Dynamic Synthesis and Release of Insulin and Proinsulin from Perifused Islets

Hiroyuki Sando, M.D., and Gerold M. Grodsky, Ph.D., San Francisco

SUMMARY

Insulin and proinsulin syntheses from leucine-H-3 were studied in glucose-stimulated perifused rat islets, with particular attention being placed on the contribution of de novo synthesis to the characteristic second phase of insulin release. At fifty to sixty minutes, when the second phase had reached approximately steady state, most of the islet radioactivity was still in the proinsulin fraction; newly synthesized insulin during this interval represented less than 0.5 per cent of the total insulin secreted. By 110 to 120 minutes, this value had increased, but to less than 17.5 per cent. The half time of conversion of proinsulin to insulin was approximately sixty minutes. At either fifty to sixty or 110 to 120 minutes, proinsulin was less than 5 per cent of the total hormone secreted. The ratio of specific activities for secreted to islet insulin was less than unity at sixty minutes and exceeded unity at 120 minutes; the comparable ratios for proinsulin were somewhat above unity at both times. Results suggest newly synthesized insulin is not responsible for the second phase of insulin release. In addition, storage of insulin is nonhomogeneous with preferential release of the older nonlabeled hormone occurring during the first hour of glucose stimulation. DIABETES *22*: 354-60, May, 1973.

Early investigations, using static incubations of pancreatic slices, showed that glucose can stimulate the de novo synthesis of insulin from labeled amino acids.[1] Subsequently dynamic studies revealed that a pancreas continuously stimulated by glucose releases insulin in two phases, the second of which is detectable within ten to fifteen minutes.[2]

Since the second and major phase of insulin secretion could be partially inhibited in vitro by puromycin[2] or cyclohexamide,[3] it was suggested that this phase of insulin secretion could have resulted from de novo synthesis of insulin.[2] However, we have emphasized that inhibition was only partial;[4,5] about 80 per cent of the second phase was not inhibited by these agents. Furthermore, in experiments using perfused pancreas with recirculating perifusate, concentrations of dinitrophenol, sufficient to block total protein synthesis, had no detectable effect on glucose-stimulated insulin release.[6]

Others have found that little de novo synthesized insulin is secreted into incubation media before two hours,[1,7-10] but these investigators used static incubation systems whose ability to secrete insulin is less than that of dynamic perfused systems[2,11] and whose rate of synthesis may also be less. Dynamic systems also permit comparing the isotopic characteristics of islet insulin or proinsulin and the secretion rates of these substances at an identical period in time.

The perifused islet system was preferred to the perfused pancreas preparation to study the contribution of insulin synthesis during the second phase of insulin secretion because (1) this method uses a tissue preparation rich in insulin, where the methods for isolation and separation of insulin and proinsulin are well defined,[7] and (2) flow rates of perifusate are approximately one-tenth that of the perfused whole pancreas preparation,[13] resulting in significant reduction in the amounts of high specific activity leucine-H-3 required.

MATERIALS AND METHODS

Partially purified collagenase was obtained from the Worthington Biochemical Corp. Hank's solution was prepared just before use.[7] L-leucine -4,5-H-3 was supplied by Swartz-Mann, Orangeburg, N. Y., at a specific activity of 51 Ci per millimole. Salt-poor human albumin, 25 per cent, obtained from Cutter Laboratories, Berkeley, Calif., and Abbott Laboratory, Pasadena, Calif., was dialyzed before dilution against distilled water at 0° C. for three days.

Pancreases were obtained from fed Long-Evans rats anesthetized with sodium pentobarbital. After expanding the pancreas by retrograde injection of Hank's solution into the bile duct, islets were isolated using colla-

From the Metabolic Research Unit and Department of Biochemistry and Biophysics, University of California, San Francisco, California 94122.

Dr. Sando's present address is: Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Hongo, Tokyo.

Accepted for publication October 31, 1972.

genase.[14] Incubation with collagenase, subsequent washes with Hank's solution, and isolation of islets from the final suspension by micropipette were all performed at 37° C. This method routinely yielded 150 to 300 islets per pancreas. Two hundred islets were perifused by a modification of the Lacy method[12] using a Millipore filter chamber (catalog No. 5 + 00013000, plastic) fitted with a 13 mm. filter (part No. 6000, metrical, Ga-1, pore size 5.0 micron, Gelman Instrument Co., Ann Arbor, Mich.). Glass tubing was used wherever possible to reduce oxygen loss from perifusate. Perifusate consisted of 1 per cent dialyzed human serum albumin in bicarbonate-phosphate buffer identical with that used in previous experiments with intact perfused pancreas.[2,13] In addition, it contained 50 mg./100 ml. glucose and the leucine-free unlabeled amino acid mixture described elsewhere (70 to 200 mEq. each).[7] Perifusate was maintained at 37° C. under an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide.

The flow rate was adjusted to 1 ml. per minute and pressure was continuously monitored. In all experiments, islets were perifused for a fifty minute equilibration period in which a high initial insulin secretion rapidly declines. Then leucine-H-3 (104.5 μC./ml.) and high glucose (final concentration: 300 mg./100 ml.) were continuously added by a side arm constant flow pump for thirty minutes. In the subsequent chase periods of thirty or ninety minutes, the perifusate via side arm was replaced with an identical solution containing unlabeled leucine (200 μM) instead of the isotope. Eluates were collected over ice at one minute intervals during the first ten minutes of high glucose and every five or ten minutes thereafter. Samples were stored frozen for direct immunoassay of insulin or, in certain instances, for extraction.[2] After termination of each experiment, the Millipore filter with its islets was immediately immersed in acid-alcohol containing 1 per cent albumin and stored overnight at 4° C. The mixture was then homogenized, allowed to stand an additional twenty-four hours at 4° C., centrifuged, and the precipitate discarded. An aliquot of eluate (8 ml.), collected during the terminal ten minutes of each chase period (fifty to sixty minutes or 110 to 120 minutes), was added to 21 ml. of cold acid-alcohol. Acid-alcohol extracts of eluate or islets were treated by a modification of the Grodsky and Tarver[15] and Davoren methods.[16] The final alcohol-ether precipitate was air dried and dissolved in 3 M acetic acid, and the insulin and proinsulin were separated on a 1 x 50 cm. Biogel P-30-column at room temperature as described earlier.[7] The first peak of this column contains proinsulin and intermediates; the second peak contains insulin and C-peptide. Fractions of 1 ml. were collected in 0.5 ml. of 0.1 per cent albumin. Samples, 1 ml., from each fraction were mixed with 10 ml. triton-toluene scintillator (Triton x—100, 100 ml.; toluene 200 ml.; PPO, 1,200 mg.; POPOP, 15 mg.) and counted in a Nuclear Chicago Liquid Scintillation Spectrometer. Recovery of radioactive material from the column ranged from 95 to 100 per cent.

The remaining 0.5 ml. in each collection sample was concentrated to dryness over KOH in vacuo and the dried material was dissolved in 1 to 2 ml. of 0.4 M glycine—1 per cent albumin (pH 8.0). Proinsulin and insulin content were measured by the radioimmunoassay of Grodsky and Forsham;[17] standards used were rat insulin and a mixture of rat proinsulin with intermediates.[7]

The specific activity of either proinsulin or insulin was calculated by dividing the total radioactivity of their respective peaks from the Biogel column by the appropriate proinsulin or insulin content. No correction for the difference in leucine of rat proinsulin (10 leucines) and rat insulin (6 leucines) was necessary in these experiments since the connecting polypeptide (4 leucines) is quantitatively recovered in the insulin peak in this extraction and gel filtration method.[7] At no time during the experiments or isolations was carrier hormone added.

RESULTS

As shown in figure 1, nonspecific insulin release occurred during the initial perifusion of islets with buffer containing 50 mg./100 ml. glucose and amino acids; however, in all cases, a forty to fifty minute equilibration period resulted in the eluates stabilizing at a constant low insulin concentration. The constant infusion of glucose (300 mg. per milliliter) caused the characteristic diphasic response.[2] There was an immediate but transient early phase; the second phase of insulin release was detectable in ten to fifteen minutes and reached a comparatively steady level in thirty minutes. Secretion rate increased slowly thereafter throughout the 120 minute stimulation.

Figure 2 shows the amounts of insulin and proinsulin in islets or eluate when experiments were terminated at either sixty or 120 minutes. On a molar basis, proinsulin was approximately 10 per cent of the total amount of proinsulin and insulin in the islets at either sixty or 120 minutes (8.5 ± 0.9 per cent at sixty minutes, 10.0 ± 0.8 per cent at 120 minutes). Of

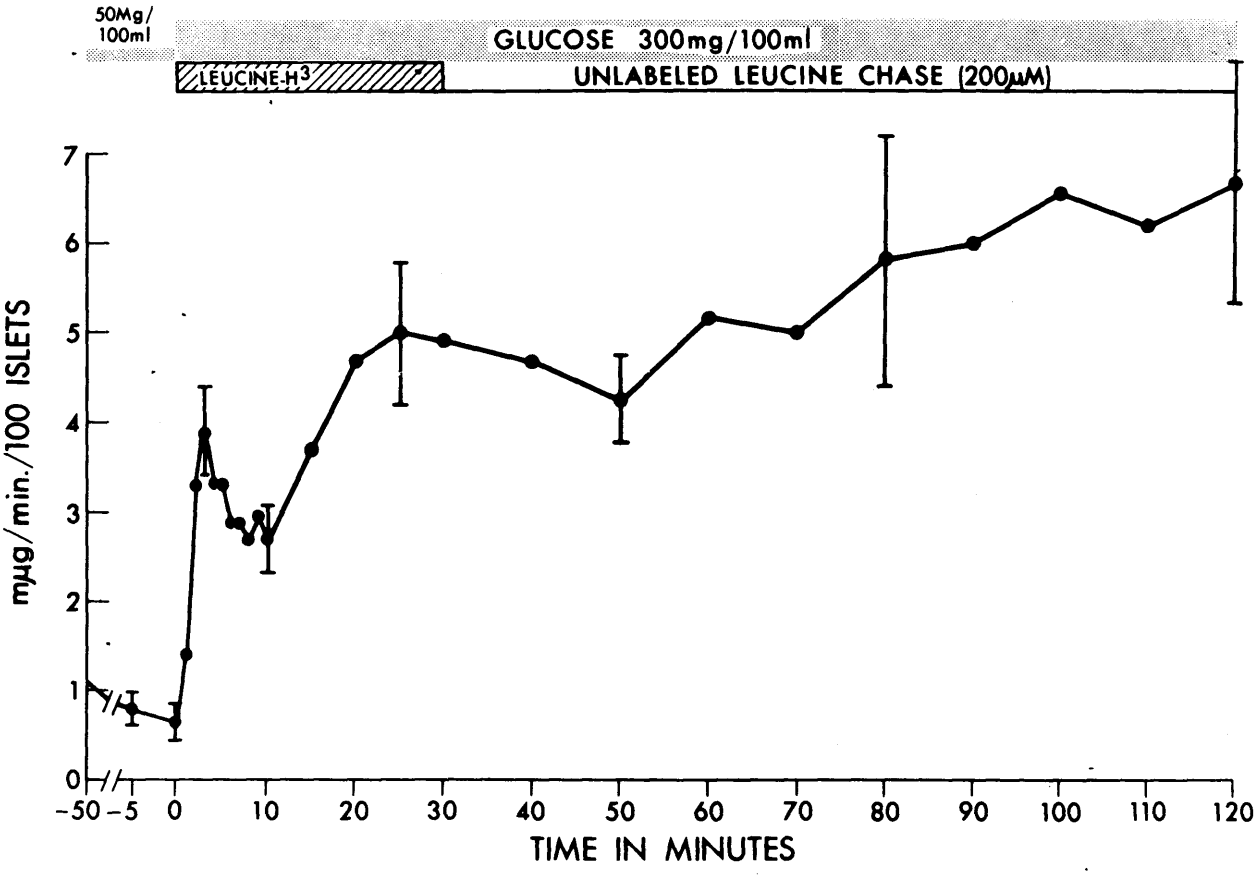

FIG. 1. Mean insulin secretion from perifused islets during glucose stimulation. Data include experiments stopped at sixty minutes after glucose (N = 9) and experiments extended to 120 minutes (N = 4). Leucine-H-3, when added, was perifused as indicated. Perifusate samples (10 ml.) from fifty to sixty or 110 to 120 minutes were used for chromatographic analysis.

the total proinsulin and insulin secreted during minutes fifty to sixty, 1.3 ± 0.4 per cent was proinsulin; this percentage increased slightly to 4.9 ± 0.3 at 120 minutes ($p < 0.001$).

The percentage of insulin in the islets that was secreted was not significantly different at either fifty to sixty minutes (8.7 ± 2.4) or 110 to 120 minutes (4.3 ± 1.7) ($0.05 < p < 0.0$). Less than 2 per cent of proinsulin in the islets was secreted during either of the terminal ten minute periods: there was no significant difference between per cent proinsulin secretion at sixty minutes (1.3 ± 0.6) and per cent proinsulin secretion at 120 minutes (1.9 ± 0.7) ($0.2 < p < 0.4$).

Although proinsulin represented a small portion of the total amount of islet proinsulin and insulin, at sixty minutes 89.9 ± 2.2 per cent of the total radioactivity was in the islet proinsulin fraction (figure 3). However, in experiments terminated at 120 minutes, radioactivity in the islets was distributed equally between proinsulin and insulin. Absolute counts in the proinsulin fraction decreased to 52.9 ± 3.3 per cent during the sixty minute differential period of the two types of experiments ($p < 0.001$). Total radioactivity in the islets at 120 minutes was still 71 per cent of that found at sixty minutes, a difference which was not statistically significant (19,890 ± 3,024 cpm/100 islets at sixty minutes, 14,117 ± 1,853 at 120 minutes) ($0.1 < p < 0.2$).

Of the radioactivity in the islet insulin fraction at sixty or 120 minutes, only 4.5 ± 0.4 rising to 7.0 ± 2.0 per cent was secreted into the eluate during each final ten minute period. This difference was not significant ($0.2 < p < 0.5$). The per cent of islet proinsulin radioactivity secreted was 1.6 ± 0.3 at sixty minutes but rose significantly to 4.9 ± 1.4 at 120 minutes ($p < 0.05$).

Figure 4 and table 1 compare the specific activities of insulin and proinsulin in islets and perifusate at the respective time periods. At sixty minutes, specific activity in the islet proinsulin fraction was 100 times that

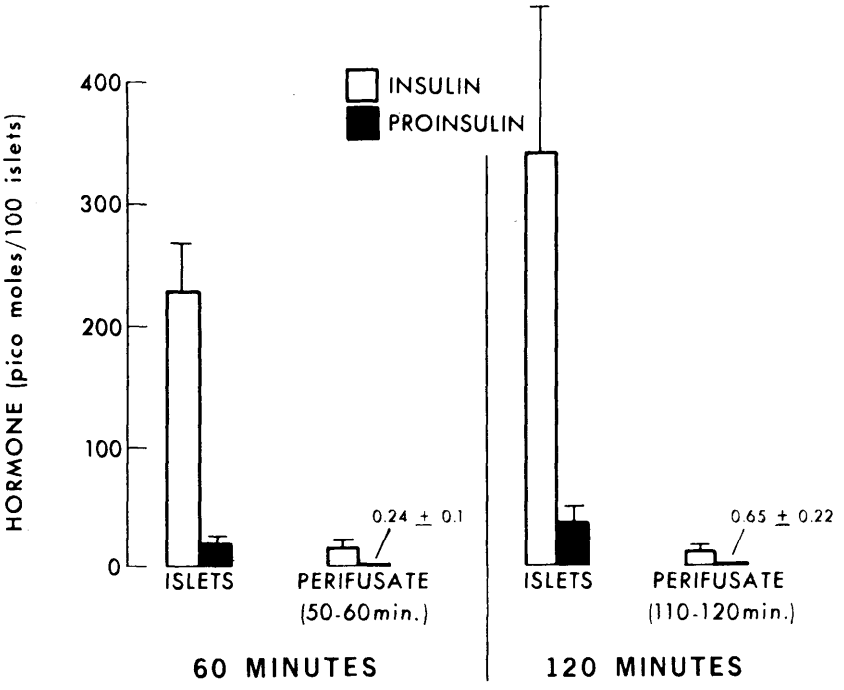

FIGURE 2

Total immunoreactive insulin and proinsulin in islets or perifusate.

of islet insulin. As shown in figure 4 and table 1, there was no evidence of preferential release of either newly synthesized proinsulin or insulin at sixty minutes. The ratio of perifusate to islet specific activities of secreted proinsulin was slightly greater than 1.0 (1.24 ± 0.35); for insulin, the ratio of perifusate to islet specific activities at sixty minutes was actually less than unity (0.64 ± 0.14) ($p < 0.1$). At 120 minutes the ratio of perifusate to islet specific activities of insulin increased above unity (1.58 ± 0.29) and significantly above the sixty minute value ($p < 0.025$). The increased ratio of perifusate to islet specific activity of proinsulin (2.73 ± 0.81) was not significantly above the sixty minute value ($0.1 > p > .05$).

Taking the specific activity of islet proinsulin to represent the minimum specific activity of newly syn-

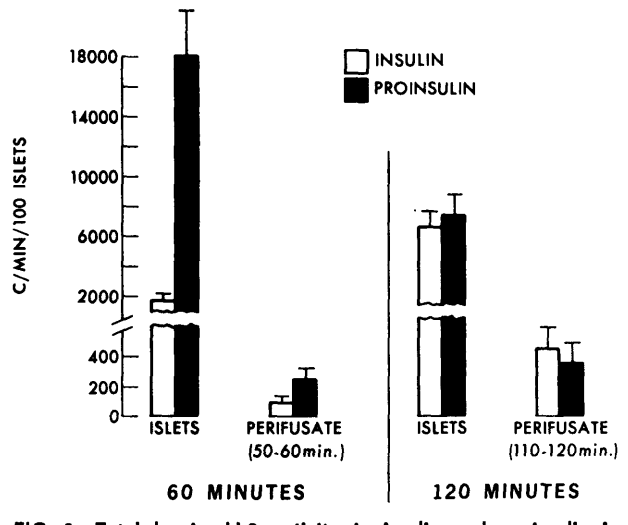

FIG. 3. Total leucine-H-3 activity in insulin and proinsulin in islets or perifusate.

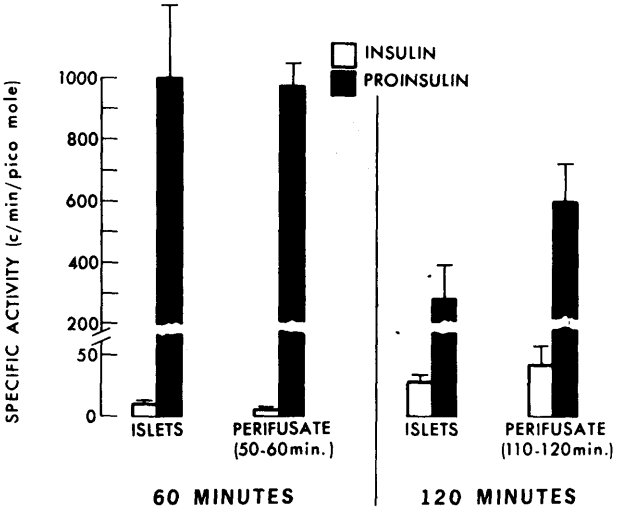

FIG. 4. Specific activity of insulin and proinsulin in islets or perifusate.

TABLE 1

Specific activities of insulin and proinsulin in perifusate and islets

Experiment	Specific activities (cpm/picomole) islets proinsulin	islets insulin	perifusate proinsulin	perifusate insulin	Ratio of specific activities (medium:islets) proinsulin	Ratio of specific activities (medium:islets) insulin	Total insulin secreted as newly synthesized (Specific activity islet insulin) / (Specific activity islet proinsulin) × 100
			60 minutes				%
1	1,900	13.4	774	3.4	0.41	0.26	0.18
2	764	13.1	972	6.2	1.27	0.47	0.81
3	895	6.4	—	4.1	—	0.65	0.46
4	907	8.7	1,061	9.3	1.17	1.07	1.03
5	524	4.4	1,109	3.3	2.12	0.74	0.63
Mean±SE	998±236	9.2±1.8	979±74	5.3±1.1	1.24±0.35	0.64±.14	0.62±0.15
			120 minutes				
1	601	55.3	955	87.0	1.59	1.57	14.48
2	182	25.8	560	34.0	3.07	1.32	18.68
3	261	14.5	360	15.4	1.38	1.06	5.9
4	108	13.8	526	32.8	4.86	2.38	30.4
Mean±SE	288±109	27.4±9.7	600±126	42.3±15.5	2.73±0.81	1.58±0.29	17.37±5.09

thesized insulin,* a maximum of only 0.6 ± 0.1 per cent of the insulin secreted at sixty minutes was newly synthesized insulin. By 120 minutes, the comparable figure was still only a maximum of 17.4 ± 5.1 per cent ($p < 0.01$).

DISCUSSION

The perifused islet preparation described by Lacy[2] is a dynamic system which produces at least some of the multiphasic insulin secretion patterns previously noted in the in vitro perfused intact pancreas.[2,4] In our hands, the perifusion system produces definite, though possibly less dramatic and less reproducible, initial release phases in response to high concentrations of glucose.[5] The characteristics of the second phase produced by the two systems (of prime interest in this study) were quite similar, secretion rising after the first ten to fifteen minutes and approaching a maximum rate after thirty to sixty minutes. The mean secretion rate of 4.2 per cent of islet insulin during the final 110 to 120 minutes is equivalent to 25.2 per cent per hour, similar to the rate noted for the in vitro perfused pancreas.[2,11] Thus, the sensitivity of perifused islets to high glucose is similar to islets in the intact pancreatic preparation. In the dynamic system, as in static islets[7] or in man,[18] proinsulin was not an important fraction of the secreted hormones during glucose stimulation, being under 5 per cent at sixty or 120 minutes. Burr et al.,[19] using a perifused pancreatic segment preparation, previously reported that proportional secretion of proinsulin increased to 17 per cent during sixty minutes of glucose stimulation. In comparable studies using the perfused pancreas (unpublished observations) we found, as with perifused islets, only small amounts of proinsulin secreted during glucose stimulation. There was no evidence of preferential release of labeled proinsulin compared to labeled insulin since the ratio of insulin to proinsulin radioactivity in the eluates was always greater than the ratios in islets.

Our present results strongly indicate that little of the first or second phase of glucose-stimulated insulin secretion reflects the secretion of de novo synthesized insulin. At sixty minutes, when the second phase was almost maximal, 91 per cent of the total radioactivity in the islet was still in the small proinsulin fraction; proinsulin specific activity was approximately 100 times that of insulin. Of the total radioactivity that had reached the insulin fraction, an equivalent of only 1.6 per cent was being secreted during the terminal ten minutes of the experiment. As described by Sando and Steiner,[7] the specific activity of newly synthesized insulin can be approximated by the specific activity of proinsulin in the islets at any given time. Based on these approximations, less than 0.5 per cent of the total insulin secreted during the terminal portions of

*Since our first measurements were made thirty minutes after terminating the leucine-H-3 pulse, it is probable that the radioactive proinsulin fraction was diluted by new unlabeled proinsulin. Thus, our calculations would represent a minimum estimate of the specific activity of the newly synthesized insulin and a maximum estimate of the amount secreted.

the experiment (fifty to sixty minutes) consisted of newly synthesized insulin. Even by 120 minutes, newly synthesized insulin made up only 15 per cent of the secreted insulin.

The rate-limiting step in the provision of newly synthesized hormone for ultimate secretion as insulin in the perifused islets may be the conversion of proinsulin to insulin.[9] Using the change of total counts in the islet proinsulin fraction between the sixty and 120 minute experiments of 57 per cent, the half time for conversion of proinsulin to insulin in the dynamic system was approximately sixty minutes, a value similar to that estimated from studies by Steiner et al. using static islets.[20] From the half time and the mean islet proinsulin level (23.7 picomoles/100 islets), the turnover for proinsulin, and therefore the maximum rate of synthesis of insulin, in glucose-stimulated islets, is approximately 12 picomoles/hr./100 islets.

Studies with inhibitors,[2,21,22] kinetic analysis,[5,23] or electronmicroscopy[24] suggest the first and second phases of glucose-stimulated insulin release reflect two different metabolic or anatomic targets for glucose action.

This dual action of glucose can be mathematically approximated by a model in which insulin is not stored in a homogeneous single compartment but as "packets" which respond in an all-or-nothing fashion when their threshold to glucose is reached or exceeded[5,23] (see figure 5). The second phase provides additional signal or insulin to the secretory system, resulting in hypersensitivity to further glucose stimulation. This provisionary or potentiating action of glucose is particularly significant for the evaluation in man of glucose/insulin relationships during glucose stimulation.[5,25,26] In the model, the source of insulin during the provisionary phase could have been from de novo synthesis or from additional stored insulin. In the former case (figure 5a), preferential release of newly synthesized insulin could occur. In the current experiments, at sixty minutes, the ratio of secreted to islet specific activity for insulin was actually less than unity (0.63); thus, instead of preferential release of new insulin at this time, a preferential release of older insulin was indicated. In view of these results and the small amount of insulin synthesis, model 5b seems more applicable. At 110 to 120 minutes, preferential release of radioactive insulin and proinsulin was detected (secreted specific activity ÷ islet specific activity = 1.6 and 2.1, respectively). These results are similar to those observed by Sando using static islet incubations[7] and may reflect cell deterioration. However, both the low ratio of secreted to islet insulin at sixty minutes and the increase to greater than unity at 120 minutes, are consistent with our concept of nonhomogeneous insulin storage in which the wave of radioactive insulin does not reach the releasable compartment until the later time. Additional studies, including a systematic variation of pulse and chase times, are necessary to resolve this question.

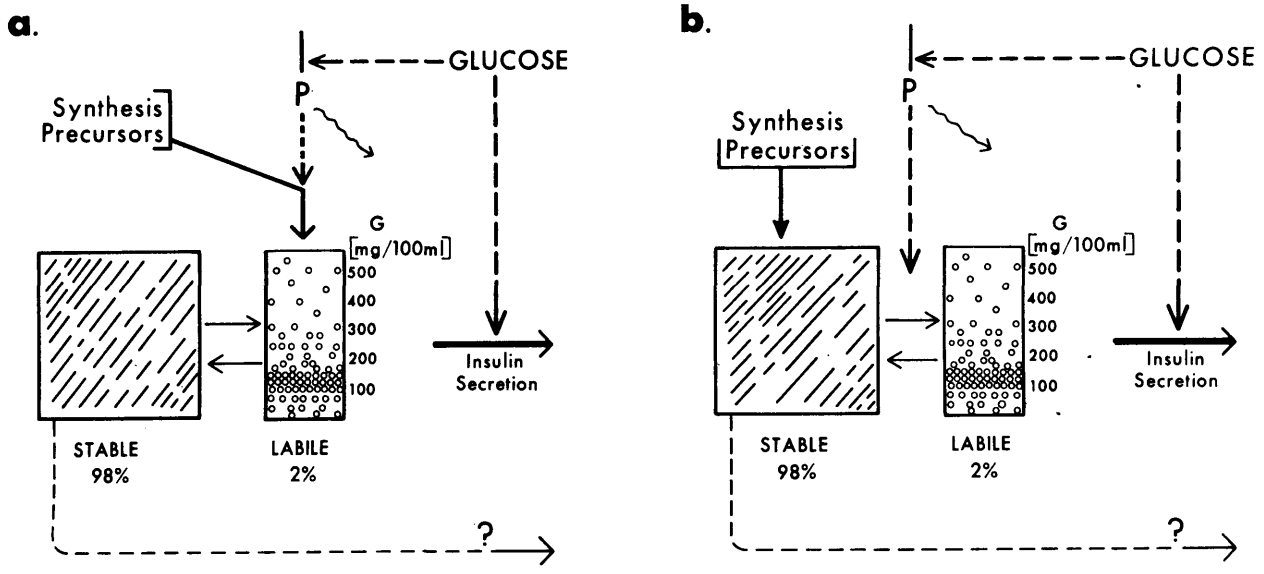

FIG. 5. Theoretical scheme for insulin secretion, incorporating the hypothesis of a distribution of insulin packets which rapidly release insulin when their thresholds to glucose are reached or exceeded. Open circles in the small compartment represent distribution of the threshold sensitive packets. Figures a and b show the form of the model if new insulin preferentially enters the small compartment (a) or the large compartment (b). (Taken from reference 5.)

ACKNOWLEDGMENT

We thank Drs. M. Mozen and A. Pappenhagen of Cutter Laboratories, Berkeley, Calif., and Dr. J. Holper of Abbott Laboratories, South Pasadena, Calif., for their generous assistance in providing human albumin necessary for these studies. We also appreciate the technical assistance of Ms. F. G. Schmid, Ms. M. Manning, and Mr. R. Fanska.

This study was supported in part by Grant AM-01410, NIAMD; the Hoechst Pharmaceutical Company, Somerville, New Jersey; the Levi J. & Mary Skaggs Foundation; and the Kroc Foundation.

REFERENCES

[1] Howell, S. L., Perry, D. G., and Taylor, K. W.: Secretion of newly synthesized insulin in vitro. Nature (Lond.) *208*:487, 1965.

[2] Curry, D. L., Bennett, L. L., and Grodsky, G. M.: Dynamics of insulin secretion by the perfused rat pancreas. Endocrinology *83*:572-84, 1968.

[3] Basabe, J., Lopez, N., Viktora, J., and Wolff, F.: Studies of insulin secretion in the perfused rat pancreas: Effect of diazoxide and A025. Diabetes *19*:271-81, 1970.

[4] Grodsky, G. M., Curry, D., Landahl, H., and Bennett, L.: Further studies on the dynamic aspects of insulin release in vitro with evidence for a two-compartmental storage system. Acta Diabetol. Lat. *6* (Suppl. 1):554-79, 1969.

[5] Grodsky, G. M.: A threshold distribution hypothesis for packet storage of insulin and its mathematical modeling. J. Clin. Invest. *51*:2047-59, 1972.

[6] Grodsky, G. M., and Bennett, L.: Insulin secretion from the isolated pancreas in absence of insulinogenesis: Effect of glucose. Proc. Soc. Exp. Biol. Med. *114*:769-71, 1963.

[7] Sando, H., Borg, J., and Steiner, D. F.: Studies on the secretion of newly synthesized proinsulin and insulin from isolated rat islets of Langerhans. J. Clin. Invest. *51*:1476-85, 1972.

[8] Tanese, T., Lazarus, N. R., Devrim, S., and Recant, L.: Synthesis and release of proinsulin and insulin by isolated rat islets of Langerhans. J. Clin. Invest. *49*:1394-1404, 1970.

[9] Lin, B. J., Nagy, B. R., and Haist, R. E.: Effect of various concentrations of glucose on insulin biosynthesis. Endocrinology *91*:309-11, 1972.

[10] Sorensen, R. L., Steffes, M. W., and Lindall, A. W.: Subcellular localization of proinsulin to insulin conversion in isolated rat islets. Endocrinology *86*:88-96, 1970.

[11] Grodsky, G. M., Batts, A. A., Bennett, L. L., Vcella, C., McWilliams, N. B., and Smith, D. F.: Effects of carbohydrates on secretion of insulin from isolated rat pancreas. Am. J. Physiol. *205*:638-44, 1963.

[12] Lacy, P.: Personal communication.

[13] Bennett, L. L., and Grodsky, G. M.: Multiphasic aspects of insulin release after glucose and glucagon. *In* Diabetes. Ostman, J., and Miller, R. D. G., editors, Amsterdam, Excerpta Medica Foundation, pp. 461-69, 1969.

[14] Lacy, P. E., and Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes *16*:35-39, 1967.

[15] Grodsky, G., and Tarver, H.: Paper chromatography of insulin. Nature (Lond) *177*:223-25, 1956.

[16] Davoren, P. R.: The isolation of insulin from a single cat pancreas. Biochim. Biophys. Acta *63*:150-53, 1962.

[17] Grodsky, G. M., and Forsham, P. H.: An immunochemical assay of total extractable insulin in man. J. Clin. Invest. *39*:1070-79, 1960.

[18] Melani, F., Rubenstein, A. H., and Steiner, D. F.: Human serum proinsulin. J. Clin. Invest. *49*:497-507, 1970.

[19] Burr, I. M., Balant, L., Stauffacher, W., Renold, A. E., and Grodsky, G.: Dynamic aspects of proinsulin release from perifused rat pancreas. Lancet *2*:882-83, 1969.

[20] Clark, J. L., Rubenstein, A. H., Oyer, P. E., Mackenzie, J. J., Cho, S., and Steiner, D. F.: Proinsulin and insulin biosynthesis. *In* The Structure and Metabolism of the Pancreatic Islets. Falkmer, S., Hellman, B., and Taljedal, I.-B., editors. New York, Pergamon, 1969, pp. 339-48.

[21] Grodsky, G. M., Sando, H., Gerich, J., Karam, J., and Fanska, R.: Synthesis and secretion of insulin in dynamic perfusion systems. *In* Advances in Metabolic Disorders. Levine, R., and Mahler, R., Eds., 1973. In press.

[22] Levin, S. R., Charles, M. A., Smith, D., and Grodsky, G. M.: Contrasting effects of diphenylhydantoin (DPH) and diazoxide (Dz) upon the first and second phases of insulin secretion in the isolated, perfused rat pancreas. Diabetes *21*: 327, 1972.

[23] Grodsky, G. M.: A threshold distribution hypothesis for packet storage of insulin: II. Effect of calcium. Diabetes *21* (Suppl. 2):584-93, 1972.

[24] Lee, J. C., Grodsky, G. M., Bennett, L. L., Smith-Kyle, D. F., and Craw, L.: Ultrastructure of β-cells during the dynamic response to glucose and tolbutamide in vitro. Diabetologia 6:542-49, 1970.

[25] Grodsky, G. M., Licko, V., and Landahl, H.: Variable sensitivity of the perfused rat pancreas to glucose. *In* Recent Advances in Endocrinology, Mattar, E., editor, Amsterdam, Excerpta Medica Foundation, Intern. Cong. Series No. 238, pp. 421-29, 1971.

[26] Cerasi, E., Luft, R., and Efendic, S.: Decreased sensitivity of the pancreatic beta cells to glucose in prediabetic and diabetic subjects. A glucose dose-response study. Diabetes *21*:224-34, 1972.