

InsP₃ are similar. Figure 2 shows fast and slow rate constants calculated for InsP₃-induced Ca²⁺ release using rat cerebellar microsomes. Both rate constants increase with [InsP₃]; however, the ratio of the fast and slow rate constants is not constant but rather increases at low [InsP₃]. This indicates that the stores are heterogeneous in their sensitivities to InsP₃. This result is unlikely to be a consequence of using a different cell system, because other studies on the kinetics of InsP₃-induced Ca²⁺ release using permeabilized hepatocytes also showed biphasic release¹². The ratios of the fast and slow rate constants when calculated from that paper also varied with [InsP₃].

In the light of data given in refs 1 and 5, we must reassess our current models for the complex kinetic behaviour of the InsP₃ receptor. Heterogeneity of InsP₃ sensitivity of the Ca²⁺ stores cannot be ruled out.

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HIROSE AND IINO REPLY — Missiaen *et al.* report that heavy loading of Fura-2/AM to Ca²⁺ stores affected the Ca²⁺ release induced by InsP₃ in permeabilized A7r5 cells. The Ca²⁺ leakage rate (in the absence of InsP₃) was not changed by Fura-2 loading in their experiments, indicating that the driving force for Ca²⁺ efflux was not changed, that is, the free luminal Ca²⁺ concentration was not altered by the dye loading. The observed effect on the InsP₃-induced Ca²⁺ release would then not result from a dependence of InsP₃-induced Ca²⁺ release on luminal Ca²⁺ concentration, but more probably from a nonspecific effect of their loading procedure. If, on the other hand, there had been a decrease in luminal Ca²⁺ concentration which was somehow cancelled by a nonspecific effect of Fura-2 on the Ca²⁺ leakage pathway, then the observed slowing of the Ca²⁺ release rate could be explained by the decreased driving force alone.

In our view, the observations of Missiaen *et al.* do not require a reinterpretation of our previous results¹ for the following reasons: (1) they used cultured cells, whereas we used intact smooth muscle cells, which react differently to dye treatment; and (2) the shape of the time course of Ca²⁺ release seemed little changed after Fura-2 loading in their experiment, indicating that the kinetics of InsP₃-induced Ca²⁺ release was not seriously affected by the Fura-2 effect.

In summary, although Fura-2 may indeed have minor nonspecific effects on Ca²⁺ release mechanisms under certain experimental conditions, the results of Missiaen *et al.* do not offer definitive proof or disproof of previous hypotheses

on the properties of InsP₃-sensitive stores.

Mezna and Michelangeli report the heterogeneity of InsP₃ sensitivity in Ca²⁺ stores of rat cerebellum. We believe that their and our analyses are mathematically equivalent, but that their experiments are subject to a common pitfall. Their results were obtained by measuring Ca²⁺ concentration released to the cytoplasm using Fluo-3 fluorometry. When cytoplasmic Ca²⁺ concentration is measured using fluorescent Ca²⁺ indicators, the Ca²⁺ concentration around the Ca²⁺ store inevitably changes during Ca²⁺ release. This change in Ca²⁺ concentration is known to seriously affect the kinetics of Ca²⁺ release¹⁴, because the rate of InsP₃-induced Ca²⁺ release is biphasically dependent on the cytoplasmic Ca²⁺ concentration¹⁵⁻¹⁷. The extent of feedback modulation of the InsP₃ receptor by Ca²⁺ on the cytoplasmic side depends on the concentration of InsP₃, which affects the

rate of Ca²⁺ concentration change. This is why analyses of InsP₃-induced Ca²⁺ release without cytoplasmic Ca²⁺ buffering, such as reported by Mezna and Michelangeli, do not allow any conclusion about the InsP₃ sensitivities.

In contrast, we buffered the cytoplasmic Ca²⁺ concentration using 10 mM of either EGTA or BAPTA to avoid the Ca²⁺-mediated feedback effect, and found no heterogeneity in the InsP₃ sensitivity¹. Cytoplasmic Ca²⁺ concentration is as important as InsP₃ concentration in the regulation of InsP₃ receptor activity. It is important to take this point into consideration in trying to understand InsP₃-induced Ca²⁺ release.

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Penguins disturbed by tourists 10.1038/376301b0

SIR — Nimon *et al.*¹ found no differences in the heart rate of gentoo penguins (*Pygoscelis papua*), measured by using an artificial egg, in the absence and presence of one person. They concluded from this observation that our recorded increases in Adélie penguin (*P. adeliae*) heart rate during human presence were primarily a consequence of our² experimental method. They suggested that our birds had been stressed while we fitted them with the recording apparatus and that they were "predisposed to extreme reaction on subsequent sighting of humans".

The attempt by Nimon *et al.* to minimize stress and provide more realistic controls, made possible by recent advances in electronic technology, is laudable. Implanted birds are indeed more likely to react adversely to humans than unhandled controls. But we believe that the interpretation of their preliminary results cannot yet be definitive with regard to the effects of tourism, and that the comparison of their data with those obtained by us on a different species is not justified.

Between November and February (during the entire reproductive season of Adélie and gentoo penguins), tourism in the Antarctic consists mainly of luxury liners landing groups of up to 100, and sometimes more, people ashore near penguin breeding colonies by means of zodiacs. Some colonies are visited as often as twice a week. It is unrealistic to expect even well-informed and well-meaning tourists somehow to spread themselves out so that only one subject can be seen by any one penguin at a time. Tourists tend to take as many photographs as they can over as wide an area as possible during the limited time they have on land.

By re-analysing the penguin heart-rate

data of Nimon *et al.*, we found that maximum values observed when humans approached were on average 19% higher than resting values of undisturbed birds. This indicates that the penguins studied by Nimon *et al.* did indeed react to the minimal human stimulus presented. There is also evidence that other bird species, for example terns (*Sterna paradisaea*), react to approaching humans by increasing their heart rates, although, as in the study of Nimon *et al.*, these birds had never been handled³.

Nimon *et al.* determined heart rates of gentoo penguins during incubation. We obtained heart rates of Adélie penguins when the chicks were in crèches, and were able to show that it is not possible to extrapolate the reaction of incubating birds to other stages of breeding⁴. Escape behaviour of unhandled Adélie penguins to an approaching human was, in fact, minimal during the incubation period from November to early December and maximal when the chicks were in crèches in late January⁴. The minimum approach distance required to elicit escape from the nest increased during this period from 0.5 to 6 m. Modification of Adélie penguin behaviour and escape reactions when approached by humans on foot have also been reported by others⁵.

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Although Adélie and gentoo penguins are congeneric, interspecific and even intraspecific differences can be substantial. For example, gentoo penguins breeding at Ardley island (South Shetlands), very near to a summer station, can easily be approached to 1 m by a single human. In this respect, they are similar to the birds studied by Nimon *et al.* However, gentoo penguins at the small, undisturbed Hope Bay (Antarctic peninsula) colony will abandon their nests when approached slowly to 20 m by a single human (our unpublished observations). The same is true for sub-Antarctic gentoo penguins⁶, a situation which presumably renders ineffective a heart rate measuring system encased in an egg. With such substantial intraspecific differences, it is premature to make interspecific generalizations.

Finally, it is not only penguins near nests that are likely to be disturbed by tourist activities. We observed that a single human approaching to within 20 m of unhandled Adélie penguins commuting between the beach and their colonies in mid-January caused the birds to deviate from their normal tracks. The birds made a 70-m detour for several hours after the human had left the area. We estimated that this single disturbance caused the 12,000 birds commuting on these tracks during the 10 h of observation to cover an extra 840 penguin-km. Observation was by remote-controlled video placed 150 m from the study site⁴.

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Oil biodegradation around roots

SIR — Four years after the Gulf War, about 50 km² of the Kuwaiti desert still suffers from the intensive oil pollution caused by the Iraqi forces. Although there is experimental evidence for some self-cleaning of this environment through the activity of indigenous microorganisms¹, heavily contaminated areas still fail to support wild plants; however, moderately to weakly contaminated areas with less than about 10% by weight of oil sediments currently support such annuals.

Here we report that roots of such plants and of crop plants grown in polluted sand in pots under field conditions are associated with hundreds of millions of oil-utilizing microorganisms per gram of fresh roots. These microorganisms take up and metabolize various aliphatic and aromatic hydro-

carbons rather quickly, thus detoxifying and bioremediating the soil in the immediate vicinity of the roots. This may explain the plant survival mechanism in oil-contaminated soils. Further, this finding paves the way for a bioremediation approach which depends on densely cultivating oil-polluted desert areas with selected crop plants that tolerate oil and whose rhizospheres are rich in oil-degraders. Heavily contaminated areas would first have to be mixed with clean sand to dilute the oil to levels tolerable by the plants.

We initially observed that Kuwaiti desert plants, belonging predominantly to the family Compositae, although growing in black, polluted sand, always possessed white, clean roots. Even the sand adhering to the roots, the rhizosphere, was always clean whereas the sand just apart was black and polluted. We took complete desert plant samples to the laboratory, and also potted three crop plants, corn, tomato and termis, in sand we had previously polluted with 10% by wt crude oil. The pots were kept for 4 weeks in the Botanical Garden, under open conditions.

Whole roots of desert and crop plants, together with the adhering soil particles, were blended for 30 s in sterile water and the suspensions used for plate-counting and isolating oil-utilizing microorganisms using a solid inorganic medium containing 2% crude oil as a sole source of carbon². Representative oil-utilizing strains were isolated, purified and identified, and their potential to take up and oxidize individual alkanes and aromatic hydrocarbons was investigated using GLC analysis of hydrocarbons and cell total fatty acids, as we have described previously^{2,3}.

Seeds of the three crop plants showed a germination rate of 90–100% in the polluted pots, comparable to seeds in control clean pots. However, 4-week plants in the polluted pots, although quite healthy,

grew more weakly than the control plants, reaching 60–75% of their optimum height.

The rhizosphere samples of all plants were rich in oil-utilizing microorganisms. Respective bacterial numbers for corn, tomato and termis were 3.0×10^8 , 8.1×10^8 and 5.2×10^8 cells per g fresh roots, and fungal numbers were 4.4×10^5 , 1.5×10^5 and 1.4×10^5 propagules per g fresh roots. In all samples, filamentous actinomycetes, probably *Streptomyces*, were present at several thousand propagules per g root. One bacterial genus, *Arthrobacter*, was predominant (>95%) in the rhizosphere of all plants, whereas in the polluted non-rhizospheric soil several other genera, *Rhodococcus*, *Pseudomonas* and *Bacillus*, predominated, with *Arthrobacter* making up <5% of the total. The predominant fungi in the rhizosphere samples belonged to *Penicillium* and *Fusarium*, but in the polluted non-rhizospheric soil *Trichoderma* predominated. In a few cases an oil-utilizing yeast was found as a minor constituent of the rhizospheric microflora.

A total of eight strains of *Arthrobacter*, two of *Penicillium* and two of *Fusarium*, isolated from the rhizosphere of various plants, grew well, utilizing individual even- and odd-chain *n*-alkanes with C₁₀ to C₄₀ chains and three representative aromatic hydrocarbons, benzene, naphthalene and phenanthrene, as sole sources of carbon and energy. Four representative predominant *Arthrobacter* strains in the rhizospheres of various plants could quickly consume the *n*-alkanes dodecane (C₁₂), hexadecane (C₁₆) and decosane (C₂₂) and the aromatic hydrocarbons naphthalene and phenanthrene from their growth media. GLC analysis of the *n*-alkane-incubated biomass revealed that cells accumulated in their lipids fatty acids equivalent in chain length to the substrate alkanes: that is, the cells could metabolize oil constituents further after their uptake.

In conclusion, the rich oil-utilizing microflora around roots of crop plants obviously clean oil-polluted soil just adjacent to the roots. Our new work consolidates a few earlier reports^{4,5} by suggesting densely cultivating suitable plants in polluted Kuwaiti desert areas as a promising approach for their bioremediation. Heavily polluted areas would first have to be mixed with sand from adjacent clean areas to reduce the oil content to 10% by wt or less, the concentrations tolerable by the crops.

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Individual *Senecio glaucus* plants growing along the polluted border of an oil lake in the Kuwaiti desert. The plant roots and adhering sand particles are white and clean. In contrast, the surface of the transitional zone between the root and shoot is black and polluted.