$InsP_3$ are similar. Figure 2 shows fast and slow rate constants calculated for $InsP_3$-induced Ca^{2+} release using rat cerebellar microsomes. Both rate constants increase with $[InsP_3]$; however, the ratio of the fast and slow rate constants is not constant but rather increases at low $[InsP_3]$. This indicates that the stores are heterogeneous in their sensitivities to $InsP_3$. This result is unlikely to be a consequence of using a different cell system, because other studies on the kinetics of $InsP_3$-induced Ca^{2+} release using permeabilized hepatocytes also showed biphasic release[12]. The ratios of the fast and slow rate constants when calculated from that paper also varied with $[InsP_3]$.

In the light of data given in refs 1 and 5, we must reassess our current models for the complex kinetic behaviour of the $InsP_3$ receptor. Heterogeneity of $InsP_3$ sensitivity of the Ca^{2+} stores cannot be ruled out.

Mokdad Mezna
Francesco Michelangeli
School of Biochemistry,
Birmingham University, Edgbaston,
Birmingham B15 2TT, UK

HIROSE AND IINO REPLY — Missiaen *et al.* report that heavy loading of Furaptra/AM to Ca^{2+} stores affected the Ca^{2+} release induced by $InsP_3$ in permeabilized A7r5 cells. The Ca^{2+} leakage rate (in the absence of $InsP_3$) was not changed by Furaptra loading in their experiments, indicating that the driving force for Ca^{2+} efflux was not changed, that is, the free luminal Ca^{2+} concentration was not altered by the dye loading. The observed effect on the $InsP_3$-induced Ca^{2+} release would then not result from a dependence of $InsP_3$-induced Ca^{2+} release on luminal Ca^{2+} concentration, but more probably from a nonspecific effect of their loading procedure. If, on the other hand, there had been a decrease in luminal Ca^{2+} concentration which was somehow cancelled by a nonspecific effect of Furaptra on the Ca^{2+} leakage pathway, then the observed slowing of the Ca^{2+} 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 results[1] 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 Ca^{2+} release seemed little changed after Furaptra loading in their experiment, indicating that the kinetics of $InsP_3$-induced Ca^{2+} release was not seriously affected by the Furaptra effect.

In summary, although Furaptra may indeed have minor nonspecific effects on Ca^{2+} 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 $InsP_3$-sensitive stores.

Mezna and Michelangeli report the heterogeneity of $InsP_3$ sensitivity in Ca^{2+} 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 Ca^{2+} concentration released to the cytoplasm using Fluo-3 fluorometry. When cytoplasmic Ca^{2+} concentration is measured using fluorescent Ca^{2+} indicators, the Ca^{2+} concentration around the Ca^{2+} store inevitably changes during Ca^{2+} release. This change in Ca^{2+} concentration is known to seriously affect the kinetics of Ca^{2+} release[14], because the rate of $InsP_3$-induced Ca^{2+} release is biphasically dependent on the cytoplasmic Ca^{2+} concentration[15–17]. The extent of feedback modulation of the $InsP_3$ receptor by Ca^{2+} on the cytoplasmic side depends on the concentration of $InsP_3$, which affects the rate of Ca^{2+} concentration change. This is why analyses of $InsP_3$-induced Ca^{2+} release without cytoplasmic Ca^{2+} buffering, such as reported by Mezna and Michelangeli, do not allow any conclusion about the $InsP_3$ sensitivities.

In contrast, we buffered the cytoplasmic Ca^{2+} concentration using 10 mM of either EGTA or BAPTA to avoid the Ca^{2+}-mediated feedback effect, and found no heterogeneity in the $InsP_3$ sensitivity[1]. Cytoplasmic Ca^{2+} concentration is as important as $InsP_3$ concentration in the regulation of $InsP_3$ receptor activity. It is important to take this point into consideration in trying to understand $InsP_3$-induced Ca^{2+} release.

Kenzo Hirose
Masamitsu Iino
Department of Pharmacology,
Faculty of Medicine,
The University of Tokyo,
Tokyo 113, Japan

Penguins disturbed by tourists

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 our[2] 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 handled[3].

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 breeding[4]. 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 January[4]. 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 others[5].

1. Nimon, A. J., Schroter, R. C. & Stonehouse, B. *Nature* **374,** 415 (1995).
2. Culik, B., Adelung, D. & Woakes, A. J. in *Antarctic Ecosystems: Ecological Change and Conservation* (eds Kerry, K. R. & Hempel, G.) 177–182 (Springer, Berlin, 1990).
3. Neebe, B. & Hüppop, O. *Artenschutzreport* **4,** 8–14 (1994).
4. Wilson, R. P. *et al. Polar Biol.* **11,** 363–370 (1991).
5. Aguirre C. & Acero, J. M. in *Workshop on Researcher-Seabird Interactions* (eds Fraser, W. L. & Trivelpiece, W. Z.) 41 (Fraser, Montana State Univ., 1994).
6. Bost, C. A. & Clobert, J. *Acta oecol.* **13,** 593–605 (1992).