studies on changes in 24 h eating rhythmicity during hypothalamic hyperphagia in rats[1,2], we developed simple, inexpensive food hoppers provided with approach detectors so that food approach can be monitored continuously. This method has the advantage of being relatively inexpensive and avoids time-consuming measurement of food intake. However, it will be clear that food approaches are not necessarily representative of food intake. Unless each entry into the food hopper is followed by ingestion of the same amount of food and no spillage occurs, the method does not provide reliable data on actual food intake, and gives only information on the temporal pattern of feeding behaviour. On the other hand, if food approach can be monitored and is found to correspond to conventionally measured food intake, the method can give detailed information on the feeding behaviour in rats.

In order to evaluate the experimental set-up, we studied 15 rats for a period of about a year, continuously monitoring food approach and measuring food intake by weighing the food at intervals varying from 0.5 to 6.0 days.

Methods. 15 adult female Wistar rats (body weight at the start of the experiment 285 ± 23 g) were individually housed in a light and temperature controlled room with an average humidity of 60%. Each cage contained a pellet box constructed as described elsewhere[1,2].

In short, for each approach to the food a perspex flap had to be pushed away by the animal. The movement of the flap resulted in the activation of an approach detector (Pepperl and Fuchs, Mannheim FRG), the output of which was fed into a microcomputer type DEC LSI-11 configuration for temporary storage. In addition to the eating activity, a number of items of general information (number of

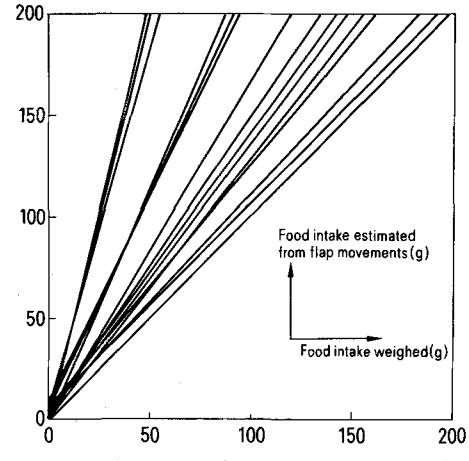

Fig. 1. Regression lines for each rat weighed food intake and food intake calculated from the number of food hopper flap movements and average pellet weight. The slopes of the regression lines range from 1.03 to 5.49, indicating differences in food approach behaviour between rats.