Demonstration and Partial Characterization of Insulin Receptors in Human Platelets

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ABSTRACT Recently, evidence has been reported to suggest that human platelets like several other circulating blood cells may bind insulin. To examine whether human platelets contain specific insulin receptors, washed human platelets suspended in Hepes buffer were incubated at 24°C with ^{125}I-insulin in the presence and absence of unlabeled insulin and specific insulin binding was determined. Insulin binding by platelets increased progressively with time of incubation to reach a maximum at 3 h and was proportional to the number of platelets in the incubation mixture. Maximum insulin binding was observed at pH 8. Insulin degradation by platelets as assessed by TCA precipitability and reincubation studies was minimal. Scatchard analysis of the binding data and dissociation studies revealed evidence of negative cooperativity of the platelet insulin receptor. A high affinity dissociation constant of $\cong 3 \times 10^9$ M^{-1} was determined and the concentration of platelet insulin receptors was estimated as 25 binding sites/μn^2 platelet surface area. Binding of ^{125}I-insulin by platelets was inhibited by unlabeled porcine insulin and to a lesser extent by catfish insulin and porcine proinsulin but not by glucagon, prolactin, growth hormone, and thrombin. The findings indicate that human platelets contain specific insulin receptors. The significance of the platelet insulin receptor, particularly with respect to altered platelet function in diabetes mellitus, remains to be determined.

INTRODUCTION

There is evidence that a number of different cells contain specific insulin receptors, including certain formed elements of the circulating blood such a monocytes (1, 2), lymphocytes (3), and erythrocytes (4–6). Recently, Beck-Nielsen et al. (2) examined the binding of insulin to different cells contaminating peripheral blood monocyte preparations and found evidence to suggest that human platelets also may bind insulin. In this paper the results of a detailed study of the binding of ^{125}I-insulin to isolated human platelets are presented that illustrate the specific nature and some of the properties of the human platelet insulin receptor.

METHODS

Materials. Crystalline porcine monocomponent insulin (615-08E-199, 25.4 U/mg), porcine proinsulin (615-D63-267), and glucagon (NDL 0002-0N51-01) were generously supplied by Eli Lilly & Co., Indianapolis, Ind. Catfish was a gift of Dr. A. M. Permutt, Washington University School of Medicine. Human prolactin (HPR VLS-3) and human growth hormone (2002 F) were obtained from the National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Md., and diisopropyl fluorophosphate (DIP)[1]-treated purified bovine thrombin was a gift of Dr. P. W. Majerus, Washington University School of Medicine.

Preparation of platelet suspensions. Blood was collected from healthy male and female volunteers by clean venipuncture and two-syringe technique into a one-seventh volume of acid citrate dextrose solution. Written informed consent was obtained from all subjects before blood collection. Platelets were isolated by differential centrifugation according to the method of Mustard et al. (7) and resuspended in Tyrode's solution containing 0.35% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) and potato apyrase (prepared as described [8]) (3 U/ml). The platelet suspension was again centrifuged at 2,500 *g* for 10 min and the sedimented platelets were resuspended in Hepes buffer (0.1 M Hepes, 0.12 M NaCl, 1.2 mM $MgSO_4$, 2.5 mM KCl, 10 mM glucose, 1 mM EDTA, 1% bovine serum albumin), pH 8.0. Leukocyte contamination was monitored by phase-contrast microscopy (9) and found to be one leukocyte or less per 100,000 platelets. The final platelet suspensions were stored in plastic tubes at room temperature.

Platelet size estimation. Mean platelet volume was determined after appropriate dilution of aliquots of the final platelet suspensions with isotonic saline using a Coulter

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[1] *Abbreviation used in this paper*: DIP, diisopropyl flurophosphate.

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model H4 channelyzer (Coulter Electronics Inc., Hialeah, Fla.) (without hydrodynamic focussing) as described (10).

Insulin binding assay. Porcine insulin was iodinated by a modification of the chloramine T procedure (11). The specific activity of the biologically active material was 120–300 μCi/μg (0.4–0.5 iodine atoms per insulin molecule). The labeled insulin was purified by Sephadex G-50 chromatography (Pharmacia Fine Chemicals, Piscataway, N. J.) and was used within two wk of preparation. ^{125}I-insulin (120–650 pg/ml) was added to suspensions of platelets in Hepes buffer (0.5–2.0 × 10^6/μl) at pH 8.0, unless stated otherwise, in the presence or absence of unlabeled insulin (0–10 μg/ml). The mixtures were incubated at 24°C. At various time intervals 0.5-ml aliquots of the mixtures were transferred to plastic tubes containing 0.5 ml Hepes buffer (4°C) and immediately centrifuged for 5 min in a Beckman microfuge B (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The supernatant fluid was removed and the radioactivity of the platelet sediment was determined in a Packard model 3001 Tri-Carb gamma counter (Packard Instrument Co., Inc., Downers Grove, Ill.) at 50% counting efficiency. Specific binding of ^{125}I-insulin by platelets was determined by subtracting the counts per minute associated with the platelets in the presence of 10 μg/ml of unlabeled insulin from the total counts per minute determined in the platelet sediments in the absence of unlabeled hormone. In some experiments binding of ^{125}I-insulin was studied in the presence of either unlabeled insulin or porcine proinsulin, catfish insulin, glucagon, growth hormone, or DIP thrombin.

RESULTS

The time-course of binding of ^{125}I-insulin by platelets in the absence of unlabeled insulin (total insulin binding) and presence of excess unlabeled insulin (nonspecific binding) is illustrated in Fig. 1. Total insulin binding increased progressively to reach a maximum between 2 and 3 h of incubation. Half-maximum binding was observed at 30 min. Nonspecific insulin binding was 10–15% of total binding and remained relatively constant throughout the incubation period.

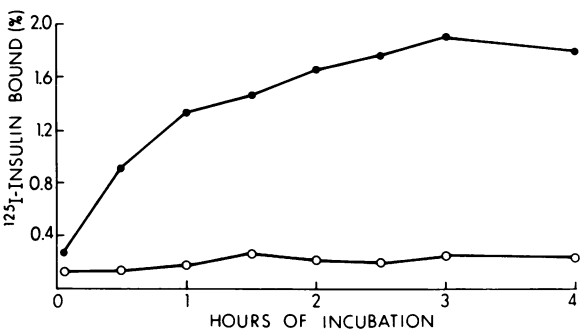

FIGURE 1 Time-course of binding of ^{125}I-insulin to washed human platelets. Aliquots of a platelet suspension (800,000/μl) were incubated with ^{125}I-insulin (450 pg/ml) at 24°C in the absence (●) and presence (○) of unlabeled insulin (10 μg/ml). Total ^{125}I-insulin binding (in the absence of unlabeled insulin) and nonspecific binding (in the presence of excess unlabeled insulin) are expressed as percentage of total radioactivity determined in aliquots of the incubation mixture after subtraction of the amount of radioactivity bound to the centrifuge tube and trapped by the platelet pellet (0.19%).

All binding experiments were performed at pH 8.0 because that pH yielded maximum specific binding. Insulin degradation in the medium by platelets was studied by both TCA precipitability and rebinding of the labeled hormone after incubation with platelets for 3 h and was found to be negligible (results not shown).

The amount of ^{125}I-insulin bound to platelets during a 3-h incubation period was found to be directly proportional to the concentration of platelets in the incubation mixture (Fig. 2).

The specific binding of ^{125}I-insulin tended toward saturation when increasing amounts of radioiodinated insulin were added to platelets (Fig. 3). However, complete saturation was not observed in this and one other experiment (not shown) at free ^{125}I-insulin concentrations of up to 7 pmol/ml, the maximum concentration readily attainable with ^{125}I-insulin. Similar results, i.e., lack of saturation, have been reported for insulin binding to fat cells (12). For this reason studies were conducted using more traditional competitive binding techniques, with a fixed concentration of ^{125}I-insulin and increasing concentrations of unlabeled hormone.

There was a progressive decrease in the amount of ^{125}I-insulin bound to the platelets with increasing concentrations of unlabeled hormone in the medium (Fig. 4). Half-maximum displacement of ^{125}I-insulin was observed at 3.5 ng/ml of unlabeled insulin.

A Scatchard (13) plot derived from the data of competitive binding studies is shown in Fig. 5. A high affinity dissociation constant of ≅3 × 10^9 M^{-1} was determined. The curvilinear pattern of the plot appears to be consistent with either the presence of two classes

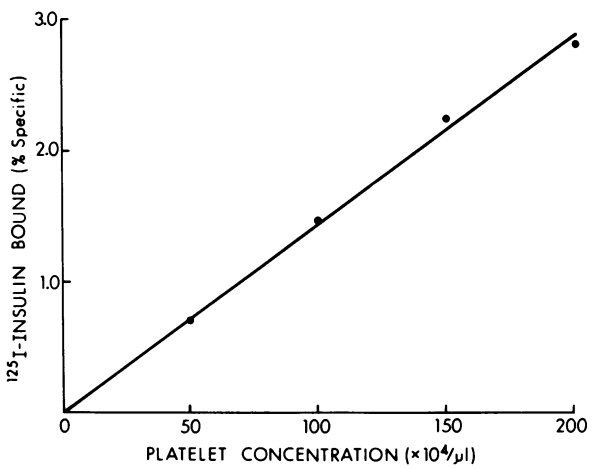

FIGURE 2 Effect of platelet concentration on specific binding of ^{125}I-insulin by platelets. Aliquots of a platelet suspension containing different concentrations of platelets were incubated with ^{125}I-insulin (238 pg/ml) in the presence and absence of unlabeled insulin (10 μg/ml) at 24°C. Specific binding was determined as described in the text and expressed as a percentage of the ^{125}I-insulin-related radioactivity measured in an aliquot of the total incubation mixture.

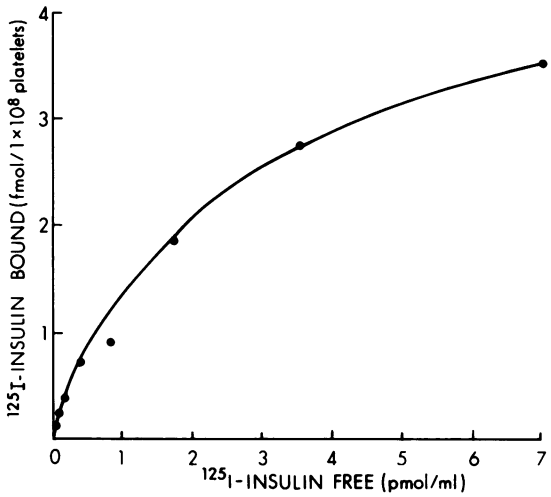

FIGURE 3 Binding of ^{125}I-insulin by human platelets. Increasing amounts of radio-iodinated insulin were incubated with a platelet suspension (0.5 ml) (736,000/μl) for 3 h at 24°C in the presence and absence of unlabeled insulin (10 μg/ml) and the specific binding of ^{125}I-insulin was determined.

of platelet binding sites for insulin with different affinities or the presence of a single population of insulin receptors that display enhanced dissociation of labeled

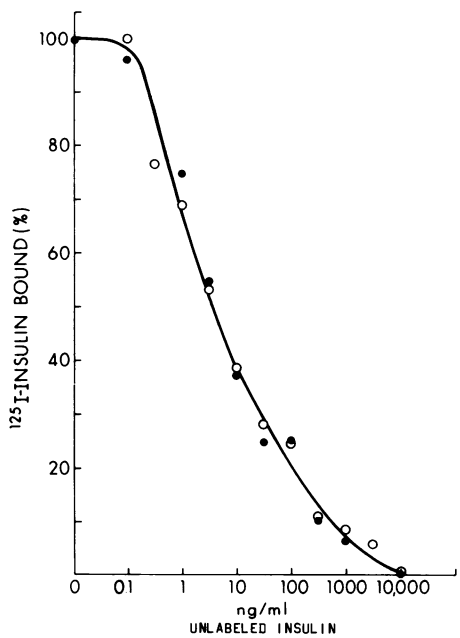

FIGURE 4 Effect of unlabeled insulin on binding of ^{125}I-insulin by human platelets. In two separate experiments aliquots of two platelet suspensions (●, 454,000/μl; ○, 828,000/μl) were incubated with ^{125}I-insulin (●, 304 pg/ml; ○, 126 pg/ml) in the presence of different concentrations of unlabeled insulin for 3 h at 24°C. The amount of labeled insulin bound in the presence of unlabeled insulin was expressed as a percentage of that determined in the absence of unlabeled insulin (●, 1.6%; ○, 1.36%).

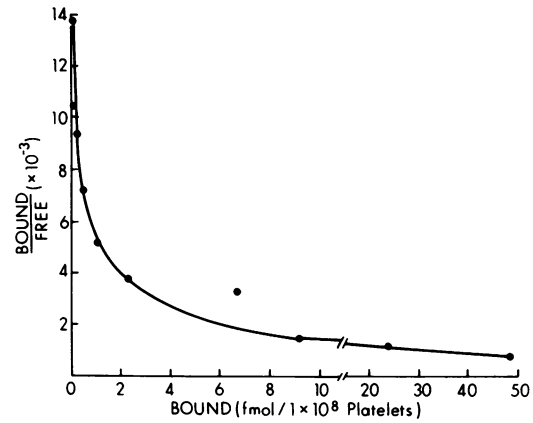

FIGURE 5 Scatchard plot of the insulin binding data derived from one of the experiments (○) shown in Fig. 4.

insulin in the presence of excess unlabeled hormone, after the model of negative cooperativity proposed by De Meyts et al. (14). To further test for negative cooperativity of the platelet insulin receptor, the dissociation of ^{125}I-insulin bound to platelets was studied in the absence and presence of excess unlabeled hormone. Platelets were incubated with ^{125}I-insulin in the presence and absence of unlabeled insulin (10 μg/ml) for 3 h and specific binding was determined. The platelets were then sedimented at 2,000 *g* for 10 min and resuspended in Hepes buffer with or without the addition of unlabeled insulin (1 μg/ml). The mixtures were incubated at 24°C. Aliquots (0.5 ml) were removed at certain time intervals and ^{125}I-insulin binding was determined and expressed as percentage of that observed at the end of the initial 3-h incubation period. The results shown in Fig. 6 indicate that the rate of dissociation of ^{125}I-insulin is enhanced in the presence of unlabeled insulin.

The specificity of the platelet insulin receptor was defined further by experiments in which the displacement of ^{125}I-insulin by unlabeled porcine insulin (100 ng/nl) was compared with that induced by unlabeled porcine proinsulin, catfish insulin, and several other unrelated peptides. The data shown in Table I indicate that catfish insulin and porcine proinsulin, both of which are less biologically active than porcine insulin (3, 15), are also less able to displace ^{125}I-insulin from the platelet receptor. Neither glucagon, prolactin, growth hormone, nor DIP thrombin caused measurable displacement of ^{125}I-insulin.

The mean concentration of insulin-binding sites in human platelets estimated from the *x*-intercept of the Scatchard plots of four experiments was 570±100 (SD) per platelet. This concentration is appreciably less than that reported for some of the other insulin-binding cells such as cultured human lymphocytes (16), human (6, 17) and turkey (5) erythrocytes, and rat adipocytes

TABLE I

Effect of Insulin-Related and -Unrelated, Biologically Active Peptides on Binding of ^{125}I-Insulin by Human Platelets

		Percentage of inhibition of ^{125}I-insulin binding
	μg/ml	%
Porcine insulin	0.1	100
Catfish insulin	0.1	85
Porcine proinsulin	0.1	49
Porcine glucagon	0.1	0
Human prolactin	3.0	0
Human growth hormone	10.0	0
Bovine DIP thrombin	0.5	0

Suspensions of platelets (454,000–1,238,000/μl) were incubated with ^{125}I-insulin (382–624 pg/ml) in the presence and absence of unlabeled peptides at 24°C for 3 h and platelet-associated ^{125}I-insulin-related radioactivity was determined. The inhibition of ^{125}I-insulin binding by unlabeled peptides was expressed as a percentage of that observed with unlabeled porcine insulin.

(18) (Table II). When the concentration of insulin receptors was calculated per mean platelet unit surface area (calculated as 23 μm^2 on the basis of a mean platelet volume of 10.3 μm^3, estimated with the use of a Coulter model H4 channelyzer), the results were indeed remarkably similar to those reported for the other insulin-binding cells listed (Table II).

DISCUSSION

The data presented in this paper indicate that human platelets contain binding sites for insulin as suggested by the work of others (2). Although the platelet suspensions used in this study were not entirely free of leukocytes, it seems highly unlikely that leukocyte contamination of the platelet preparations could account for a significant portion of the insulin binding observed. The results of competitive binding studies indicate that binding of ^{125}I-insulin occurs at physiologic concentrations (Fig. 4) and illustrate the specific nature of the platelet insulin receptor (Table I). Thus, unlabeled porcine insulin and to a lesser extent the biologically less active catfish insulin and porcine proinsulin were able to displace ^{125}I-insulin, whereas no displacement was observed with glucagon, prolactin, growth hormone, and DIP thrombin. The failure of DIP thrombin to displace labeled insulin from platelets is of particular interest because the presence of specific thrombin receptors in human platelets has been demonstrated (19) and insulin does not inhibit binding of DIP thrombin by human platelets.[2]

TABLE II

Comparison of Estimated Number of Insulin Binding Sites in Human Platelets with that Reported for Some Other Cell Types

Cell type	Binding sites	
	per cell	*per μm^2 cell surface area*
Human platelet (this study)	570	25
Human lymphocyte (12, 18)	12,500	20
Human erythrocyte (13, 14)	2,000	14
Turkey erythrocyte (15)	3,000	29
Rat adipocyte (16)	170,000	23

The numbers in parentheses indicate references.

Certain properties of the human platelet insulin receptor defined in this study, such as alkaline pH-optimum, high affinity binding constant, and curvilinear Scatchard plot, were found to closely resemble those described for other insulin binding cells (2–6, 16–18, 20, 21). The results of the Scatchard analysis of the data from the competitive binding studies appear to be consistent with the presence of several classes of insulin binding sites with different affinities. However, the data can also be interpreted as indicating a single population of insulin receptors exhibiting negative cooperativity with increasing occupancy of receptors (14). Indeed, our finding of enhanced dissociation of ^{125}I-insulin from platelets in the presence of unlabeled insulin seems to support the possibility of negative cooperativity of the platelet insulin receptor (14, 22). However, the interpretation of enhanced dissociation of bound labeled ligand with increasing concentrations of unlabeled ligand in the medium as indicating negative cooperativity has recently been questioned (23). In this study (23), the dissociation rate of ^{125}I-insulin bound to cultured human lymphocytes appeared to be virtually independent of receptor occupancy.

It is possible that a portion of the platelet-associated ^{125}I-insulin reflects uptake of specifically bound insulin bound by the platelet. This is likely if one considers the relatively long period of incubation required for insulin binding to reach steady state (Fig. 1), and the finding that a significant portion of the labeled insulin did not dissociate from the platelets (Fig. 6). One would expect such a finding because at the temperature at which the experiments were carried out (24°C) uptake may still occur but intracellular degradation might be suppressed leading to a build-up of internalized insulin. This is consistent with recent observations demonstrating an uptake or compartmentalization of specifically bound insulin by IM9-lymphocytes (24), hepatocytes (25), and adipocytes (26).

The concentration of insulin receptors per platelet in this study was found to be substantially less than

[2] Majerus, P. W. Personal communication.

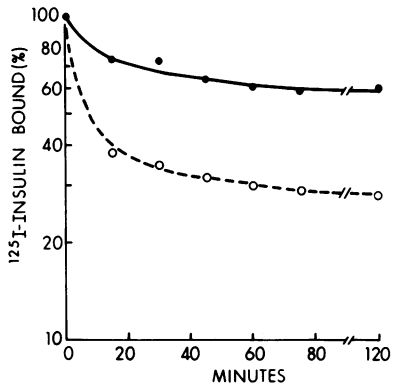

FIGURE 6 Dissociation of bound ^{125}I-insulin from the platelets in the presence and absence of unlabeled insulin. Aliquots of a platelet suspension (680,000/μl) were incubated with ^{125}I-insulin (382 pg/ml) in the presence and absence of unlabeled insulin (10 μg/ml) at 24°C for 3 h and specific insulin binding was determined. The platelets were then resuspended in Hepes buffer containing no unlabeled insulin (●) or containing 1 μg/ml of unlabeled insulin (○). The mixtures were incubated at 24°C and duplicate 0.5-ml aliquots were removed at certain time intervals for determination of specific platelet-associated radioactivity. The results are expressed as percentage of the total specific binding (1.3%) determined at the end of the initial 3-h incubation period.

that reported for other cells, a finding that could be readily explained by the comparably small size of the platelet. Indeed, the concentration of insulin receptors per platelet unit surface area appeared to be remarkably similar to that described for other cells. However, the calculation of the platelet unit surface area was based on the assumption of a smooth, continuous outer cell surface. Because intact disk-shaped platelets, as used in this study, appear to have an extensive sponge-like, internal, surface-connecting, canalicular system (27), the actual surface area of the platelet potentially accessible for insulin binding is probably much greater as indicated by the work of others (28). Thus, the concentration of insulin receptors per platelet unit surface area may have been grossly overestimated. However, this error is also inherent to a variable extent in previous estimations of insulin receptors in other cells that have irregular surfaces.

The biological significance of the platelet insulin receptor demonstrated in our study is not clear. There is some evidence that insulin may affect platelet energy metabolism. Thus, insulin was found to increase lactate formation by human platelets in the presence of glucose (29). However, the concentrations of insulin used in this study (0.004–0.4 U/ml) were far in excess of plasma insulin concentrations observed in man, leaving the significance of this finding in doubt. In addition, potentiation of glucose-induced inhibition of collagen-induced platelet aggregation in citrated human platelet-rich plasma has been reported (30). However, in this in vitro study a commercial insulin preparation was used and the possible effects of contaminants and the preservative contained in this preparation were not investigated. Inhibition of ADP- and calcium-induced platelet aggregation in citrated platelet-rich plasma from diabetic patients after intravenous injection of insulin has also been reported (31), but this finding was quite inconsistent. Accumulating evidence from a number of laboratories (31–34) indicates that in vitro and in vivo platelet function may be altered in diabetes mellitus, which may have important implications with respect to the development and progression of premature atherosclerotic disease in this disorder. Because of this evidence and the present demonstration of specific insulin receptors in human platelets, the effects of insulin on platelet metabolism and platelet function seem to warrant further study.

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REFERENCES

1. Schwartz, R. A., A. R. Bianco, B. S. Handwerger, and C. R. Kahn. 1975. Demonstration that monocytes rather than lymphocytes are the insulin binding cells in preparations of human peripheral blood mononuclear leucocytes: implications for studies of insulin-resistant states in man. *Proc. Natl. Acad. Sci. U. S. A.* **72:** 474–478.
2. Beck-Nielsen, H., O. Pederson, K. Kragballe, and N. Schwartz-Sorensen. 1977. The monocyte as a model for the study of insulin receptors in man. *Diabetologia.* **13:** 563–569.
3. Gavin, J. R., III, P. Gorden, J. Roth, J. A. Archer, and D. N. Buell. 1973. Characteristics of the human lymphocyte insulin receptor. *J. Biol. Chem.* **248:** 2202–2207.
4. Gambhir, K. K., J. A. Archer, and L. Carter. 1977. Insulin radioreceptor assay for human erythrocytes. *Clin. Chem.* **23:** 1590–1595.
5. Ginsberg, B. H., R. C. Kahn, and J. Roth. 1977. The insulin receptor of the turkey erythrocyte: similarity to mammalian insulin receptors. *Endocrinology.* **100:** 82–90.
6. Gambhir, K. K., J. A. Archer, T. J. Robinson, L. Carter, C. Bradley, and V. W. Hollis, Jr. 1978. Erythrocyte insulin receptors: characterization in normal, diabetic, and uremic man. *Clin. Res.* **26:** 415A. (Abstr.)
7. Mustard, J. F., D. W. Perry, N. G. Ardlie, and M. A. Packham. 1972. Preparation of suspensions of washed platelets from humans. *Br. J. Haematol.* **22:** 193–204.
8. Molnar, J., and L. Lorand. 1961. Studies on apyrases. *Arch. Biochem. Biophys.* **93:** 353–363.
9. Brecher, G., and C. P. Cronkite. 1954. Morphology and enumeration of human blood platelets. *J. Appl. Physiol.* **3:** 365–372.
10. Mundschenk, D. D., D. P. Connelly, J. G. White, and R. D. Brunning. 1976. An improved technique for the electronic measurement of platelet size and shape. *J. Lab. Clin. Med.* **88:** 301–315.

11. Freychet, P., C. R. Kahn, J. Roth, and D. M. Neville, Jr. 1972. Insulin interactions with liver membranes. Independence of binding of the hormone and its degradation. *J. Biol. Chem.* **247:** 3953–3961.
12. Hammond, J. M., L. Jarett, I. K. Mariz, and W. H. Daughaday. 1972. Heterogeneity of insulin receptors on fat cell membranes. *Biochem. Biophys. Res. Commun.* **49:** 1122–1128.
13. Scatchard, G. 1949. The attraction of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51:** 660–675.
14. De Meyts, P., J. Roth, D. M. Neville, Jr., J. R. Gavin, and M. A. Lesniak. 1973. Insulin interactions with its receptors: experimental evidence for negative cooperativity. *Biochem. Biophys. Res. Commun.* **55:** 154–161.
15. Freychet, P., J. Roth, and D. M. Neville. 1971. Insulin receptors in the liver: specific binding of ^{125}I-insulin to the plasma membrane and its relation to insulin bioactivity. *Proc. Natl. Acad. Sci. U. S. A.* **68:** 1833–1837.
16. Gavin, J. R., III. 1977. Immunopharmacology. Chapter 17. Plenum Publishing Corporation, New York. 357.
17. Gambhir, K. K., J. A. Archer, and C. J. Bradley. 1978. Characteristics of human erythrocyte insulin receptors. *Diabetes.* **27:** 701–708.
18. Jarett, L., and R. M. Smith. 1975. Ultrastructural localization of insulin receptors on adipocytes. *Proc. Natl. Acad. Sci. U. S. A.* **72:** 3526–3530.
19. Tollefsen, D. M., J. R. Faegler, and P. W. Majerus. 1974. The binding of thrombin to the surface of human platelets. *J. Biol. Chem.* **249:** 2646–2651.
20. Rechler, M. M., and J. M. Podskalny. 1976. Insulin receptors in cultured human fibroblasts. *Diabetes.* **25:** 250–255.
21. Podskalny, J. M., J. Y. Chou, and M. M. Rechler. 1975. Insulin receptors in a new human placenta cell line: demonstration of negative cooperativity. *Arch. Biochem. Biophys.* **170:** 504–513.
22. De Meyts, P., A. R. Bianco, and J. Roth. 1976. Site-site interaction among insulin receptors: characterization of the negative cooperativity. *J. Biol. Chem.* **251:** 1877–1888.
23. Pollet, R. J., M. L. Standaert, and B. A. Hasse. 1977. Insulin binding to the human lymphocyte receptor: evaluation of the negative cooperativity model. *J. Biol. Chem.* **252:** 5828–5834.
24. Goldfine, I. D., A. L. Jones, G. T. Hradek, K. Y. Wong, and J. S. Mooney. 1978. Entry of insulin into human cultured lymphocytes: electron microscope autoradiographic analysis. *Science (Wash. D. C.).* **202:** 760–761.
25. Gorden, P., J. L. Carpentier, P. Freychet, A. LeCam, and L. Orci. 1978. Intracellular translocation of iodine-125-labeled insulin: direct demonstration in isolated hepatocytes. *Science (Wash. D. C.).* **200:** 782–785.
26. Kahn, C. R., and K. Baird. 1978. The fate of insulin bound to adipocytes: evidence for compartmentalization and processing. *J. Biol. Chem.* **253:** 4900–4906.
27. White, J. G. 1972. Interaction of membrane systems in blood platelets. *Am. J. Pathol.* **66:** 295–312.
28. Feagler, J. R., T. W. Tillack, D. D. Chaplin, and P. W. Majerus. 1974. The effect of thrombin on phytohemagglutinin receptor sites in human platelets. *J. Cell Biol.* **60:** 541–553.
29. Karpatkin, S. 1967. Studies of human platelet glycolysis: effect of glucose, cyanide, insulin, citrate, and agglutination and contraction on platelet glycolysis. *J. Clin. Invest.* **46:** 409–417.
30. Wamhoff, D., and G. Panzram. 1976. The influence of insulin, glucose and other monosaccharides on the platelet functions. *Klin. Wochenschr.* **54:** 1137–1141.
31. Hassanein, A. A., T. A. El-Garf, and Z. El-Baz. 1972. Platelet aggregation in diabetes mellitus and the effect of insulin *in vivo* on aggregation. *Thromb. Diath. Haemorrh.* **27:** 114–120.
32. Colwell, J. A., P. V. Halushka, K. Sarji, J. Levine, J. Sagel, and R. M. G. Nair. 1976. Altered platelet function in diabetes mellitus. *Diabetes.* **25**(Suppl. 2): 826–831.
33. Joist, J. H., R. K. Baker, and M. Smith. 1978. Platelet function in diabetes mellitus. *In* Platelet Function Testing. J. H. Day, H. Holmsen, and M. B. Zucker, editors. Department of Health, Education, and Welfare, Publication No. 78-1087.662-669.
34. Preston, F. E., J. D. Ward, B. H. Marcola, N. R. Porter, W. R. Timperly, and B. C. O'Malley. 1978. Elevated β-thromboglobulin levels and circulating platelet aggregates in diabetic microangiopathy. *Lancet.* **I:** 238–240.