In view of the recent demonstration of a structural coupling between alpha and beta cells (*4*), we wonder whether the paradoxical behavior here disclosed may not be somehow related to a functional uncoupling of these cells as the result of calcium deprivation (*15*). If so, the present protocol might prove useful for further studies on the significance of intercellular bridging in the physiology and pathology of islet tissue.

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References and Notes

1. W. J. Malaisse, in *Handbook of Physiology*, sect. 7, vol. 1: *Endocrine Pancreas*, N. Freinkel and D. F. Steiner, Eds. (American Physiological Society, Washington, D.C., 1972), pp. 237–260; P. J. Randle and C. N. Hales, in *ibid.* pp. 219–235; R. P. Rubin, *Pharmacol. Rev.* **22**, 389 (1970); K. Wakabashi, I. W. Kamberi, S. M. McCann, *Endocrinology* **85**, 1046 (1969); A. J. Steiner, G. T. Peake, R. D. Utiger, I. E. Karli, D. M. Kipnis, *ibid.* **86**, 1354 (1970).
2. R. M. Buckle, A. D. Care, C. W. Cooper, H. J. Gitelman, *J. Endocrinol.* **42**, 529 (1968); L. M. Sherwood, I. Herrman, C. A. Bassett, *Nature (Lond.)* **225**, 1056 (1970); S. B. Oldham, J. A. Fisher, C. C. Capen, G. W. Sizemore, C. D. Arnaud, *Am. J. Med.* **50**, 650 (1971); J. T. Potts, Jr., T. M. Murray, M. Peacock, H. D. Niall, G. W. Tregear, H. T. Keutman, D. Powell, L. J. Deftos, *ibid.*, p. 639.
3. V. Leclercq-Meyer, J. Marchand, W. J. Malaisse, *Endocrinology* **93**, 1360 (1973); J. C. Edwards and S. L. Howell, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **30**, 89 (1973).
4. L. Orci, R. H. Unger, A. E. Renold, *Experientia* **29**, 1015 (1973); L. Orci, *Diabetologia* **10**, 163 (1974).
5. A. Loubatières, M. M. Mariani, G. Ribes, H. de Malbosc, J. Chaptal, *Diabetologia* **5**, 1 (1969).
6. H. Brunengraber, M. Boutry, Y. Daikuhara, L. Kopelovich, J. M. Lowenstein, *Methods Enzymol.* **35**, 597 (1974).
7. The total calcium was estimated with the cresolphthalein method using a SMA 12 Technicon analyzer. We thank R. Leclercq (I. M. C. Anderlecht; chief, P. Mascart) for these determinations.
8. Trasylol was a gift from G. Schnells and G. Wald of Bayer, Federal Republic of Germany, and Bayer-Pharma, Brussels,
9. We are currently using a combined radioimmunoassay for glucagon and insulin, based on a charcoal-dextran separation technique (V. Leclercq-Meyer, J. Marchand, O. Rebolledo, W. J. Malaisse, R. Leclercq, in preparation). The glucagon (lot 258-234-B-167-1) and insulin (lot R 170) standards were donated by M. Root (Lilly, Indianapolis) and J. Schlichtkrull (Novo, Copenhagen), respectively.
10. R. H. Unger and P. J. Lefebvre, in *Glucagon, Molecular Physiology, Clinical and Therapeutic Implications*, P. J. Lefebvre and R. H. Unger, Eds. (Pergamon, Oxford, England, 1972), pp. 213–244.
11. J. E. Gerich, B. J. Frankel, R. Fanska, L. West, P. H. Forsham, G. M. Grodsky, *Endocrinology* **94**, 1381 (1974).
12. R. H. Unger, in *Glucagon, Molecular Physiology, Clinical and Therapeutic Implications*, P. J. Lefebvre and R. H. Unger, Eds. (Pergamon, Oxford, England, 1972), pp. 245–257; ———, L. L. Madison, W. A. Müller, *Diabetes* **21**, 301 (1972).
13. E. F. Pfeiffer, R. Fussgänger, S. Raptis, *Acta Diabetol. Lat.* **9** (Suppl. 1), 233 (1972); W. A. Müller, G. R. Faloona, R. H. Unger, *J. Clin. Invest.* **50**, 1992 (1971); J. T. Braaten, G. R. Faloona, R. H. Unger, *ibid.* **53**, 1017 (1974).
14. H. Laube, R. J. Fussgänger, V. Maier, E. F. Pfeiffer, *Diabetologia* **9**, 400 (1973); B. J. Frankel, J. E. Gerich, R. Hagura, R. E. Fanska, G. C. Gerritsen, G. M. Grodsky, *J. Clin. Invest.* **53**, 1637 (1974).
15. Calcium deprivation causes uncoupling of cells in various tissues [W. R. Lowenstein, *Ann. N.Y. Acad. Sci.* **137**, 441 (1966)]. In monolayer cultures of rat endocrine pancreatic cells, monitored by time-lapse cinematography, calcium deprivation also causes a rapid loss of the normal contiguity of adjacent cells [L. Orci, B. Blondel, F. Malaisse-Lagae, M. Ravazzola, C. Wollheim, W. J. Malaisse, A. E. Renold, *Diabetologia* **10**, 382 (1974)].
16. Supported in part by grants from the Fonds National de la Recherche Scientifique Médicale (Belgium), and a contract of the Ministère de la Politique Scientifique (Belgium) within the framework of the Association Euratom Universities of Pisa and Brussels. O.R. was supported by a fellowship from the Consejo Nacional de Investigaciónes Cientificas y Técnicas de la República Argentina.

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Prostaglandins and the Pulmonary Vasoconstrictor Response to Alveolar Hypoxia

Said and co-workers (*1*) reported a frequent appearance of prostaglandins or prostaglandin-like biologically active substances in the venous effluent from isolated, perfused lungs during hypoxic ventilation. Furthermore, aspirin, which is a potent inhibitor of prostaglandin biosynthesis (*2*), was found to reduce the pulmonary vasoconstrictor response to hypoxic breathing in cats. Several things make this work difficult to assess. Alveolar hypoxia was induced in cat lungs perfused with the extremely low flow of 10 ml/min (*3*). No information was given about pulmonary arterial pressure (PAP), but assuming a normal pressure range, pulmonary vascular resistance (PVR) must have been increased by a factor of 25 or more, indicating grossly abnormal lungs. Furthermore, PAP-rises of as little as 1 mm-Hg were taken as evidence of a true hypoxic response. The average increase in PAP also seemed to be very small (*4*).

The effluent pulmonary perfusate superfused a series of specific smooth muscle assay organs. Whenever alveolar hypoxia elicited pulmonary hypertension, one or more of the assay organs contracted in all but four cases. When used, the rat stomach strip, the rat colon, and the chick rectum contracted in 38, 31, and 19 percent of the cases, respectively. Vane and co-workers recommend the *simultaneous* contraction of rat stomach strip, rat colon, and chick rectum for the bioassay of prostaglandin-like substances (*5*). The incidence of simultaneous contraction of the above muscle organs in the work of Said and co-workers cannot have been higher than 19 percent, which is the lowest percentage given for contraction of one of the assay organs. Other prostaglandin-sensitive tissues such as the guinea pig ileum and guinea pig trachea contracted in 29 and 71 percent of the cases used. As stated by Said and co-workers, some of the organ responses could not be attributed solely to prostaglandins. Addition of aspirin-like drugs to the perfusate might have indicated whether prostaglandins actually were released. An irregular release of prostaglandins from the perfused lungs might well have been an artifact caused by the experimental situation. Prostaglandins are released by various chemical and mechanical stimuli with distortion of cell membranes (*5*). Since the lungs perfused were not normal in the control situation, even slight additional changes might induce prostaglandin-release as a secondary and not as a causal event.

After administration of aspirin to cats a significant reduction of the pulmonary hypoxic response was reported. However, aspirin by itself elevated PVR, whereas the PVR level obtained during hypoxic breathing was identical with that in the control situation. When an aspirin-related reduction in the vasoconstrictor response was claimed, it was based on calculations in percent of the new PVR baseline levels, assuming a linear system. No tests with a pulmonary vasoconstrictor agent were carried out. General and nonspecific depression of vascular smooth muscles has earlier been described for rabbit lungs following the administration of aspirin-like drugs (*6*).

For these several reasons we do not feel convinced that the experiments of Said and co-workers are conclusive as regards a frequent prostaglandin release, and its possible role in the mediation of the pulmonary vascular response to hypoxia. In the notes of their report Said and co-workers also state that another inhibitor of prostaglandin biosynthesis, indomethacin, "sometimes even enhanced" the pulmonary arterial pressor response to hypoxia in cats (*1*).

This last observation is in agreement with recent results obtained in our laboratory (*7*). We used an isolated, ventilated rat lung preparation perfused with blood at constant volume inflow (*8*). Pressor responses elicited by repeated episodes of alveolar hypoxia (*9*) followed a characteristic pattern (*8*). In all experiments PVR was within normal limits for rats. In eight experiments addition to the perfusate (100 μg/ml) of either indomethacin, sodium meclofenamate, or aspirin never gave a reduction in the pressor responses to alveolar hypoxia, but sometimes a moderate increase in these responses was observed. This increase was significant ($P < .04$, Wilcoxon two-sided test). The baseline level of the pulmonary arterial pressure was not in-

fluenced by the drugs. The general reactivity of the vascular smooth muscles was unchanged, since intra-arterial injections of kallidin gave identical pressor responses before and after the administration of drugs. That aspirin-like drugs actually enhanced the pulmonary hypoxic response might be due to a secondary release of vasodilatory prostaglandins during pulmonary hypertension induced by alveolar hypoxia. Our results are supported by a short, recent communication from Weir *et al.* (*10*).

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References and Notes

1. S. I. Said, T. Yoshida, S. Kitamura, C. Vreim, *Science* **185**, 1181 (1974).
2. J. R. Vane, *Proceedings of the Vth International Congress of Pharmacology, San Francisco, 1972* (Karger, Basel, 1973), vol. 5, pp. 352–378.
3. Cats weighing between 2.0 and 5.0 kg used in our laboratory have a cardiac output varying between 250 and 500 ml/min with the chest opened.
4. Average increase was 31.6 percent for perfusions with 5 percent dextran in Krebs solution, and 38.7 percent when a mixture of dextran-Krebs and blood was used as a perfusate.
5. S. H. Ferreira and J. R. Vane, *Nature (Lond.)* **216**, 868 (1967); P. J. Piper and J. R. Vane, *ibid.* **223**, 29 (1969); *Ann. N.Y. Acad. Sci.* **180**, 363 (1971); P. J. Piper, *Pol. J. Pharmacol. Pharm.* **26**, 61 (1974).
6. A. Hauge, P. K. M. Lunde, B. A. Waaler, *Acta Physiol. Scand.* **66**, 269 (1966).
7. J. Vaage, L. Bjertnaes, A. Hauge, *ibid.*, in press.
8. A. Hauge, *ibid.* **72**, 33 (1968); *Circ. Res.* **22**, 371 (1968).
9. "Hypoxic tests" consisted of ventilation for 3 minutes with a gas mixture containing 2 percent O_2, 5 percent CO_2, and 93 percent N_2. The interval between each hypoxic episode was 9 minutes.
10. E. K. Weir, J. T. Reeves, R. F. Grover, *Physiologist* **17**, 355 (1974).

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Vaage and Hauge raise a number of good questions dealing with virtually every aspect of the report (*1*). We hope that the additional information and clarification we are here providing will answer these questions.

In the isolated lung experiments, the control pulmonary arterial pressure (PAP) averaged 11.8 mm-Hg, and airway pressure, 4.8 mm-Hg. The lungs were indeed normal to external examination. The low perfusion rate need not imply any hidden abnormalities; such low flow rates are often necessary when the isolated lung is perfused with blood-free solutions, in order to delay the onset of pulmonary edema. We omitted blood from the perfusing fluid to ensure that any active substance appearing in the perfusate could have come only from the lung. There are several examples of the use of similarly low perfusion rates (approximately 2 to 5 percent of predicted normal flow in vivo) by other investigators examining the pulmonary metabolism and release of biologically active substances (*2*).

The pressor response to hypoxia in our isolated lungs was relatively small when compared with the same response in living animals or when isolated lungs are perfused with blood. This finding is in keeping with observations by earlier students of the pulmonary vascular reaction to hypoxia (*3*). Whatever the explanation for the enhancement of the pressor response in the presence of blood, the release of biologically active materials from the lung should assume even greater significance if it could be demonstrated with only moderate rises in pulmonary arterial pressure.

The specificity of the responses of the smooth-muscle organs is questioned. We are fully aware of the limitations, as well as the considerable advantages, of this bioassay technique. One of these limitations is the difficulty in making a positive identification of the active substance or substances eliciting a given set of responses. For this reason, we repeatedly referred to prostaglandins or prostaglandin-like materials (*1*), because the responses were more suggestive of these compounds than of any other known agents. We added, however, that some of the smooth-muscle reactions could not be explained by prostaglandins alone. From more recent observations on newly extracted lung peptides (*4*), to which we alluded in our report (*1*), we are persuaded that even the simultaneous contraction of rat stomach, rat colon, and chick rectum does not necessarily signify the presence of prostaglandins.

Questions about the hypoxic pressor response in vivo and its attenuation with aspirin concern the magnitude and meaning of the aspirin effect. This protective action of aspirin has now been confirmed in a total of 17 anesthetized cats (*5*). Ventilation with 8 percent O_2 raised pulmonary vascular resistance (PVR) from 30.9 ± 3.9 to 50.0 ± 6.8 (an increase of 61.8 ± 9.6 percent) before aspirin, and from 34.1 ± 4.8 to 46.3 ± 7.1 (an increase of 35.8 ± 2.5 percent), after 50 mg of aspirin per kilogram. The corresponding increases in PAP were 13.0 mm-Hg (70.3 ± 6.5 percent) and 8.6 mm-Hg (41.2 ± 7.4 percent), respectively. The reduction in the pressor response to hypoxia was significant at $P < .001$, whereas the slight increase in control PVR after aspirin was nonsignificant ($P > .10$). The protective effect of aspirin was dose-related, being progressively greater at doses of 30, 50, and 100 mg/kg ($P < .05$). A nonspecific depression of pulmonary vascular reactivity by aspirin is unlikely, since $PGF_{2\alpha}$ (1.0 μg/kg), given in six cats, raised PVR by a mean of 97.6 percent before, and 107.1 percent after aspirin.

Indomethacin (up to 100 mg/kg) exerted a different effect, lowering PAP during air breathing (13.9 ± 0.9 versus 16.7 ± 0.9 mm-Hg) and during hypoxia (23.7 ± 0.9 versus 26.9 ± 1.2), but causing an increase in percent rise in PVR (94.9 ± 13.6 versus 57.5 ± 4.8 percent). The difference in action of these two inhibitors of prostaglandin biosynthesis is unexplained, but a similar discrepancy has been noted in their ability to inhibit prostaglandin synthesis by sheep seminal vesicles (*6*). The negative result with aspirin reported by Vaage and Hauge in isolated rat lung is possibly attributable to differences between the humoral mediators that the lungs of these two species are capable of releasing during hypoxia. The identities of these mediators at present are only partially known.

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References

1. S. I. Said, T. Yoshida, S. Kitamura, C. Vreim, *Science* **185**, 1181 (1974).
2. P. Piper and J. Vane, *Ann. N.Y. Acad. Sci.* **180**, 363 (1971); G. N. Gillis and Y. Iwasawa, *J. Appl. Physiol.* **33**, 404 (1972); M. Hamberg and B. Samuelsson, *Biochem. Biophys. Res. Commun.* **61**, 942 (1974).
3. H. N. Duke and E. M. Killick, *J. Physiol. (Lond.)* **117**, 303 (1952); A. Hauge, *Acta Physiol. Scand.* **72**, 33 (1968).
4. S. I. Said, V. Mutt, T. Yoshida, N. Hara, *Clin. Res.* **23**, 351A (1975).
5. S. I. Said, N. Hara, T. Yoshida, *Fed. Proc.* **34**, 438 (1975).
6. A. Raz, H. Stern, R. Kenig-Wakshal, *Isr. J. Med. Sci.* **9**, 556 (1973).

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