3 days) results of Gilon et al. [1]. In subsequent studies, the length of culture period ranged from 3–12 days [7, 8, 12], and no change in glucose responsiveness over this time period of the type reported by Gilon et al. was detected. Our studies were carried out using islets isolated from the C57BL/KsJ strain of mouse, whereas those of Gilon et al. utilized NMRI mice, raising the possibility that strain-specific differences were responsible for the divergent results. As shown in Figure 1, we extended these observations to another strain of mouse, the C57BL/6J. Even in this mouse strain, we were unable to observe the alterations in long-term culture reported by Gilon et al. [1]. The relevance of their findings even with respect to the NMRI mice is brought into question by previous studies demonstrating no effect of 7-day culture in 10 mmol/l glucose on insulin secretory responsiveness of NMRI islets [13]. In fact, the only situation in which we were able to observe the reported types of aberrant $[Ca^{2+}]_i$ responses to glucose was in islets isolated from the diabetic db/db mouse [14] and to a lesser extent in islets from the obese hyperglycaemic ob/ob mouse (Fig. 1 B and D). As in the case of the control islets, the abnormal responsiveness to glucose was observed immediately (1–2 days), and remained consistent throughout the period of study (10 days).

The isolation and culture conditions employed by Gilon et al. [1] were essentially identical to our own. The only experimental difference was the concentration of stimulatory glucose employed; 15 mmol/l glucose vs the 11–12 mmol/l glucose utilized by most groups. Our studies have shown that at 20 mmol/l glucose (again irrespective of culture duration) a sustained rise in Ca^{2+} is produced [14], due to the non-oscillatory depolarization induced by this supramaximal concentration of glucose (normal glucose levels under fed conditions in mice and man rarely exceed 12 mmol/l). Therefore, it is conceivable that a subtle increase in glucose sensitivity over the culture period might partially explain the loss of Ca^{2+} oscillations reported by Gilon et al. [1]. It would not, however, explain the disappearance of the phase 0 response, which in db/db mice is associated with a pathological decrease in endoplasmic reticulum Ca^{2+} ATPase levels [4]. Thus, the similarity of the changes in $[Ca^{2+}]_i$ in response to glucose reported by Gilon et al. [1] in the NMRI islets to those we have reported in ob/ob and db/db islets would suggest that the NMRI islets are metabolically compromised.

Yours sincerely,
M. W. Roe, B. Spencer, M. E. Lancaster, R. J. Mertz, J. F. Worley III, I. D. Dukes

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Response from the authors

Dear Sir,
Drs. Roe et al. disagree with our recent report [1] that glucose-induced oscillations of $[Ca^{2+}]_i$ in beta cells are altered when the islets (from NMRI mice) are cultured for 3–4 days. In their experience, the pattern of $[Ca^{2+}]_i$ changes induced by glucose is highly reproducible in islets (from C57BL/KsJ and C57BL/6J mice) cultured for 3–12 days, and similar to what we have observed with overnight cultured NMRI mouse islets [1–3]. Because the response of NMRI mouse islets cultured for 4 days resembles that of ob/ob and db/db mouse islets cultured for only 1–2 days [4], Roe et al. suggest that NMRI mouse islets are metabolically compromised and that our findings are pertinent only to the islets of the particular strain that we use.

We have tested this hypothesis in experiments using one strain studied by Roe et al., C57BL/6J mice. The islets were

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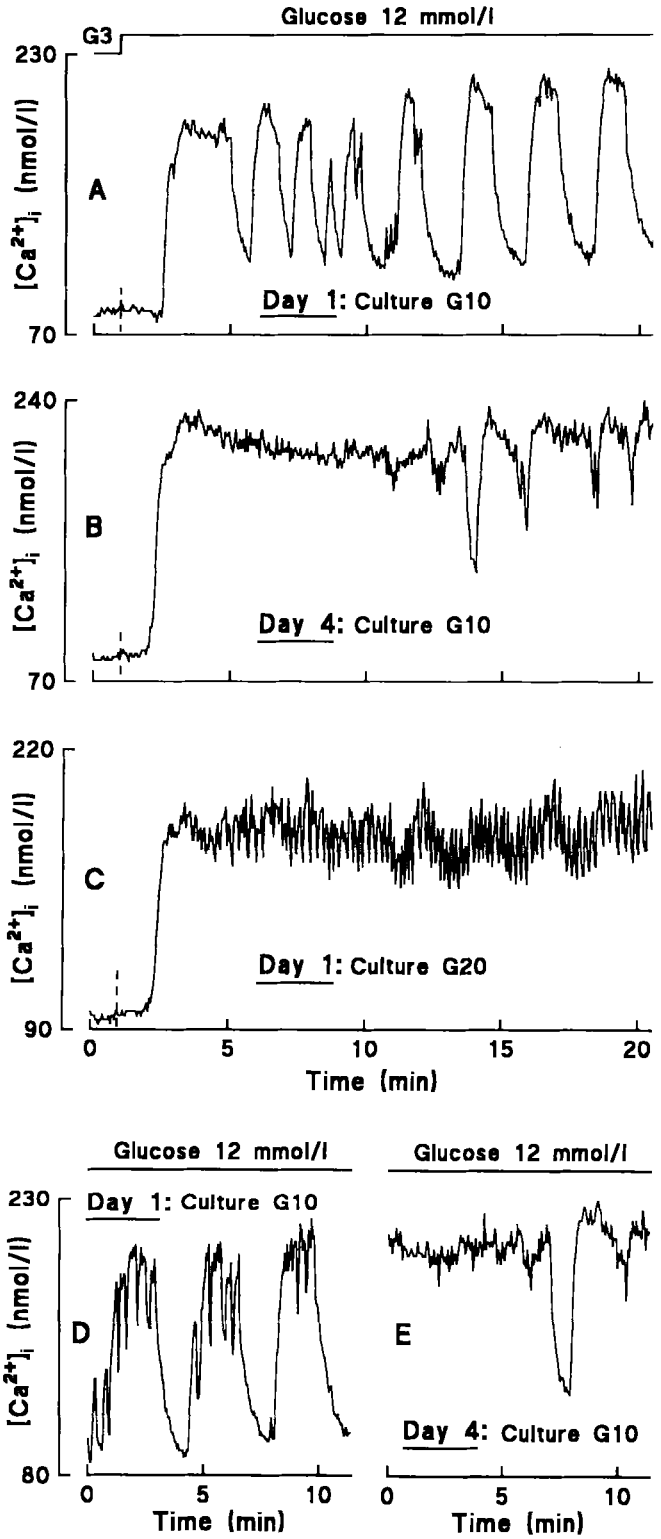

Fig. 1. (A–E) Influence of culture duration and conditions on glucose-induced $[Ca^{2+}]_i$ changes in islets from C57BL/6J mice. The islets were cultured in RPMI 1640 medium containing 10 (G10: **A, B, D, E**) or 20 (G20: C) mmol/l glucose for 1 or 4 days. They were then loaded with fura-2 (1 μmol/l) during 40 min of incubation in the presence of 10 mmol/l glucose before being transferred to the recording chamber. **A–C,** the islets were first perifused for about 10 min with a medium containing 3 mmol/l glucose before the glucose concentration was increased to 12 mmol/l. **D** and **E** the perifusion medium contained 12 mmol/l glucose throughout. Representative of results obtained in 7 **(C)** 9 **(A, D)** and 10 **(B, E)** islets

cultured for 1–4 days in RPMI medium and loaded with fura-2 (40 min in 10 mmol/l glucose), before measurement of $[Ca^{2+}]_i$ [2]. In islets cultured overnight in 10 mmol/l glucose, a rise of glucose in the perifusion medium (from 3 to 12 mmol/l) caused a small initial fall in $[Ca^{2+}]_i$ followed by a typical increase, with oscillations during steady-state stimulation (Fig. 1 A). After 4 days of culture, the elevation of $[Ca^{2+}]_i$ was almost continuous (Fig. 1 B). The influence of culture duration was also clearly visible in islets that were kept in 12 mmol/l glucose continuously following loading. After 1 day of culture, $[Ca^{2+}]_i$ oscillations were present in all islets, with a mixture of rapid oscillations superimposed on slow oscillations (Fig. 1 D). In contrast, on day 4, $[Ca^{2+}]_i$ usually remained steadily elevated with only occasional and transient decreases (Fig. 1 E). The response was also influenced by the glucose concentration during culture. After one night in 20 mmol/l glucose, small and fast oscillations of $[Ca^{2+}]_i$ were seen upon stimulation with 12 mmol/l glucose (Fig. 1 C). These fast oscillations disappeared after 4 days of culture (not shown). The influence of culture (duration and glucose concentration) was even more apparent when the islets were stimulated with 15 mmol/l glucose, with or without initial exposure to 3 mmol/l glucose (data not shown, 6–12 islets for each experimental protocol).

Old timers in the islet field have long known that both the rapidity and the intensity of the ionic, electrical and secretory responses to glucose can be influenced by the preincubation and preperifusion conditions [5]. The $[Ca^{2+}]_i$ changes are no exception. When islets are maintained long enough in a low-glucose medium before being stimulated with high glucose, the initial $[Ca^{2+}]_i$ fall is more marked nad the subsequent rise is delayed [P. Gilon, unpublished data]. This is what happens after loading of the islets in 2 mmol/l glucose, as in the experiments of Roe et al. The first $[Ca^{2+}]_i$ rise does not occur until about 4–5 min of stimulation and the initial fall is easily detected [2]. When we load the islets in 10 mmol/l glucose and only briefly perifuse them with 3 mmol/l glucose, the rise in $[Ca^{2+}]_i$ brought about by high glucose occurs within 2 min (Fig. 1) [1]. The initial fall is smaller and more difficult to see because it is masked by the rapid rise. However, it remains detectable even after 4 days of culture if the rise in $[Ca^{2+}]_i$ is prevented, e. g. by omission of extracellular Ca^{2+} [1]. This is not a sign of beta-cell dysfunction, but the probable functional expression of an acceleration of glucose metabolism after culture [6].

The influence of culture on $[Ca^{2+}]_i$ in islet cells is not an artefact introduced by some odd habit in our laboratory. F. Martin and B. Soria have also noted that the characteristics of glucose-induced $[Ca^{2+}]$ changes in islets isolated from OF1 mice are modified by the duration (1–4 days) of the culture and by the concentration of glucose (5–11 mmol/l) during the culture period (personal communication). The pattern of $[Ca^{2+}]_i$ changes induced by 11 mmol/l glucose in ob/ob mouse islets was also strongly influenced by overnight culture in different glucose concentrations [7]. The duration of culture is also known to affect the oscillations of $[Ca^{2+}]_i$ induced by glucose in single beta cells from ob/ob mice [8] and albino rats [9].

In conclusion, the results from our and several other laboratories indicate that the pattern of glucose-induced $[Ca^{2+}]_i$ changes in beta cells from various normal strains can be reversibly influenced by culture. These changes do not reflect intrinsic abnormalities of these cells, but their ability to adapt to novel environmental conditions. Our conclusion [1] that caution should be exercised when drawing conclusions from $[Ca^{2+}]_i$ measurements obtained from cultured preparations remains valid.

Yours sincerely,
P. Gilon, J. C. Jonas, J. C. Henquin

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