Of 23 normal sibling (+/+ or +/*c*) stage 35 hearts explanted into culture, 21 (or 91 percent) beat vigorously within 15 minutes and continued beating throughout the culture period. Of the 21 mutant (*c*/*c*) stage 35 hearts cultured with stage 30 normal anterior endoderm, 17 (or 81 percent) began beating throughout their lengths within 12 hours and by 24 hours in culture were contracting as vigorously as the normal controls. Control mutant hearts in culture did not contract vigorously although within 15 minutes of explanting there were a few slight contractions visible in the conus regions of some hearts. These slight conus contractions were observed in in vivo hearts as well and are presumed not to be the result of the culture conditions. Even after 48 hours in culture the 44 control mutant hearts failed to contract in their ventricular, atrial, or sinal portions and therefore closely resembled in vivo mutant hearts (*3, 4, 10*).

After 48 hours, the cultures were prepared for study in the transmission electron microscope. The tissues were fixed initially in a mixture of glutaraldehyde, formaldehyde, and picric acid buffered with 0.10*M* phosphate to *p*H 7.3, postfixed in 2 percent osmium tetroxide buffered to *p*H 7.2 with 0.10*M* phosphate, dehydrated in ethanol, and embedded in Epon. Thick sections were made for orientation and to locate the heart ventricles. Thin sections of the ventricles, cut with a diamond knife, were mounted on bare copper grids, stained with uranyl acetate and lead citrate, and viewed at 80 kV in an AEI 801 electron microscope.

The ventricles of seven of the cultured normal control hearts were examined in the electron microscope and, as was expected (*3, 5, 7, 9, 19*), were composed of myocytes containing numerous well-organized sarcomeric myofibrils (Fig. 1a). The stage 35 mutant hearts cultured with stage 30 normal anterior endoderm also contained numerous myofibrils of normal morphology (Fig. 1b). In fact, of the 12 corrected mutant hearts examined by electron microscopy, all were identical in appearance to the normal control hearts. In contrast, when electron microscopy was used to analyze 18 of the control mutant heart ventricles (that is, those cultured alone or in epidermal vesicles with or without posterior endoderm or myotomes), no organized sarcomeric myofibrils could be found (Fig. 1c). These cells contained instead amorphous proteinaceous collections, along with a few thick (15 nm) and thin (6 nm) filaments and thus their ultrastructure closely approximated mutant hearts in vivo at corresponding developmental stages (*3, 4*).

Our results (Table 1) demonstrate that normal anterior endoderm (prior to the onset of heart beat) corrects the heart defect in cardiac lethal mutant axolotl embryos and essentially transforms mutant hearts into normal ones. We believe that this is accomplished because normal anterior endoderm provides an essential "final inductive influence" to cause the mutant hearts to form organized sarcomeric myofibrils and begin beating. Since mutant hearts do not beat normally or form myofibrils when cultured alone, it seems improbable that a general inhibitory effect causes the defect, as was once considered a possibility (*3, 10, 20*). In the unlikely event that there are inhibitory influences operating in cardiac mutant embryos, such effects can be overcome by the addition of normal anterior endoderm. The mechanism of the inductive abnormality in mutants and its relation to gene *c* and to the heart mesoderm remains moot at this time and requires further study. Our study suggests that gene *c* results in an absence of heart function because there is an alteration of the normal heart inductive processes from the anterior endoderm and this prevents final differentiation of the heart.

LARRY F. LEMANSKI
DANIEL J. PAULSON
CRAIG S. HILL
Department of Anatomy and
Muscle Biology Laboratories,
University of Wisconsin, Madison 53706

References and Notes

1. R. R. Humphrey, *Anat. Rec.* **162**, 475 (1968).
2. G. M. Schreckenberg and A. G. Jacobson, *Dev. Biol.* **42**, 391 (1975).
3. L. F. Lemanski, *ibid.* **33**, 312 (1973).
4. _____, M. S. Mooseker, L. D. Peachey, M. R. Iyengar, *J. Cell Biol.* **68**, 375 (1976).
5. L. F. Lemanski, *Am. J. Anat.* **136**, 487 (1973).
6. _____, *J. Supramol. Struct.* **5**, 221 (1976).
7. _____, *Am. Zool.* **18**, 327 (1978); *J. Cell Biol.*, in press.
8. _____, X. Joseph, M. R. Iyengar, *J. Cell Biol.* **67**, 239a (1975).
9. L. F. Lemanski and R. A. Fuldner, *ibid.* **75**, 327 (1977).
10. R. R. Humphrey, *Dev. Biol.* **27**, 365 (1972).
11. A. G. Jacobson and J. T. Duncan, *J. Exp. Zool.* **167**, 79 (1968); S. L. Fullilove, *ibid.* **175**, 323 (1970).
12. O. Mangold, *Naturwissenschaften* **43**, 287 (1956); *ibid.* **44**, 289 (1957); H. Amano, *Doshisha Eng. Rec.* **8**, 203 (1958).
13. F. Orts-Llorca, *Arch. Entwicklungsmech. Org.* **154**, 533 (1963); _____ and D. R. Gill, *ibid.* **156**, 368 (1965).
14. O. R. Hommes, *Primary Entodermal Defects. Development of Body Form and Genital Organs of Acardii in Uni-vitelline Twins* (Van Campen, Amsterdam, 1957).
15. L. F. Lemanski, B. S. Marx, C. S. Hill, *Science* **196**, 894 (1977).
16. R. Rugh, *Experimental Embryology* (Burgess, Minneapolis, 1962).
17. A. G. Jacobson, in *Methods in Developmental Biology*, F. H. Wilt and N. K. Wessels, Eds. (Crowell, New York, 1967).
18. V. Hamburger, *A Manual of Experimental Embyrology* (Univ. of Chicago Press, Chicago, 1960).
19. C. S. Hill and L. F. Lemanski, *J. Cell Biol.* **75**, 49a (1977); *J. Exp. Zool.*, in press.
20. R. R. Kulikowski and F. J. Manasek, *J. Exp. Zool.* **201**, 485 (1977).
21. This study was supported by grant HL 22550 from the National Institutes of Health and by a grant-in-aid from the American Heart Association. The work was done during the tenure of an Established Investigatorship from the American Heart Association awarded to L.F.L. C.S.H. is supported by a National Science Foundation Predoctoral Fellowship. We thank Dr. G. Malacinski, Dr. A. J. Brothers, F. Bacher, and L. Lawrence for providing some of the embryos used in this study, and Dr. F. J. Manasek, Dr. R. R. Kulikowski, and Dr. J. T. Justus for stimulating discussions on the cardiac lethal mutation.

14 August 1978; revised 22 January 1979

Intercellular Communication in Pancreatic Islet Monolayer Cultures: A Microfluorometric Study

Abstract. *Single islet cells in monolayer cultures of neonatal rat pancreas were microinjected with fluorescein and scanned topographically by microfluorometry. Fluorescein spread from an injected islet cell directly into neighboring islet cells, and, in the presence of 16.7 millimolar glucose, significantly more islet cells communicated with the injected cell than in glucose-free medium. Islet cells were also microinjected with glycolytic substrates and activators that produced transient changes in cellular levels of reduced pyridine nucleotides—nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate [NAD(P)H]. Changes in NAD(P)H fluorescence were observed in islet cells incubated first for 18 hours in very low glucose concentrations and then in a glucose-free medium and injected with glycolytic substrates and activators; however, little change of fluorescence occurred in adjacent islet cells. In contrast, after adding 16.7 millimolar glucose to the medium, injection of glycolytic substrates and activators produced transient changes in NAD(P)H fluorescence in the injected cell and in neighboring cells.*

The presence of gap junctions (*1*) between insulin-containing B cells (and other endocrine cells) in the pancreatic islets of Langerhans (*2–4*), as well as electrophysiological studies (*5*), suggests that cell-to-cell communication (*6–11*) may be important for secretory functions of islet cells. The objectives of this study were to determine whether intercellular communication can be directly demon-

0036-8075/79/0525-0862$00.50/0

strated between living pancreatic islet cells and, if so, to test the effects of glucose, the principal regulator of insulin secretion, on cell-to-cell communication in islets.

Monolayer cultures of neonatal rat pancreatic islet cells (*12-14*) were prepared in open microchambers designed for microfluorometry and micromanipulation. Single islet cells were injected, by microelectrophoresis, with either a fluorescein tracer (*15-19*) or glycolytic substrates and activators that lead to transient changes in concentrations of pyridine nucleotides—nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate [NAD(P) $\rightleftharpoons$ NAD(P)H], thereby producing transient changes in intensity of blue NAD(P)H fluorescence (*20*).

The fluorescence changes produced by either exogenous (fluorescein) or endogenous [NAD(P)H] fluorochromes were recorded by multichannel microspectrofluorometry (*21-26*). A unilinear array of about 90 to 100 adjacent cell regions (each about 0.5 to 0.8 μm wide by 5 to 15 μm high) in at least two contiguous cells was scanned every 64 to 128 msec. The scans were recorded on magnetic tape and computer-processed. A matrix of numbers (fluorescence counts) was obtained, each row representing a single scan and each column a cell region. Two types of plots were derived: topographic plots showing the level of fluorescence in adjacent cells for selected time scans, and time plots showing the time course of the fluorescence change in selected cell regions. A special dichroic mirror (Optisk Laboratorium, Lingby, Denmark) provided near total reflection of red light toward the eyepiece of the microfluorometer and enabled us to see the cell clusters and microinstrument; it also provided a 50 percent reflection of blue-green-yellow light and enabled us to observe fluorescence changes in the injected cell and neighbors at the same time that these changes were recorded by an optical multichannel detector. Two to three adjacent cells in a cluster of islet cells were viewed by the detector channels through a rectangular slit in the image plane, while the entire cluster of islet cells and surrounding fibroblasts or other islet cell clusters were viewed through the ocular. Thus, although fluorescence changes were recorded quantitatively only from two or three islet cells, the spread of tracer could be followed visually over an entire cluster of islet cells.

The monolayers were cultivated for 5 days in bicarbonate-buffered medium 199 (Gibco) containing 10 percent fetal calf serum and 16.7 m*M* glucose, then transferred to Hepes-buffered medium 199 containing 10 percent dialyzed calf serum and 0 or 16.7 m*M* glucose (*27*). Single islet cells were injected with a fluorescein dye, and the fluorochrome spread throughout the injected cell, including its cytoplasmic projections, and further on into neighboring islet cells in the same cluster.

The kinetics of intercellular transfer of 6-carboxyfluorescein (*26*) from an injected islet cell into an adjacent islet cell are shown in Fig. 1. Intercellular transfer of fluorescein is evidenced by an increase in fluorescence in the injected islet cell, followed, after 0.9 second, by the same increase in its neighbor, and also by the observation that fluorescence decreased in the injected cell during the time interval (0.4 second) that it increased in its neighbor. There was no significant difference between intercellular transit times for islet cells placed in glucose-free medium (0.4 $\pm$ 0.1 second, $N = 9$) or medium containing 16.7 m*M* glucose (0.5 $\pm$ 0.1 second, $N = 17$).

However, during incubation of the cells in 16.7 m*M* glucose, fluorescein spread into significantly more neighboring islet cells (3.7 $\pm$ 0.4, $N = 30$ cells injected) than when the cells were incubated in glucose-free medium (2.2 $\pm$ 0.3, $N = 19$, $P < .001$). Furthermore, in 16.7 m*M* glucose, the communicant islet cells were clustered around the injected cell, whereas in glucose-free medium, fluorescein often spread to more remote islet cells in the cluster via long cytoplasmic projections of the injected cell.

Although fluorescent dyes can help in the evaluation of intercellular transfer kinetics and mapping of communicating territories, the possible metabolic regulation of intercellular communication is better studied by measuring transient changes in levels of endogenous coenzymes [NAD(P) $\rightleftharpoons$ NAD(P)H] in cells injected with glycolytic substrates and ac-

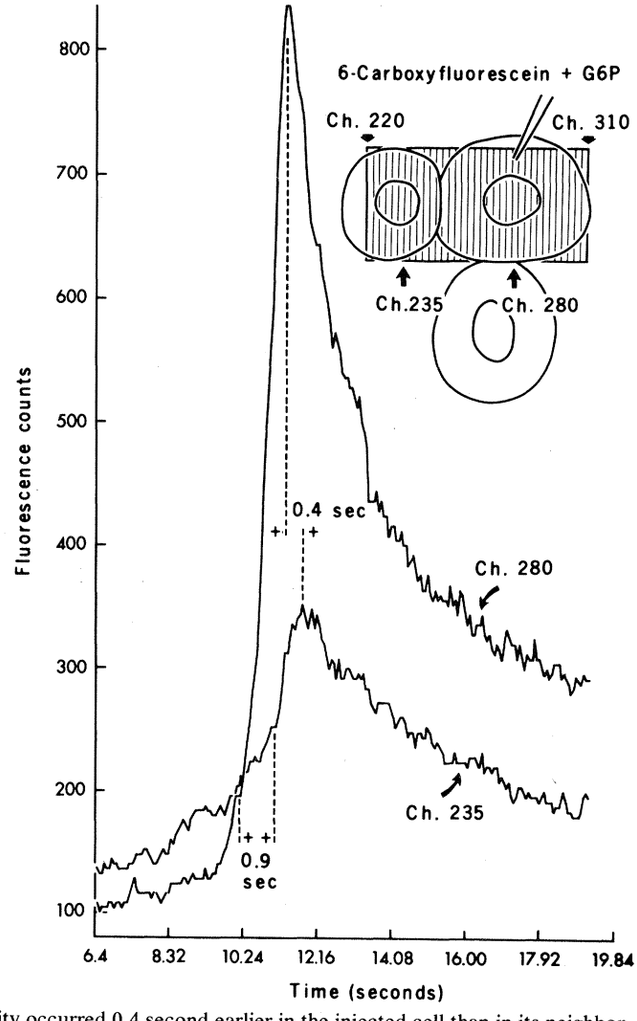
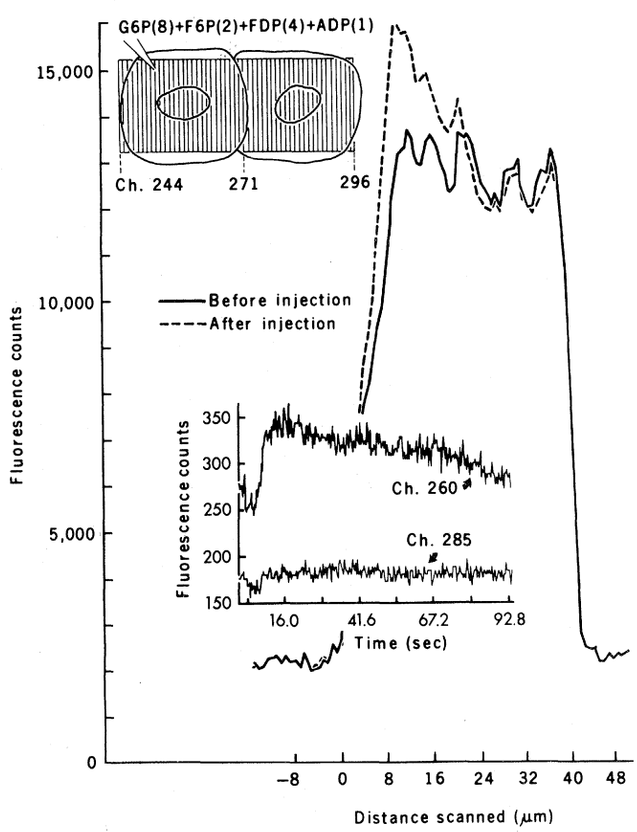


Fig. 1. Time kinetics of intercellular transfer of 6-carboxyfluorescein in monolayer cultures of pancreatic islet cells. The cells were maintained in 16.7 m*M* glucose for 5 days and were then observed microfluorometrically, also in 16.7 m*M* glucose. The portions of two islet cells viewed by the multichannel detector are indicated by an overlying grid (right upper corner). 6-Carboxyfluorescein and glucose 6-phosphate (*G6P*) (to minimize clot formation) were injected by microelectrophoresis into the cell to the right. Fluorescence intensity for two channels, one at the center of the injected cell (channel 280) and the other at the center of its neighbor (channel 235) is plotted as a function of time, each unilinear scan along the multichannel array being completed in 0.064 second. The intercellular transit time (0.9 second) is the interval between the beginning of a change in the slope of fluorescence in the injected cell and that in the neighboring cell. Peak fluorescence intensity occurred 0.4 second earlier in the injected cell than in its neighbor. Similar results were observed by injecting either fluorescein (~ 300 daltons) or fluorescein isothiocyanate glutamine acid (~ 500 daltons). Fluorescence counts were obtained in an optical multichannel analyzer (1205A, Princeton Applied Research Corporation); 1 count is equivalent to two photoelectrons.

tivators. Observations in several cell lines (*28*) reveal that cells maintained in standard tissue culture media (containing about 5 m*M* glucose) are not easily perturbed out of their steady-state NAD(P)H fluorescence by microinjection of glycolytic intermediates. This has been attributed to endogenous stores of glycogen or different metabolic intermediates (*28*). Therefore, controlled depletion of cellular concentrations of these compounds, tailored to each cell line, is required to enhance the ability of injected glycolytic intermediates to trigger NAD(P) $\rightleftharpoons$ NAD(P)H fluorescence transients.

A similar approach was found necessary in monolayer cultures of pancreatic islet cells. Thus, the largest NAD(P) $\rightleftharpoons$ NAD(P)H fluorescence transients were observed in islet cells maintained in very low glucose concentrations (0.05 to 0.25 m*M*) for 18 hours, then transferred to a glucose-free medium and injected with a combination of glycolytic substrates and activators. However, under such conditions, there was no or little change of NAD(P)H fluorescence in neighboring islet cells (Fig. 2). In contrast, injection of glycolytic substrates and activators within the first minute after adding 16.7 m*M* glucose to the medium resulted in a transient increase in NAD(P)H fluorescence in the injected islet cell and in its neighbor. When the injection was made more than 1 minute after adding 16.7 m*M* glucose to the medium, a decrease in NAD(P)H fluorescence was observed in both the injected islet cell and its neighbor (Fig. 3). Observation of NAD(P)H fluorescence changes in the neighbor or neighbors of a cell injected with metabolites has been demonstrated in other cell lines (*26, 28*); such changes are thought to be due to the intercellular transfer of glucose-6-phosphate or other glycolytic intermediates.

According to Hess (*29*), glycolysis is normally in a "bistable" state, and stimulation of the upper glycolytic pathway [NAD(P) reduction] or the lower glycolytic steps [NAD(P)H reoxidation] may predominate, depending on the concentrations of various regulatory intermediates. Therefore, in islet cells presumably depleted of glycogen and glycolytic intermediates by incubation in glucose-deficient media, injection of gly-



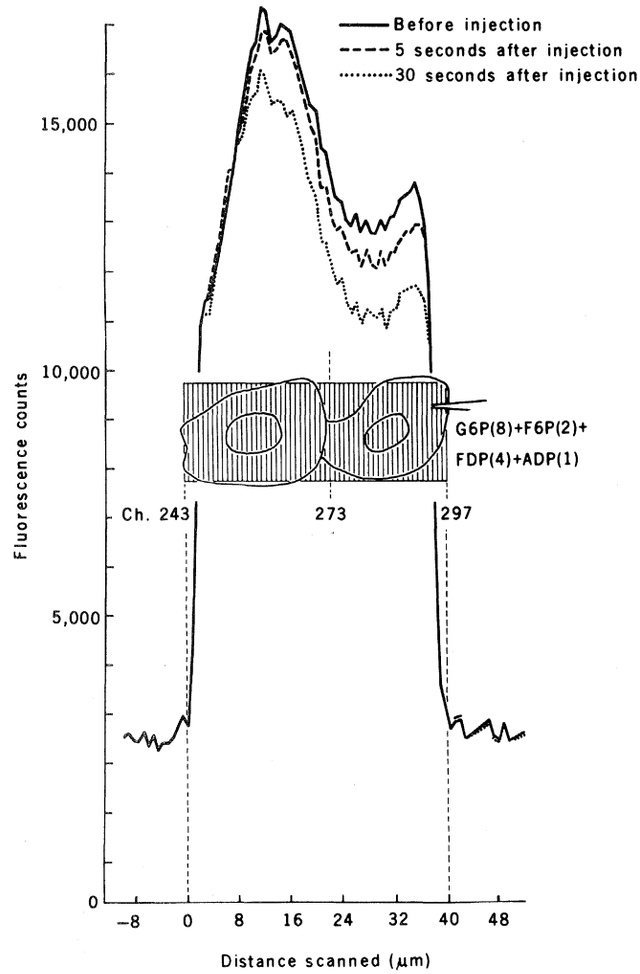


Fig. 2 (left). Monolayer cultures of pancreatic islet cells were maintained in 16.7 m*M* glucose for 4 days, in 0.05 m*M* glucose for 18 hours, and then incubated and studied in a glucose-free medium. The portions of two islet cells viewed by the multichannel detector are indicated by an overlying grid (left upper corner). A solution of glucose 6-phosphate (*G6P*), fructose 6-phosphate (*F6P*), fructose-1,6-diphosphate (*FDP*), and adenosine diphosphate (ADP) in the molar ratios of 8:2:4:1, respectively, was injected by microelectrophoresis into the cell to the left. Fluorescence [NAD(P)H] intensity before and about 6 seconds after injection is plotted as a function of topography, from 0 to 40 μm being the distance scanned from channel 244 to channel 296. After the injection, there is increased fluorescence limited to the left portion of the topographic plot, and this corresponds to the injected cell (channels 244 to 271). The inset in the lower central portion of the figure shows the time course of fluorescence changes at the center of an injected islet cell (channel 260) and the lack of any effect on the fluorescence level at the center of its neighbor (channel 285). Fig. 3 (right). Topographic plots of NAD(P)H fluorescence changes in monolayer cultures of pancreatic islet cells prepared as indicated in Fig. 2, except that microelectrophoretic injections of glycolytic substrates and activators was carried out more than 1 minute after adding 16.7 m*M* glucose to the incubation medium. At 5 seconds after injection, there is a decrease in NAD(P)H fluorescence [reoxidation of NAD(P)H to NAD(P)] mainly in the injected islet cell (channels 273 to 297), and, at 30 seconds, there is a further decrease in fluorescence in the injected cell as well as an appreciable decrease in fluorescence in its neighbor (channels 243 to 273), thereby indicating a transfer process from injected to adjacent cell. It should be noted that these NAD(P)H fluorescence changes occur from different steady-state levels of fluorescence in the injected cell and its neighbor, and that the steady-state fluorescence levels are influenced by cell geometry and thickness.

colytic substrates and activators would favor the upper glycolytic pathway, that is, NAD(P) reduction to NAD(P)H, resulting in an increase in fluorescence (Fig. 2). Upon reintroducing a high glucose concentration (16.7 m*M*) in the incubation medium, intracellular concentrations of glycolytic intermediates may be rapidly replenished. Steady-state concentrations of NAD(P)H, at least, are elevated in islets incubated in high glucose concentrations (*30, 31*). This may preclude any further NAD(P) reduction in response to injection of glycolytic substrates and activators and NAD(P)H reoxidation; that is, a decrease in NAD(P)H fluorescence would be observed (Fig. 3).

We have demonstrated transfer of fluorescein, as well as other signals (possibly glycolytic intermediates), between pancreatic islet cells. Furthermore, these cell-to-cell transfer processes were promoted by glucose. Since glucose is the main physiological regulator of insulin secretion, these results support the hypothesis (*2–4*) that intercellular communication and metabolic cooperation may play a role in islet cell secretory activity. However, intercellular communication was examined only at glucose concentrations (0 and 16.7 m*M*) that are extreme in terms of the threshold concentration of glucose (5 to 6 m*M*) which stimulates insulin secretion (*32*). Therefore, dose-response characteristics of glucose-induced intercellular communication in islets must be determined before more definite conclusions can be drawn regarding the actual contribution of intercellular communication to insulin secretion. Also, as in other dye-coupled cell systems (*33*), it is not known from the present study whether gap junctions between islet cells (*2–4*) are necessarily the sites of transfer of either fluorescein or signals that elicit transient changes in cellular NAD(P)H concentrations. It is, therefore, of interest that a recent ultrastructural study (*34*) has revealed an association between islet B cell secretory activity and the number and size of gap junctions in the plasma membranes of these insulin-containing islet cells.

ELLI KOHEN
CAHIDE KOHEN
Papanicolaou Cancer Research Institute, University of Miami School of Medicine, Miami, Florida 33123
BO THORELL
Department of Pathology, Karolinska Institute, Stockholm, Sweden
DANIEL H. MINTZ
ALEXANDER RABINOVITCH
Department of Medicine, University of Miami School of Medicine

References and Notes

1. L. A. Staehelin, *Int. Rev. Cytol.* **39**, 191 (1974).
2. L. Orci, A. A. Like, M. Amherdt, B. Blondel, Y. Kanazawa, E. B. Marliss, A. E. Lambert, C. B. Wollheim, A. E. Renold, *J. Ultrastruct. Res.* **43**, 270 (1973).
3. L. Orci, R. H. Unger, A. E. Renold, *Experientia* **29**, 1015 (1973).
4. L. Orci, F. Malaisse-Lagae, M. Ravazzola, D. Rouiller, A. E. Renold, A. Perrelet, R. Unger, *J. Clin. Invest.* **56**, 1066 (1975).
5. H. P. Meissner, *J. Physiol. (Paris)* **72**, 757 (1976).
6. J. H. Shubak Sharpe, R. R. Bürk, J. D. Pitts, *J. Cell Sci.* **4**, 353 (1969).
7. T. Friedman, J. E. Seegmiller, J. H. Shubak Sharpe, *Nature (London)* **220**, 272 (1968).
8. J. D. Pitts, in *Ciba Symposium on Growth Control in Cell Cultures*, G. Wolstenholme and J. Knight, Eds. (Churchill & Livingston, London, 1971), p. 89.
9. R. P. Cox, M. R. Krause, M. E. Balis, J. Damcis, in *Cell Communication* (Wiley, New York, 1971), p. 67.
10. Y. Kannon and W. R. Loewenstein, *Nature (London)* **212**, 629 (1966).
11. W. R. Loewenstein and Y. Kanno, *J. Cell Biol.* **22**, 565 (1964).
12. I. Hilwig, S. Schuster, W. Heptner, E. V. Wasielewski, *Z. Zellforsch. Mikrosk. Anat.* **90**, 333 (1968).
13. W. L. Chick, V. Lauris, J. H. Felwelling, K. A. Andrews, J. M. Woodruff, *Endocrinology* **92**, 212 (1973).
14. J. T. Braaten, M. Greider, J. E. McGuigan, D. H. Mintz, *ibid.* **99**, 684 (1976).
15. R. Azarnia and W. R. Loewenstein, *J. Membr. Biol.* **6**, 368 (1971).
16. R. Azarnia, W. Larsen, W. R. Loewenstein, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 880 (1974).
17. R. Azarnia and W. R. Loewenstein, *J. Membr. Biol.* **30**, 175 (1976).
18. I. Simpson, B. Rose, W. R. Loewenstein, *Science* **195**, 294 (1977).
19. B. Rose, I. Simpson, W. R. Loewenstein, *Nature (London)* **267**, 625 (1977).
20. B. Chance, in *Modern Trends in Physiology and Biochemistry*, E. D. Guzman Barron, Ed. (Academic Press, New York, 1952), p. 25.
21. G. H. Pollack, *J. Physiol. (London)* **255**, 275 (1976).
22. E. Kohen, B. Thorell, C. Kohen, J. M. Salmon, *Adv. Biol. Med. Phys.* **15**, 271 (1974).
23. E. Kohen, J. G. Hirschberg, C. Kohen, A. Wouters, H. Pearson, J. M. Salmon, *Biochim. Biophys. Acta* **396**, 149 (1975).
24. E. Kohen, C. Kohen, B. Thorell, *Exp. Cell Res.* **101**, 47 (1976).
25. E. Kohen and C. Kohen, *ibid.* **107**, 261 (1977).
26. ———, in *Miami Winter Symposia*, Vol. 15, *Differentiation and Development*, F. Ahmad, T. R. Russell, J. Schultz, R. Werner, Eds. (Academic Press, New York, 1978).
27. Hepes: 4-(2-hydroxyethyl)-1-piperazineethanesulfonate.
28. B. Thorell, E. Kohen, C. Kohen, *Medical Biology (Helsinki)* **56**, 386 (1978).
29. B. Hess, *Symp. Soc. Exp. Biol.* **27**, 105 (1973).
30. U. Panten, J. Christians, E. V. Kriegstein, W. Poser, A. Hasselblatt, *Diabetologia* **9**, 477 (1973).
31. B. Thorell and B. Hellman, unpublished observations.
32. W. Malaisse, F. Malaisse-Lagae, P. H. Wright, *Endocrinology* **80**, 99 (1967).
33. W. W. Stewart, *Cell* **14**, 741 (1978).
34. P. Meda, A. Perrelet, L. Orci, *Eur. J. Clin. Invest.* (Abstr.), in press.
35. This work was supported by HEW grant 1 R01 AM 21330-01 from the Institute of Arthritis, Metabolism, and Digestive Disease. We thank C. Quigley for preparation of the pancreatic islet monolayer cultures; J. D. Shelley for preparing the programs for processing the tape-recorded microfluorometric data; B. Rose, I. Simpson, and W. R. Loewenstein for useful discussions; G. Newton Flagg, B. Rose, and I. Simpson for provision of fluorescein tracers; J. Blicharska for data evaluation and plotting; and G. Ondricek and B. Reitberg for preparing drawings and prints.

25 September 1978; revised 20 November 1978

Immunoassay by Differential Pulse Polarography

Abstract. *An immunological method based on labeling an antigen with an electroactive group detectable by differential pulse polarography has been demonstrated. Estriol labeled with mercuric acetate is electroactive, giving a reduction wave at −300 millivolts versus a standard calomel electrode. Addition of estriol antibody to 4-mercuric acetate estriol diminishes the peak current as a result of the antigen-antibody binding reaction. Separation of free-labeled estriol from antibody-bound-labeled antigen is unnecessary. The method is potentially useful as an analytical immunological technique.*

The development of immunological methods has had a significant impact on clinical diagnostic tests. A wide variety of conditions such as pregnancy (*1*), drug overdose (*2*), metabolic birth defects (*3*), hormonal disorders (*4*), and diabetes (*5*) can be diagnosed immunologically. The reason for using an antibody-antigen reaction as the basis for an analytical technique is its inherent selectivity. Combining this selectivity with the sensitivity of detecting a radiolabel gives the very powerful technique of radioimmunoassay (RIA). Although radiolabels detected by radioactive counting techniques are used most frequently (*6*), other labels including radicals detected by electron spin resonance (*7*), enzymes detected by subsequent enzymatic reaction (*8*), and fluorescing species (*9*) have been explored.

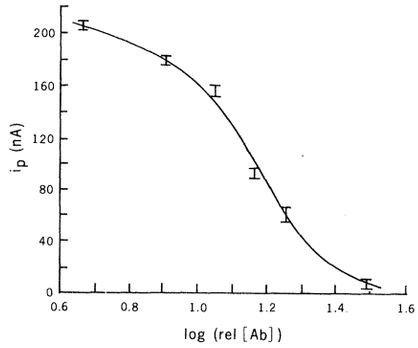


Fig. 1. Labeled antigen-antibody binding curve for 4-mercuric acetate estriol (8.0 μg/ml) with estriol antibody (relative concentration).

We report here an immunological technique based on the use of an electroactive label that can be detected electrochemically by a voltammetric technique such as differential pulse polarography. The method has been demonstrat-

0036-8075/79/0525-0865$00.50/0