which could be expected to result from the temperature rise at the Antarctic Convergence.

In core E45-74 (Fig. 2d) the IRD accumulation rate and the water temperature are positively correlated in most of the lower 500 cm. This segment of the core is therefore consistent with a location south of the GDCR, the approximate geographic limits of which are added to Fig. 2a. Similar strong positive correlations (between percentage of glacial quartz and inferred water temperature) have been documented by Margolis and Kennett (*4*) for two Pleistocene cores just north of the present Antarctic Convergence in the south-central Pacific. These occurrences, and their contrast with the data for E50-12 and E49-30, must be accepted as strong support for the major concepts involved in the model. The upper 300 cm of core E45-74 (Fig. 2d) exhibits a strong inverse relation between IRD and temperature, consistent with the core location being north of the GDCR. This requires the GDCR to have migrated southward at the time involved which, if constant sedimentation rates are assumed, is estimated paleomagnetically as about 0.33 million years ago.

In summary, we believe that our model (Fig. 1) may provide a means to more fully utilize the IRD signal recorded in all subpolar sediments, although we realize that it may be subject to modification (*19*). The limited tests we have applied and the earlier published data merely support the principles employed. Simple correlations between IRD maxima in deep-sea sediments (*11*) may not be valid unless the cores involved are all located on one side of the GDCR. Previously noted incoherence in correlations between cores (*6*) may be at least partially explained by the latitudinal range of the cores involved. It will be unrewarding to attempt to make paleoclimatic inferences from IRD abundance variations in cores spanning large latitude ranges. On the other hand, detailed and diverse analyses of closely spaced south-north traverses may yield definitive data on the timing, rate of change, extent, and intensity of the glacial and interglacial periods in the Southern Hemisphere (*20*).

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References and Notes

1. J. Murray and A. F. Renard, *Proc. R. Soc. Edinb.* **12**, 474 (1884); E. Phillipi, *Geol. Geogr.* **2**, 415 (1910).
2. G. H. Denton, R. L. Armstrong, M. Stuiver, in *The Late Cenozoic Glacial Ages*, K. Turekian, Ed. (Yale Univ. Press, New Haven, Conn., 1971), p. 267.
3. K. R. Geitzenauer, S. V. Margolis, D. S. Edwards, *Earth Planet. Sci. Lett.* **4**, 173 (1968).
4. S. V. Margolis and J. P. Kennett, *Am. J. Sci.* **271**, 1 (1971).
5. J. R. Connolly and M. Ewing, *Science* **150**, 1822 (1965); *Nature (Lond.)* **208**, 135 (1965); *Geol. Soc. Am. Mem.* **126**, 219 (1970); N. D. Opdyke, B. Glass, J. D. Hays, J. Foster, *Science* **154**, 349 (1966); J. P. Kennett and C. A. Brunner, *Geol. Soc. Am. Bull.* **84**, 2043 (1973); Goodell *et al.* (*6*); Hays and Opdyke (*7*). It is now becoming clear that the terms "glacial" and "interglacial" may not be satisfactory, particularly when applied to Antarctica, since it is virtually certain that the continent has been almost entirely covered with ice for most of the upper Tertiary.
6. H. G. Goodell, N. D. Watkins, T. T. Mather, S. Koster, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **5**, 41 (1968).
7. J. D. Hays and N. D. Opdyke, *Science* **158**, 1001 (1967).
8. R. H. Fillon, *Nat. Phys. Sci.* **238**, 40 (1972).
9. J. B. Anderson, *ibid.* **240**, 189 (1972).
10. D. A. Warnke, *Am. J. Sci.* **269**, 276 (1970).
11. D. Kent, N, D. Opdyke, M. Ewing, *Geol. Soc. Am. Bull.* **82**, 2741 (1971); R. Von Huene, J. Crouch, E. Larson, *Geol. Soc. Am. Mem.*, in press. The latter authors specifically state that between-core correlation of IRD horizons is a critical means of evaluating data quality.
12. D. H. Krinsley and J. Donahue, *Geol. Soc. Am. Bull.* **79**, 743 (1968); D. H. Krinsley and S. V. Margolis, *Trans. N.Y. Acad. Sci.* **31**, 457 (1969); W. B. Whalley and D. H. Krinsley, *Sedimentology* **21**, 87 (1974).
13. J. Keany, *Nature (Lond.)* **246**, 139 (1970).
14. J. P. Kennett, *Deep-Sea Res.* **17**, 125 (1970); J. Keany and J. P. Kennett, *ibid.* **19**, 529 (1972).
15. M. G. Petrushevskaya, in *Biological Reports of the Soviet Antarctic Expedition, 1955–1958*, A. P. Andriyaskev and P. V. Ushakov, Eds. (Program for Scientific Translations, Jerusalem, Israel, 1968), vol. 3, p. 2.
16. A. McIntyre and R. Jantzen, *Resumes Commun. 7th Int. Quaternary Assoc. Congr.* **11**, 68 (1969).
17. W. F. Ruddiman, *Geol. Soc. Am. Bull.* **82**, 283 (1971).
18. S. V. Margolis, in *Initial Reports of the Deep-Sea Drilling Project, Leg 29* (Government Printing Office, Washington, D.C., in press).
19. We subscribe to the philosophy that there are three natural stages in the compilation of models: an initial simple stage, to provide the essential working model; a second stage involving the emergence of data which frequently clash with the initial model requirements, thus forcing changes in the model; and a final stage where data and model blend in a more complex fashion, or where the model collapses. In this report, we describe our first steps in the second long stage.
20. See the models illustrated as figure 8 of Denton *et al.* (*2*, p. 293).
21. A. L. Gordon, in *Studies in Physical Oceanography—A Tribute to G. Wüst on His 80th Birthday*, A. L. Gordon, Ed. (Gordon & Breach, New York, in press).
22. A. Cox, *Science* **163**, 237 (1969). For the methods used, see N. D. Watkins and J. P. Kennett, *Antarct. Res. Ser.* **19**, 273 (1972).
23. J. D. Hays, in *Progress in Oceanography*, M. Sears, Ed. (Pergamon, Oxford, 1967), p. 117.
24. Supported by NSF grant GV25400.

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Anomeric Specificity of Glucose-Stimulated Insulin Release: Evidence for a Glucoreceptor?

Abstract. *The effects on insulin secretion of α and β anomers of* D-*glucose were studied in the in vitro perfused rat pancreas. Both phases of insulin release showed consistent stereospecificity for α-glucose; this specificity indicates an action of glucose independent of intracellular glucose metabolism.*

D-Glucose in solution or in the circulation is heterogeneous, consisting of approximately 64 and 36 percent of the β and α anomers, respectively (*1*). Nevertheless, the relative effectiveness of these naturally occurring anomers of D-glucose on secretion of insulin is unknown. The present studies, in which the perfused rat pancreas was used, show that regulation of insulin release has a stereospecificity favoring α-glucose. Since intracellular glucose metabolism may not be affected by glucose anomers (*2*), the observed stereospecificity suggests a glucose effect on insulin secretion localized at the cell membrane.

Specific details for perfusion of the rat pancreas in vitro and the immunoassay for rat insulin have been previously described (*3*, *4*). In short, the pancreas, with adjacent stomach, spleen, and part of the duodenum, was removed from fasted rats. The preparation was placed in the perfusion apparatus, and perfusion medium consisting of 1 percent albumin–3 percent T-40 dextran in bicarbonate buffer (*p*H 7.4) was introduced into the celiac artery. Complete effluent was collected from the portal vein every minute after a single passage through the pancreas; flow rates were 10 ml/min. For the 5-minute pulse experiments, α, D(+)-glucose (anhydrous, Mallinckrodt) and β, D(+)-glucose (Sigma) were rapidly dissolved and introduced into the pancreatic preparation by way of a side-arm syringe. For longer perfusion experiments, the freshly prepared anomers were introduced from side-arm syringes kept at 4°C in an ice bath (*5*). The concentration of the α or β anomers in the the effluent was determined within 1 minute with the Beckman glucose analyzer. This system employs fungal

glucose oxidase which is almost completely specific for the β anomer, thus permitting direct estimation of β-glucose. (Actual readings, standardized for equilibrated glucose, were multiplied by 0.64.) The samples were then equilibrated for 2 hours at 25°C followed by 20 hours at 4°C and reassayed to give total glucose. α-Glucose was determined by the difference.

The comparative effects of the anomers on insulin secretion were studied at two glucose concentrations, the first of which (110 mg/100 ml) has previously been shown (*6*) to be slightly above the threshold level for glucose stimulation of insulin release in this system (90 to 100 mg/100 ml). At this concentration there is little second phase secretion of insulin or potentiation by glucose of subsequent stimulations. It was therefore possible to compare the different anomers as a series of stimulations in the same pancreas. Figure 1 shows that β-glucose had only a marginal effect on insulin secretion, while the α anomer was four times more effective than the same concentration of equilibrated α,β-glucose. When the series of stimulations was repeated in the same pancreas, identical responses occurred. Thus, the

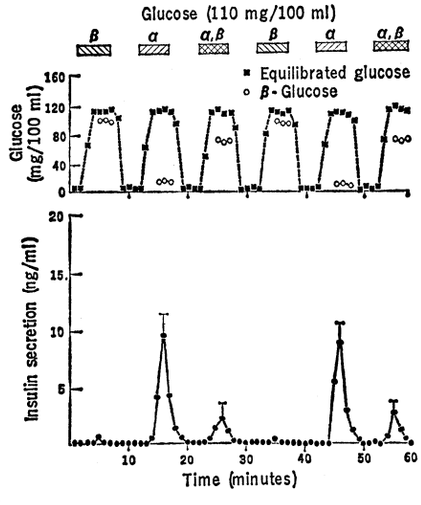

Fig. 1. Effect of glucose anomers (110 mg/100 ml for 5 minutes) on insulin secretion. Lower graph shows insulin levels in samples collected at 1-minute intervals. Standard errors are shown at representative points; $N = 4$. Upper graph shows glucose concentrations determined within 60 seconds in effluents from pancreas collected over ice. β-Glucose (○ - - - ○) was measured by stereospecific glucose oxidase (see text). Equilibrated total glucose (■ - - - ■) was determined in the same sample after 2 hours equilibration at 25°C and 20 hours at 4°C.

β anomer had little activity, whether introduced as the first stimulation in the series or subsequent to stimulation by equilibrated glucose; these results indicate a difference in anomeric specificity rather than an effect of the choice of sequences. In other experiments (not illustrated) in which α-glucose was used as the initial stimulus, results were essentially the same with, if anything, an even greater proportional effect of the α anomer.

The second glucose concentration (150 mg/100 ml) was selected to evaluate the effects of the two forms of glucose on both the first and second phases of insulin release. This concentration approximates a physiologic challenge and approaches the half-maximum (160 to 180 mg/100 ml) for equilibrated glucose stimulation of both phases of insulin release in vitro (*6*) or in vivo (*7*). As shown in Fig. 2, the β anomer at this concentration stimulated both first and second phase insulin release; however, the α anomer was still more effective on both phases than the β anomer was. The difference between α- and β-glucose stimulation of first phase insulin release ($\alpha = 0.306$ μg; $\beta = 0.190$ μg) and total insulin released ($\alpha = 0.771$ μg; $\beta = 0.350$ μg) was highly significant ($P < .01$ and $P < .02$, respectively). Comparable stimulation with equilibrated glucose gave results intermediate between α- and β-glucose, but in this comparatively small series the differences from results with either anomer were not statistically significant.

Our data show that the greatest difference in anomeric activity occurs in the lower range of glucose-stimulating concentrations, suggesting that the half-maximum for the different forms of glucose anomers will be $\alpha >$ equilibrated $> \beta$, but that they approach similar maximum activities. Complete dose-response studies will be required to establish the exact quantitative nature of the activities of each anomer on insulin release.

There was little spontaneous equilibration of either of the anomers during passage through the pancreatic preparation (see Figs. 1 and 2), which includes the stomach, the spleen, and a bit of the duodenum. Thus, under these experimental conditions, tissue mutarotase activity (*8*), if present, is of insufficient activity to influence equilibration during the short time of exposure of the tissues to the glucose medium.

At the technical level, the present studies, showing a difference in the activities of α- and β-glucose, emphasize that caution must be exerted in using freshly prepared glucose solutions for the study of insulin secretion and possibly in other glucose-modulated systems. Almost all commercially available crystalline D-glucose is in the α form. In our perfusion medium, the half-time for equilibration at 25°C was approximately 20 minutes and was much longer at 4°C.

Contrasting the anomeric specificity of glucose-stimulated insulin release with the anomeric specificity of enzyme systems involved with intracellular glucose metabolism may establish the relative importance of the various pathways or the existence of a unique specific membrane receptor for nonmetabolized glucose. Since glucose 6-phosphate, formed from glucose, equilibrates rapidly at 37°C (half-life about 1.5 seconds), all subsequent metabolic steps are believed to be insensitive to the initial glucose anomer (*2*). Thus, the anomer-sensitive secretion mechanism apparently occurs before glucose 6-phosphate and is not a result of energy production from glucose. The pentose pathway is also not implicated for the same reason; in addition, this pathway, at least in the liver, is β-specific

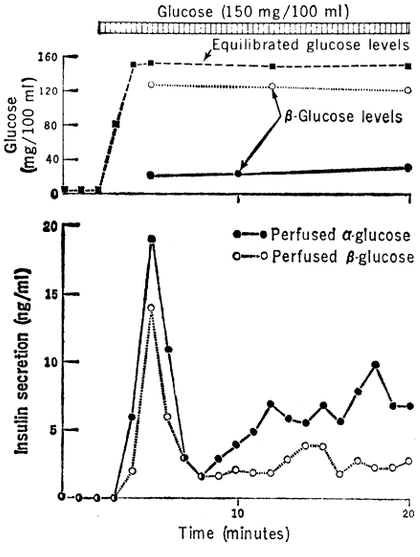

Fig. 2. Effect of glucose anomers (150 mg/100 ml for 20 minutes) on insulin secretion; α- and β-glucose were perfused in separate experiments. Lower graph shows insulin levels in samples collected at 1-minute intervals. α-Glucose perfusion, ●—●; β-glucose perfusion, ○ · · · ○. Upper graph shows glucose concentrations determined as described in Fig. 1; β-glucose, ● or ○; total glucose, ■ - - - ■.

(*9*). Hexokinase has a stereospecificity which could be similar to the observed glucose-stimulated insulin release (*2, 9, 10*), but the Michaelis constant (K_m) for this enzyme in islets (*11*) seems too low to represent the site of the initiating signal. The stereospecificity (at submaximal concentrations) of islet glucokinase is unknown, and, until more is learned of the stereospecificity of this enzyme, an initiating activity limited to this step cannot be excluded. Also, a nonhexose phosphate pathway could be involved. With these reservations, the observed specificity for insulin secretion is consistent with the concept that the initial trigger for glucose-stimulated release is by nonmetabolized glucose at the cell membrane (*12*). Both phases of insulin release seem similarly controlled—an observation consistent with the earlier demonstration that both phases have similar quantitative sensitivity to glucose (*6*). Concurrent experiments show that protection against alloxan toxicity by glucose, presumably a surface membrane effect, also has α-stereospecificity (*13*). Possibly these membrane activities may be distinct from active carrier systems for glucose which, at least in ascites tumor and red blood cells, are β-glucose specific (*14, 15*).

Pure anomers of glucose introduced into the circulation of animals require 7 minutes or longer to reach equilibration (*2*). If an acute endogenous metabolic permutation results in an anomeric specificity for either a glucose production or glucose uptake process, transient changes in the relative concentration of circulating glucose anomers may have to be considered, particularly in regard to insulin secretion and other glucose-regulating endocrine systems. In such a case, errors of interpretation could be compounded by glucose measurements in which β-specific glucose oxidase is used. Although Hill (*16*) found that neither glucagon, insulin, nor epinephrine caused even a transient change in the equilibrium ratio of circulating glucose, comparable and more extensive studies are required in diabetes and other pathologic states.

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References and Notes

1. C. Lundsgaard and S. A. Holboll, *J. Biol. Chem.* **65**, 305 (1925).
2. J. M. Bailey, P. H. Fishman, P. G. Pentchev, *ibid.* **243**, 4827 (1968).
3. G. M. Grodsky, L. L. Bennett, D. F. Smith, F. G. Schmid, *Metabolism* **16**, 222 (1967).
4. D. L. Curry, L. L. Bennett, G. M. Grodsky, *Endocrinology* **83**, 572 (1968).
5. This was rapidly diluted (25:1) in the inflowing perfusate and was introduced immediately before total perfusate entered a 37°C heating coil.
6. G. M. Grodsky, *J. Clin. Invest.* **51**, 2047 (1972).
7. E. Cerasi, R. Luft, S. Efendic, *Diabetes* **21**, 224 (1972).
8. A. S. Keston, *J. Biol. Chem.* **239**, 3241 (1964).
9. M. Salas, E. Vinuela, A. Sols, *ibid.* **240**, 561 (1965).
10. The α-glucose K_m for hexokinase is at most only twice that for β-glucose (*3*). However, the concentration curve for glucose stimulation of insulin release is sigmoidal; at marginally stimulating concentrations, a small change in K_m could cause an exaggerated change in insulin release (*6*).
11. S. J. H. Ashcroft and P. J. Randle, *Biochem. J.* **119**, 5 (1970).
12. R. Landgraf, J. Kotler-Brajtburg, F. M. Matschinsky, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 536 (1971).
13. A. A. Rossini, M. Berger, J. Shadden, G. F. Cahill, Jr., *Science* **183**, 424 (1974).
14. R. G. Faust, *J. Cell Exp. Physiol.* **56**, 103 (1966).
15. P. H. Fishman and J. M. Bailey, *Nat. New Biol.* **243**, 59 (1973).
16. J. B. Hill, *J. Appl. Physiol.* **20**, 749 (1965).
17. Supported in part by grant AM-01410, NIAMD; a joint grant from Hoechst Pharmaceutical Company of Somerville, N.J., and Upjohn Pharmaceutical Company of Kalamazoo, Mich.; and the Kroc Foundation.

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Pulsatile Growth Hormone Secretion: Suppression by Hypothalamic Ventromedial Lesions and by Long-Acting Somatostatin

Abstract. *Sequential blood samples, obtained from freely behaving, nonstressed male rats, showed a pulsatile pattern of growth hormone secretion with a mean interval between peaks of 68 minutes. The bursts of secretion were blocked by lesions of the hypothalamic ventromedial nuclei and by administration of a long-acting preparation of synthetic somatostatin.*

Physiologic studies of growth hormone (GH) regulation in the rat have been hindered by the wide variability in plasma GH levels observed (range, between 1 and > 200 ng/ml) (*1*). Recent evidence has indicated that other rat pituitary hormones are secreted in episodic bursts (*2*). We now report that abrupt changes in blood GH occur in freely behaving rats and that these bursts of secretion are dependent on hypothalamic mechanisms.

Male Sprague-Dawley rats (350 to 450 g) were prepared with permanent indwelling jugular cannulas and adapted to small isolation chambers to permit repeated sampling without disturbance to the animal (*3*). Such a sampling procedure is important since minor stress, such as handling, inhibits GH secretion (*4*). Cage adaptation was carried out for a minimum of three periods of 4 to 6 hours over a 7- to 10-day period. After adaptation, individual rats were placed in the isolation box at 0800 hours, and sampling was begun 2 hours later. Blood samples (0.35 ml) were removed every 15 minutes, and the plasma was separated and frozen; red blood cells were resuspended in saline and returned to the animal after the succeeding sample was removed. This technique prevented a fall in the hematocrit reading and permitted removal of multiple samples without hemodynamic disturbance. Plasma GH was measured in duplicate samples by radioimmunoassay; the materials used were supplied by the National Institute of Arthritis and Metabolic Diseases. Plasma corticosterone was determined by a competitive protein binding assay (*5*).

Table 1. Integrated plasma GH levels in control, VMN-lesioned, and PZ-GRIF–treated rats.

Experimental group	*N*	Serum GH (ng/ml per hour)
Intact controls	12	40.2 ± 5.39*
Sham lesions	5	30.5 ± 4.72
Hypothalamic VMN lesions	7	10.3 ± 2.49†
PZ control	6	38.5 ± 4.90
PZ-GRIF	4	9.2 ± 2.02‡

* Mean ± S.E.M. † $P < .01$ compared to sham lesions. ‡ $P < .001$ compared to PZ control.

In the first experiment, 12 normal cage-adapted rats were sampled for periods of 4 to 8 hours. Episodic GH release was identified in each animal. The secretory bursts were characterized by sudden rises in plasma GH from less than 10 ng/ml to concentrations which often exceeded 100 ng/ml (Fig. 1, a and b). The mean peak-to-peak interval of the bursts was 68.0 ± 4.04 (S.E.M.) minutes. The rapid fall in GH from the highest values (Fig. 1, a and b) indicated a half-life of endogenous GH of approximately 5 to 7 minutes,