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.

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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.
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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.

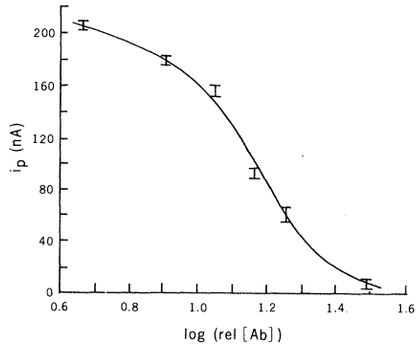

Fig. 1. Labeled antigen-antibody binding curve for 4-mercuric acetate estriol (8.0 μg/ml) with estriol antibody (relative concentration).

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-

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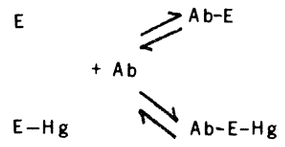
ed with estriol labeled with electroactive mercuric acetate. A small amount of estriol antibody (Ab) is added to a solution containing estriol (E) and a known amount of labeled estriol (E-Hg). The competitive binding reaction between Ab, E, and E-Hg can be expressed generally as



The amount of E-Hg in equilibrium is determined by reduction of E-Hg at a dropping mercury electrode by differential pulse polarography. The magnitude of the polarographic wave for E-Hg reduction is proportional to the concentration of E in a manner analogous to RIA.

4-Mercuric acetate estriol (E-Hg) was synthesized from estriol (Sigma Chemical Co.) by a reported procedure (*10*). A differential pulse polarogram of 4-mercuric acetate estriol in a water-ethanol mixture (90:10) with a 0.15*M* phosphate buffer (*p*H 8.0) as the supporting electrolyte gives a distinct reduction peak at −300 mV versus a standard calomel electrode (SCE). This labeled estriol is distinguishable from unlabeled estriol, which is nonelectroactive over the potential range −200 to −900 mV.

The feasibility of electrochemical immunoassay has been demonstrated by measuring the polarographic current for E-Hg in the presence of increasing amounts of specific Ab. Figure 1 shows the labeled antigen-antibody binding curve obtained when the peak current (i_p) for the reduction wave of 4-mercuric acetate estriol at −300 mV versus SCE is plotted as a function of increasing Ab concentration. The Ab concentration was varied by adding successive 100-μl portions of estriol antiserum (NEA-059A, New England Nuclear). As shown in Fig. 1, the peak current decreases as E-Hg binds to the increasing amount of Ab present in solution. The curve shape is analogous to that obtained by RIA (*11*). In a separate experiment, the addition of unlabeled estriol to a solution of Ab-E-Hg caused an increase in peak current due to competitive displacement of E-Hg by E from the antibody-antigen complex. Thus, binding of E-Hg with Ab is reversible. In addition, no change in peak current was seen when portions of nonspecific Ab were added sequentially in the absence of specific Ab.

Separation of free estriol from estriol bound to the antibody was unnecessary (*12*). The reduction wave for Ab-E-Hg occurred at a more negative potential than the wave for E-Hg. Consequently, polarograms could be performed directly on the mixture. This is advantageous, since most labels require separation of free-labeled antigen from bound-labeled antigen.

The low detection limits of modern electroanalytical techniques (*13*) such as differential pulse polarography ($\sim 10^{-8}M$) and differential pulse anodic stripping voltammetry ($\sim 10^{-10}M$) make electroactive labels potentially useful for immunoassay. Relatively inexpensive and reliable instrumentation for such techniques is commercially available.

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References and Notes

1. J. G. Grudzinskas, D. Jeffrey, Y. B. Gordon, T. Chard, *Lancet* **1977-I**, 333 (1977).
2. J. Landon and A. C. Moffat, *Analyst (London)* **101**, 225 (1976); A. Castro and H. Malkus, *Res. Commun. Chem. Pathol. Pharmacol.* **16**, 291 (1977).
3. R. H. Williams, in *Textbook of Endocrinology* (Saunders, Philadelphia, 1968), p. 1208; G. B. Pastorfide, D. P. Goldstein, T. S. Kosasa, *Am. J. Obstet. Gynecol.* **120**, 1025 (1974); T. Saito and B. B. Saxena, *J. Lab. Clin. Med.* **85**, 497 (1975).
4. I. A. Hughes and J. S. D. Winter, *J. Pediatr.* **88**, 766 (1976); B. M. Jaffe and H. R. Behrman, Eds. *Methods of Hormone Radioimmunoassay* (Academic Press, New York, 1974).
5. S. P. Bessman, P. M. Beigelman, L. J. Thomas, Jr., *Biochem. Med.* **7**, 97 (1973); C. N. Hales and P. J. Randle, *Biochem. J.* **88**, 137 (1963).
6. W. H. C. Walker, *Clin. Chem.* **23**, 384 (1977); D. S. Skelley, L. P. Brown, P. K. Besch, *ibid.* **19**, 146 (1973); R. S. Yalow, *Prog. Anal. Chem.* **7**, 1 (1974).
7. R. K. Leute, E. F. Ullman, A. Goldstein, L. A. Herzenberg, *Nature (London) New Biol.* **236**, 93 (1972).
8. E. Engvall and P. Perlmann, *Immunochemistry* **8**, 871 (1971).
9. C. M. O'Donnell and S. C. Suffin, *Anal. Chem.* **51**, 33A (1979).
10. C.-C. Chin and J. C. Warren, *J. Biol. Chem.* **243**, 5056 (1968); J. C. Warren, F. Arias, F. Sweet, *Methods Enzymol.* **36**, 374 (1975).
11. G. E. Abraham and F. S. Manlimos, in *Handbook of Radioimmunoassay*, G. E. Abraham, Ed. (Dekker, New York, 1977), p. 610.
12. C. W. Anderson, H. B. Halsall, W. R. Heineman, in preparation.
13. J. B. Flato, *Anal. Chem.* **44** (No. 11), 75A (1972); T. R. Copeland and R. K. Skogerboe, *ibid.* **46**, 1257A (1974).
14. Supported by the National Science Foundation and a National Institutes of Health Biomedical Research Fellowship.
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β_1- and β_2-Adrenergic Receptors in Rat Cerebral Cortex Are Independently Regulated

Abstract. *Repeated administration of the tricyclic antidepressant desmethylimipramine to adult rats for 10 days caused a 40 percent decrease in the density of β_1-adrenergic receptors in the cerebral cortex but had no effect on the density of β_2-adrenergic receptors. Conversely, destruction of noradrenergic neurons by administration of 6-hydroxydopamine to neonatal rats caused a 64 percent increase in the density of β_1-adrenergic receptors in adult cerebral cortex with no change in the density of β_2-adrenergic receptors. These results suggest that the β-adrenergic receptors in rat cortex involved in neuronal function are primarily of the β_1 subtype.*

The density of β-adrenergic receptors in rat cerebral cortex is controlled by the noradrenergic input to these receptors (*1*). A reduction in this input by destruction of adrenergic neurons with 6-hydroxydopamine (6-OHDA) or by receptor blockade during treatment with the β-adrenergic antagonist propranolol leads to an increase in the density of receptors (*1*). Conversely, pharmacologically induced increases in the amount of norepinephrine having access to the receptor by repeated treatment with the inhibitor of norepinephrine uptake desmethylimipramine (DMI) or the monoamine oxidase inhibitor pargyline decreases the density of β-adrenergic receptors in this brain area (*1*). These changes in receptor

Table 1. Affinity of IHYP and zinterol for β_1- and β_2-adrenergic receptors after repeated drug treatment. The K_d values for IHYP were determined by Scatchard analysis of IHYP binding. The K_d values for zinterol were calculated by a computerized analysis of biphasic Hofstee plots, as previously described (*3*). Animals were treated as described in Fig. 1. Each value is the mean ± standard error for ten animals (neonatal 6-OHDA) or 15 animals (DMI).

Treatment	K_d for IHYP ($\times 10^{-10}M$)	K_d for zinterol (μM)	
		β_1	β_2
Control	0.84 ± 0.054	2.2 ± 0.24	0.042 ± 0.0066
Desmethylimipramine	0.90 ± 0.056	2.06 ± 0.24	0.033 ± 0.0041
Control	0.72 ± 0.034	1.52 ± 0.09	0.023 ± 0.004
Neonatal 6-hydroxydopamine	0.86 ± 0.027	1.71 ± 0.16	0.040 ± 0.008

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