In the skin, nuclei of cells of the epidermis (Fig. 2C) were labeled, most strongly in the basal layers (Fig. 2, C and D), in the external root sheath of the hair (Fig. 2D), and in the basal layer of the sebaceous glands.

In the pituitary, radioactivity was observed in nuclei of certain cells (Fig. 2E) in the pars distalis (*11*). In addition, pituicytes in the infundibular process were weakly labeled as in animals injected with $^3$H-labeled estradiol (*6*). In the parathyroid most of the nuclei of parenchymal cells were labeled (Fig. 2F). This provides evidence of a direct action by $1,25(OH)_2D_3$ on the parathyroid hormone system as hypothesized previously (*1*).

The localization of target cells in the pituitary, parathyroid, and stomach indicates that calcium homeostasis is regulated by central as well as peripheral endocrine mechanisms. Although nothing is known about the involvement of the pituitary, the cells in the stomach may be hormone (probably gastrin) producing cells, because gastrin has been shown to modulate the concentration of calcitonin in the blood and vice versa (*12*). The occurrence of radioactivity in presumed endocrine cells of the stomach suggests that $1,25(OH)_2D_3$ is a modulator in the interaction between gastrin and calcitonin.

Our results demonstrate the potential of the histological approach. Not only were we able to confirm and cytologically define biochemically characterized target tissues, but we were also able to add several new cell types to those already described. With the discovery of previously unidentifed target cells of $1,25(OH)_2D_3$ or metabolites of the compound, modifications must be made in the present models for calcium homeostatic mechanisms. Although we do not know what effects the hormone has on podocytes, cells of the macula densa, cells of the pars distalis, pituicytes, and endocrine cells of the stomach, extensive studies of other tissues, such as bone and thyroid, as well as of the cell types identified herein, should help to answer this question.

WALTER E. STUMPF
MADHABANANDA SAR
FREDERIC A. REID
*Departments of Anatomy* and
*Pharmacology, University of North Carolina, Chapel Hill 27514*
YOKO TANAKA
HECTOR F. DELUCA
*Department of Biochemistry, University of Wisconsin, Madison 53706*

### References and Notes

1. C. A. Frolik and H. F. DeLuca, *J. Clin. Invest.* **52**, 543 (1973); H. F. DeLuca and H. K. Schnoes, *Annu. Rev. Biochem.* **45**, 631 (1976).
2. M. Zile, E. C. Gunge, L. Barsness, S. Yamada, H. Schnoes, H. F. DeLuca, *Arch. Biochem. Biophys.* **186**, 15 (1978); P. G. Jones and M. R. Haussler, *Endocrinology* **104**, 313 (1979).
3. F. H. Wezeman, *Science* **194**, 1069 (1976). We note that these results were obtained after rats (75 to 100 g) were injected with 3 $\mu$Ci of $^3$H-labeled 25-hydroxyvitamin $D_3$ with a low specific activity (1.1 Ci/mmole), and that the animals were killed after 48 hours. The tissues were decalcified and embedded in paraffin and the autoradiograms were exposed for only 14 days. No controls were mentioned.
4. W. E. Stumpf and L. J. Roth, *J. Histochem. Cytochem.* **14**, 274 (1966); W. E. Stumpf, in *Methods in Cell Biology*, D. M. Prescott, Ed. (Academic Press, New York, 1976), vol. 13, pp. 171-193; W. E. Stumpf and M. Sar, *Methods Enzymol.* **36**, 135 (1975).
5. W. E. Stumpf and L. D. Grant, *Anatomical Neuroendocrinology* (Karger, Basel, 1975); W. E. Stumpf, *Am. J. Anat.* **129**, 207 (1970); ——— and M. Sar, *Am. Zool.* **18**, 435 (1978); M. Sar and W. E. Stumpf, *Science* **197**, 77 (1977); W. E. Stumpf and M. Sar, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 1973 (1977).
6. W. E. Stumpf and M. Sar, in *Receptors and Mechanism of Action of Steroid Hormones*, J. Pasqualini, Ed. (Dekker, New York, 1976), pp. 41-84; M. Sar and W. E. Stumpf, *Endocrinology* **94**, 1116 (1974).
7. T. Suda, H. F. DeLuca, Y. Tanaka, *J. Nutr.* **100**, 1049 (1970).
8. This pregnant rat was from another study of $1,25(OH)_2D_3$ target sites in the fetus.
9. R. H. Wasserman and R. A. Corradino, *Vitam. Horm.* **31**, 43 (1973).
10. K. Wake, *Am. J. Anat.* **132**, 429 (1971); *J. Cell Biol.* **63**, 683 (1974); K. Hirosawa and E. Yamada, *ibid.* **70**, 269 (1976).
11. These cells are identified as thyrotropes (M. Sar, W. E. Stumpf, H. F. DeLuca, in preparation) with a combined autoradiography-immunohistochemistry technique (M. Sar and W. E. Stumpf, *Cell Tiss. Res.*, in press) that was developed in our laboratory and enables us to determine the distribution of steroid and peptide hormones simultaneously in the same preparation.
12. C. W. Cooper, R. M. Bolman III, W. M. Linehan, S. A. Wells, Jr., *Rec. Prog. Horm. Res.* **34**, 259 (1978).
13. Supported by grants NS09914 and AM14881 from the Public Health Service and by contract EY-76-S-02-1668 with the Department of Energy. We thank Dr. R. Narbaitz for advice.

11 July 1979; revised 27 August 1979

## A Glucose-Controlled Insulin-Delivery System: Semisynthetic Insulin Bound to Lectin

Abstract. *A stable, biologically active glycosylated insulin derivative that is complementary to the major combining site of concanavalin A has been synthesized. Hormone release is proportional to the quantity of glucose present. Glucose regulation of exogenous insulin delivery could have important applications in the therapy of diabetes mellitus.*

Although injectable insulin has been available for the treatment of diabetes mellitus for more than 50 years, the simple replacement of the hormone is not sufficient to prevent the pathological sequelae associated with this disease. The development of these sequelae is believed to reflect an inability to provide exogenous insulin proportional to the patients' varying concentrations of blood glucose (*1*). In fact, recent surveys in which hemoglobin $A_{1c}$ measurements were used have revealed that a large percentage of diabetic outpatients have poor diabetic control (*2*). In recent years, several biological (*3, 4*) and bioengineering (*5*) approaches have been suggested for the development of a more physiological insulin delivery system and these are in various stages of exploration. In this report we propose a chemical approach to the problem. The principle is to synthesize insulin derivatives with attached oligosaccharides, which are complementary to the binding site of lectins. The release of bound insulin derivative would be a function of the derivative's binding constant to the lectins and of the glucose concentration.

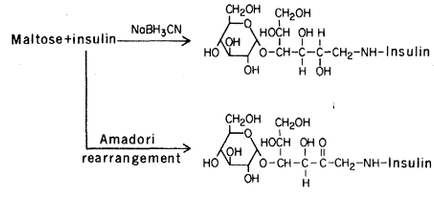


Fig. 1. Formation of insulin derivatives having terminal $\alpha$-D-glucose residues. The Schiff base adduct between maltose and insulin undergoes an Amadori rearrangement to form the 1-deoxyfructosyl-$\alpha$-1,4-glucopyranoside. In the presence of sodium cyanoborohydride, the imminium moiety is reduced to the corresponding glycitol.

Insulin derivatives complementary to the major combining site of the lectin concanavalin A were synthesized by using modifications of previously described methods for coupling aldehydes to primary amines (*6, 7*) (Fig. 1). Oligosaccharides, selected according to their differential affinities for binding to concanavalin A (*8*), were incubated with porcine insulin at molar ratios ranging from 12:1 to 650:1 for periods of 1 to 14 days at 37°C in 0.1*M* sodium phosphate buffer, *p*H 8.0. Incubations were carried out in the presence and in the absence of 0.25*M* sodium cyanoborohydride. Unreacted oligosaccharides were removed by gel filtration on Bio-Gel P-6, and unreacted insulin was separated by affinity chromatography on concanavalin A


0036-8075/79/1207-1190$00.50/0 Copyright © 1979 AAAS

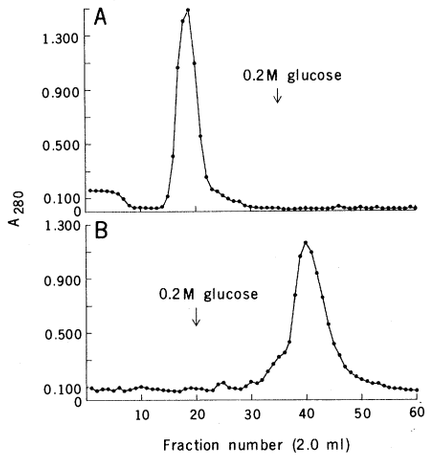


Fig. 2. Elution profiles obtained from 20 mg of unmodified insulin (A) and maltose-insulin derivative (B) applied to columns (inside diameter, 24.0 by 1.5 cm) of immobilized concanavalin A. Initial buffer was 1 m*M* $MgCl_2$, 1 m*M* $CaCl_2$, 50 m*M* NaCl, and 25 m*M* sodium phosphate, *p*H 7.4. At the point indicated by the arrow, 0.2*M* glucose in column buffer was added. Fractions (2.0 ml) were collected at a flow rate of 15 to 20 ml/hour.

coupled to Sepharose 4B. The semisynthetic insulin derivatives were stored at 4°C. Further details of the isolation procedure and the characterization of the derivatives will be described elsewhere (*9*).

Carbohydrate content was determined by the phenol-sulfuric acid method (*10*), and protein was determined by the method of Lowry (*11*). Bioactivity of the semisynthetic insulins was assessed in rats by use of the blood-glucose depression assay modified from one described earlier (*12*). Lectin-binding was evaluated by conventional affinity chromatography methods (*13*) in which concanavalin A coupled to Sepharose 4B was used.

In this report the results of the unreduced maltose-insulin derivative are given. The amount of carbohydrate that reacted with insulin increased linearly with time (*9*). After 5 days of incubation, the maltose-insulin derivative contained 1.76 moles of covalently bound carbohydrate per mole of insulin monomer. Since substitution of all three primary amino groups has been reported to result in approximately 90 percent loss of hormone activity (*14*), this incubation time was selected to maximize yield while minimizing loss of biological activity. Typical bioactivity data for this maltose-insulin derivative are presented in Table 1. Various preparations ranged from 78 to 95 percent of unmodified insulin bioactivity. Insulin derivatives were stable at 4°C for at least 7 weeks, regardless of whether reducing agent had been added (*9*).

Unmodified insulin did not bind to immobilized concanavalin A (Fig. 2A) and was completely recovered in the buffer wash; none was eluted by the addition of glucose. In contrast, all of the maltose-insulin derivative was bound to concanavalin A (Fig. 2B). None was eluted by the buffer wash, and all of this derivative could be displaced from concanavalin A binding sites by a 1000:1 molar excess of glucose. Glucose-pulse experiments with the lectin-bound maltose-insulin derivative demonstrated that hormone release is a function of the quantity of glucose present (Fig. 3).

These data demonstrate the feasibility of synthesizing stable, biologically active semisynthetic insulin derivatives that are complementary to the major combining site of lectins. Since the quantity of glucose present determines the amount of bound hormone that is liberated from the lectin, these derivatives could have important applications in the therapy of diabetes mellitus. Glucose regulation of exogenous insulin delivery might be accomplished by using soluble lectin-glycoinsulin complexes inside hollow-fiber devices of appropriate porosity. Since concanavalin A binding affinity for various saccharides varies more than tenfold (*8*), a mixture of different high- and low-affinity semisynthetic insulins could be combined to produce the most clinically desirable elution profile. Further investigation is needed to explore these possibilities.

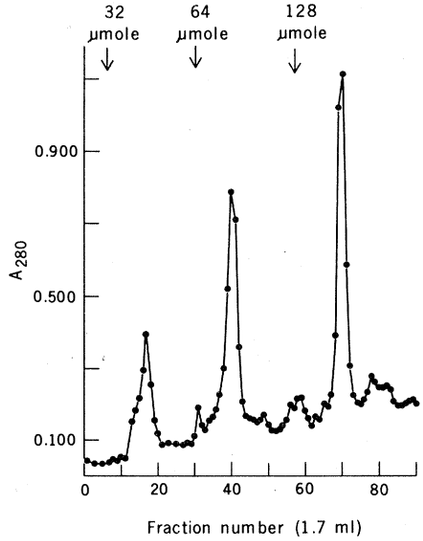


Fig. 3. Elution profile obtained from 20 mg of maltose-insulin derivative applied to a column (inside diameter, 12.0 by 1.5 cm) of immobilized concanavalin A. Initial buffer was 1 m*M* $MgCl_2$, 1 m*M* $CaCl_2$, 50 m*M* NaCl, and 25 m*M* sodium phosphate, *p*H 7.4. Displacement of hormone was accomplished by using 1.0 ml pulses of column buffer containing varying amounts of glucose. Glucose content is indicated over the appropriate arrows. Fractions (1.7 ml) were collected at a flow rate of 10 to 15 ml/hour.

Table 1. Bioactivity of maltose-insulin derivative. After an overnight fast, each animal received 0.1 mg of protein per 100 g of body weight by subcutaneous injection. Each of the animals served as its own control, receiving the maltose-insulin derivative on day 1, and control insulin on day 2. Blood glucose depression was determined 60 minutes after injection using a Beckman Glucose Analyzer 2. S.E.M., standard error of the mean.

| Animal | Blood glucose depression | | |
|---|---|---|---|
| | Maltose-insulin derivative (mg/100 ml) | Control insulin (mg/100 ml) | *P* |
| A | 61 | 75 | |
| B | 64 | 70 | |
| C | 66 | 79 | |
| D | 63 | 79 | |
| E | 69 | 64 | |
| F | 74 | 73 | |
| Mean ± S.E.M. | 66.17 ± 1.92 | 73.33 ± 2.35 | <.1 |

MICHAEL BROWNLEE*
ANTHONY CERAMI
*Laboratory of Medical Biochemistry, Rockefeller University, New York 10021*

### References and Notes

1. M. Brownlee and G. F. Cahill, Jr., *Atheroscler. Rev.* **4**, 29 (1979).
2. B. Gonen, H. Rochman, A. H. Rubenstein, *Metabolism* **28** (Suppl. 1), 448 (1979).
3. A. J. Matas, D. E. R. Sutherland, J. S. Najarian, *Diabetes* **25**, 785 (1976).
4. W. L. Chick, A. A. Like, V. Lauris, *Science* **187**, 847 (1975).
5. J. S. Soeldner, K. W. Chang, S. Aisenberg, J. M. Hiebert, R. H. Egdahl, in *Diabetes Mellitus*, S. S. Fajans, Ed. (DHEW Publ. 76-854, National Institutes of Health, Bethesda, Md., 1976), pp. 267–277; C. K. Colton, J. Giner, H. Lerner, L. Marincic, J. S. Soeldner, in *Transplantation and Clinical Immunology*, J. L. Touraine, Ed. (Excerpta Medica, Amsterdam, 1978), pp. 165–173.
6. R. F. Borch, M. D. Bernstein, H. D. Durst, *J. Am. Chem. Soc.* **93**, 2897 (1971); G. R. Gray, *Arch. Biochem. Biophys.* **163**, 426 (1974).
7. V. J. Stevens, H. Vlassara, A. Abati, A. Cerami, *J. Biol. Chem.* **252**, 2998 (1977).
8. I. J. Goldstein and C. E. Hayes, *Adv. Carbohydr. Chem. Biochem.* **35**, 128 (1978).
9. M. Brownlee and A. Cerami, in preparation.
10. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, F. Smith, *Anal. Chem.* **28**, 350 (1956).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
12. *The United States Pharmacopeia* (Mack, Easton, Pa., 1967), vol. 18, pp. 883–884.
13. C. R. Lowe and P. D. G. Dean, *Affinity Chromatography* (Wiley, London, 1974), pp. 12–90.
14. D. Brandenburg, H. G. Gattner, M. Weinert, L. Herbertz, H. Zahn, A. Wollmer, in *Diabetes: Proceedings of the 9th Congress of the International Diabetes Federation*, J. J. Bajaj, Ed. (Excerpta Medica, New York, 1977), pp. 363–376; M. J. Ellis, S. C. Darby, R. H. Jones, P. H. Sonksen, *Diabetologia* **15**, 403 (1978).
15. Supported in part by a PHS special emphasis research career award (1-K01-AM00589-01 SRC) from the National Heart, Lung, and Blood Institute and the National Institute of Arthritis, Metabolism, and Digestive Diseases.
* Reprint requests should be addressed to M.B.

22 August 1979