Review and Abstracts

Does Insulin Need a Second Messenger?

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SUMMARY

It is well established that specific binding sites for insulin are present on the plasma membranes of target tissues. In order to explain how insulin regulates a wide variety of biologic functions both on the surface of the cell as well as in its interior, it has been postulated that insulin generates a second messenger at the cell surface. To date, however, no second messenger for insulin has been identified that can carry out all of insulin's known actions. Recent studies have demonstrated that, in addition to the plasma membrane, other subcellular organelles, such as the nucleus, have specific binding sites for insulin. There is also evidence indicating that large serum proteins such as albumin, large protein hormones such as prolactin, and small protein hormones such as insulin can enter intact cells. It is hypothesized, therefore, that insulin has at least two mechanisms of action on target tissues. One mechanism entails the direct binding of insulin to the plasma membrane, which in turn leads to its well-known effects on membrane transport. The other mechanism requires the entry of insulin itself into the interior of the cell and its subsequent direct binding to subcellular organelles. This latter process then serves to mediate many of the known intracellular functions of insulin. DIABETES *26*:148-55, February, 1977.

Insulin is a potent hormone that influences a wide variety of metabolic events in many tissues (table 1). These range from rapid effects on membrane transport to delayed effects on RNA and DNA synthesis.[1-9] However, despite over 50 years of research concerning the mechanism of action of insulin, it is still not known how the hormone regulates these diverse effects on target cells. Our ignorance of the action of insulin contrasts sharply with our knowledge of the action of glucagon, a hormone discovered 10 years later than insulin whose mechanism of action via the second messenger, cyclic 3′,5′-adenosine monophosphate (cyclic AMP), has been appreciated for over a decade.

For many years investigators have searched for a *single* mechanism that would explain all of the known effects of insulin. At present there is a widely held belief that all of insulin's actions are the result of its binding to a specific receptor on the surface of target cells.[16] This theory of insulin action originated in 1949 from the studies of Levine, Goldstein, and coworkers, who demonstrated that a major effect of insulin was to accelerate the membrane transport of sugars into the interior of target tissues.[5] In addition, insulin is known to increase the membrane transport of other important metabolic substances such as ions, amino acids, and nucleotide precursors.[1-4,6] Although the stimulation of membrane transport is a major effect of insulin on target tissues, for a number of reasons the stimulation of these transport functions cannot explain all of insulin's known actions.[1-4]

With the discovery that cyclic AMP was the second messenger for glucagon, an intensive search ensued for an analogous second-messenger mechanism for insulin. It had been known that insulin can antagonize the effects of hormones that act via cyclic AMP, and it was

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TABLE 1

Examples of the diverse effects of insulin

1. Stimulation of membrane transport[1-6]
2. Activation and inhibition of both membrane-bound and soluble enzymes[7]
3. Stimulation of protein synthesis[1-4,8,9]
4. Inhibition of protein degradation[10]
5. Stimulation of messenger RNA synthesis[2-4,11]
6. Stimulation of DNA synthesis[2-4,12,13]
7. Alteration of cell morphology[14,15]

later found that under certain circumstances insulin can blunt the hormone-induced elevations of this nucleotide.[2-4,17] Further, it has been demonstrated that insulin can increase the activity of cyclic AMP phosphodiesterase,[18-20] the enzyme that degrades cyclic AMP, and that under certain circumstances insulin can inhibit adenylate cyclase,[21] the enzyme that forms cyclic AMP. For a number of reasons, the lowering of hormone-stimulated cyclic AMP levels cannot explain all of the known effects of insulin.[2-4] In fact, under certain circumstances insulin can elevate cellular levels of cyclic AMP.[22]

Several other substances have been proposed as second messengers for insulin. Larner, on the basis of his extensive studies with the enzyme glycogen synthetase, has postulated that insulin produces a unique nucleotide that is interconvertible with cyclic AMP.[23] Others have noted that under certain circumstances insulin can elevate cellular levels of the nucleotide cyclic GMP.[24] Because insulin is known to alter the ion content of target cells,[25] it also has been postulated that ions may be second messengers for insulin.[26] It has been observed that insulin can alter calcium distribution in fat cells,[27,28] and insulin has been reported to directly stimulate ATPase activity in plasma membrane fractions.[29,30] At present, however, there is not enough evidence to indicate that any of these phenomena constitute a second-messenger system that can explain all of insulin's diverse actions.

A major advance in endocrinology has been the development of techniques, employing radioiodinated hormones, to characterize directly polypeptide hormone receptors on or in target cells. The validity of this method was first unequivocally demonstrated by Lefkowitz, Roth, and co-workers[31] for ACTH in solubilized adrenal tumor membranes and was soon employed by Freychet, Roth, and Neville to identify insulin receptors in highly purified rat liver plasma membranes.[32] Subsequent studies then demonstrated that purified plasma membranes from a variety of target tissues have high-affinity specific binding sites for insulin.[33-36] At first, these observations supported the theory that insulin acts exclusively at the cell membrane; however, it is now clear that intracellular organelles also have specific binding sites for insulin. Studies from this laboratory demonstrate that highly purified nuclei isolated from both rat liver and human cultured lymphocytes have specific high-affinity binding sites for insulin that are similar in many respects to those found on the plasma membrane.[37] Other investigators have found specific binding sites for insulin on a variety of intracellular membranes,[38] including Golgi membranes,[39] and have also identified binding sites for insulin in the rough and smooth endoplasmic reticulum[36] and in mitochondria.[40] The functional significance of these intracellular binding sites, however, has not been appreciated because of the strongly held belief that insulin does not enter the intact cell.

It is well known that other families of hormones, such as the steroid and thyroid hormones, also exert diverse effects on many tissues, and it is now clear that these hormones carry out many of their actions after they enter the interior of target cells. Because insulin has a much higher molecular weight than either the steroid or thyroid hormones, it was initially thought unlikely that insulin entered the intact cell.[41] This concept of insulin acting only at the plasma membrane was first supported by those experiments employing insulin covalently coupled to beads of insoluble agarose.[42] Such insulin-agarose preparations, the particles of which are too large to enter the intact cell, were initially reported to have a biologic potency close to that of native insulin.[42] Had these experiments been valid, they would have provided proof that insulin need not enter the cell to exert its actions. However, the validity of these original experiments was questioned on technical grounds,[16,43] and it was demonstrated subsequently that the insulin-agarose complex has only about 1 per cent of the activity of native insulin.[44,45] Further, the biologic activity of insulin-agarose can be accounted for by the solubilization of free insulin from the insulin-agarose complex, which occurs when the complex comes into contact with biologic materials.[44-47]

It has been demonstrated that large proteins, such as immunoglobulins,[48] plant lectins,[48] albumin,[49] histones,[50] lipoproteins,[51] and enzymes,[52] can enter the intact cell. Also, it is known that protein hormones can enter the interior of target cells. Nordquist and Palmieri, employing both light- and electron-microscopic autoradiography, have demonstrated that

parathormone is taken up by cells of the renal tubule and that this hormone is then concentrated in the mitochondria.[53] In addition, Cohen and co-workers, employing iodinated antibodies and selective trypsinization, have found indirect evidence that epidermal growth factor, a hormone with a molecular weight of approximately 5,400, enters target cells.[54] Finally, prolactin, a large protein hormone, has been identified by immunohistochemical techniques to be present in the interior of mammary gland cells.[55] Thus, there would appear to be no a priori reason why insulin, a hormone approximately one-third the size of prolactin, cannot enter the intact cell.

There is, in fact, evidence indicating that insulin enters target cells. Over 20 years ago, Lee and Williams infused radioiodinated insulin into the portal vein of rats, homogenized the liver, prepared various subcellular fractions, and found that the insulin was associated with the nuclear, mitochondrial, and microsomal fractions.[56] These investigators concluded that insulin entered the liver cell and then bound to cytoplasmic structures and the nucleus. These studies, however, have largely been ignored. Stein and Gross, employing both binding and autoradiographic techniques, found intact insulin in the cytoplasm of muscle, liver, and lung cells.[57] From their studies on the binding and degradation of insulin by isolated hepatocytes, Terris and Steiner have deduced that insulin must enter the hepatocyte in order to be degraded.[58] Also, Arguilla and co-workers have found that immunoreactive insulin is concentrated in rat liver nuclei.[59] Finally, preliminary studies from the laboratory of this author, employing human lymphocytes in culture, indicate that insulin enters the intact cell. When these lymphocytes are incubated with radioiodinated insulin, extensively washed, and homogenized, and purified nuclei are prepared, a significant portion of the total cellular-bound insulin is associated with the nucleus.[60] In addition, autoradiographs of these cells, after incubation with radioiodinated insulin, demonstrate that insulin enters the cell and then binds to the nucleus.

It is still possible that insulin regulates all of its actions via a second messenger generated at the plasma membrane. However, since intracellular structures have specific binding sites for insulin, and since it is very likely that insulin does enter the intact cell, an alternate theory provides a simpler explanation for the diverse effects of this hormone. According to this formulation, insulin in the extracellular fluid binds to receptors on the cell surface, and as a direct result of this interaction there are changes in plasma membrane functions, such as transport and enzyme activity. In addition to binding to the plasma membrane, insulin enters the interior of the cell and then binds to the nucleus, endoplasmic reticulum, Golgi apparatus, and other structures. As a direct result of binding to these intracellular structures, insulin itself then mediates various intracellular functions leading to the synthesis of DNA, RNA, and protein.

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ABSTRACTS

(All are verbatim summaries)

Bach, Gideon; Friedman, Robert; Weissmann, Bernard; and Neufeld, Elizabeth F. (NIAMDD, NIH, Bethesda, Maryland, and Dept. of Biochem., Univ. of Illinois, Col. of Med., Chicago, Ill.): THE DEFECT IN THE HURLER AND SCHEIE SYNDROMES: DEFICIENCY OF α-L-IDURONIDASE. Proc. Natl. Acad. Sci. *69*:2048, August, 1972.

Skin fibroblasts cultured from patients affected with the Hurler or Scheie syndromes (mucopolysaccharidoses I or V, respectively) have a functional deficiency of a protein required for catabolism of sulfated mucopolysaccharide that has been designated the "Hurler corrective factor." We now show Hurler factor purified from normal human urine to be associated with α-L-iduronidase activity. Cell lines deficient in Hurler corrective factor have no detectable activity of α-L-iduronidase (less than 3% of that found in cells from individuals of other genotypes). Such correspondence indicates that Hurler corrective factor and α-L-iduronidase are the same entity. Correction of deficient cells is accompanied by an efficient uptake of α-L-iduronidase from the medium.

Bergeron, J. J. M.; Evans, W. H.; and Geschwind, I. I. (National Inst. for Med. Rsch., London, N. W. 7, England): INSULIN BINDING TO RAT LIVER GOLGI FRACTIONS. J. Cell Biol. *59*:771, 1973.

Although Golgi elements were first obtained from rat liver homogenates as a single fraction a new procedure was recently developed which yielded three fractions which accounted for all of the Golgi elements present in the microsomal fraction, as indicated by recovery of UDP-galactosyl transferase. Morphological analysis showed that the Golgi "light" and "intermediate" subfractions contained a large number of large and small vesicles filled with very low density lipoprotein (VLDL) particles, whereas in the Golgi "heavy" subfraction, flat cisternal elements derived from the cis face of the Golgi apparatus predominated. Low activities of enzymes (e.g., glucose-6-phosphatase) associated with the microsomal fraction were detected, but all fractions possessed 5′-nucleotidase activity. Cytochemical tests indicated that the presence of 5′-nucleotidase (normally considered as a plasmalemma marker) in the Golgi subfractions was not due to contamination by plasmalemma membranes, but that 5′-nucleotidase was an indigenous component of the Golgi. We now report the detection of insulin-binding activity in the Golgi subfractions.

Brown, Michael S.; and Goldstein, Joseph L. (Univ. of Texas Allied Health Sci. Centr. at Dallas, Dallas, Texas): RECEPTOR-MEDIATED CONTROL OF CHOLESTEROL METABOLISM. Science *191*:150, 1976.

Cholesterol, a major component of all mammalian plasma membranes, is vital to cell growth and survival; yet, excessive amounts of this sterol can also be lethal, as is evidenced by the cholesterol deposition in arterial cells that potentiates the development of atherosclerosis. Thus, mammalian cells are faced with the dual problem of providing sufficient cholesterol for membrane growth and replenishment and, at the same time, of avoiding excessive accumulation of this insoluble substance.

In this article we discuss a mechanism by which certain mammalian cells, such as cultured human fibroblasts, utilize a specific cell surface receptor to accomplish their dual task. This receptor, designated the low density lipoprotein (LDL) receptor, binds the major cholesterol-carrying lipoprotein of plasma and thereby regulates the rate at which this lipoprotein transfers its cholesterol into the cell. The LDL receptor itself is under feedback regulation so that its activity (and hence the amount of cholesterol that enters the cell) is inversely proportional to the cellular content of cholesterol. Thus, cultured fibroblasts obtain cholesterol by increasing the number of receptor molecules, and conversely they protect themselves against an overaccumulation of the sterol by suppressing the synthesis of LDL receptors.

Carpenter, Graham; and Cohen, Stanley (Dept. of Biochem., Vanderbilt Univ., Nashville, Tenn.): ^{125}I-LABELED HUMAN EPIDERMAL GROWTH FACTOR: BINDING, INTERNALIZATION, AND DEGRADATION IN HUMAN FIBROBLASTS. J. Cell Biol. *71*:159, 1976.

^{125}I-labeled human epidermal growth factor (hEGF) binds in a specific and saturable manner to human fibroblasts. At 37° C, the cell-bound ^{125}I-hEGF initially may be recovered in a native form by acid extraction; upon subsequent incubation, the cell-bound ^{125}I-hEGF is degraded very rapidly, with the appearance in the medium of ^{125}I-monoiodotyrosine. At 0° C, cell-bound ^{125}I-hEGF is not degraded but slowly dissociates from the cell.

The data are consistent with a mechanism in which ^{125}I-hEGF initially is bound to the cell surface and subsequently is internalized before degradation. The degradation is blocked by inhibitors of metabolic energy production (azide, cyanide, dinitrophenol), some protease inhibitors (Tos-Lys-CH_2Cl, benzyl guanidobenzoate), a lysosomotropic agent (chloroquine), various local anesthetics (cocaine, lidocaine, procaine), and ammonium chloride.

After the binding and degradation of ^{125}I-hEGF, the fibroblasts are no longer able to rebind fresh hormone. The binding capacity

of these cells is restored by incubation in a serum-containing medium; this restoration is inhibited by cycloheximide or actinomycin D.

Goldfine, Ira D.; and Smith, George J. (Metab. Section, Dept. of Med., VA Hosp. San Francisco, Calif. and Dept. of Med., Univ. of California Hosp., San Francisco): BINDING OF INSULIN TO ISOLATED NUCLEI. Proc. Natl. Acad. Sci. *73*:1427, May, 1976.

Specific binding sites for ^{125}I-labeled insulin were detected in purified nuclei isolated from rat liver. Binding was rapid, reversible, and directly proportional to the number of nuclei employed. Unlabeled native insulin, at concentrations as low as 1 ng/ml, significantly inhibited the binding of labeled hormone, whereas unlabeled proinsulin and desoctapeptide insulin were less potent. In contrast, glucagon, thyrotropin, growth hormone (somatotropin), and prolactin were without effect. Under identical incubation conditions, ^{125}I-labeled glucagon bound to liver plasma membranes 5- to 10-fold more strongly than did insulin; in contrast, glucagon did not bind to liver nuclei.

These studies demonstrate the presence of specific binding sites for insulin in purified nuclei isolated from rat liver. In addition, they suggest that the nucleus may be an intracellular site of insulin action.

Horvat, Agnes; Li, Edith; and Katsoyannis, Panayotis G. (Dept. of Biochem., Mt. Sinai Sch. of Med., City Univ. of New York, New York, New York): CELLULAR BINDING SITES FOR INSULIN IN RAT LIVER. Biochim. Biophys. Acta *382*:609, 1975.

A study of the sites of insulin binding in subcellular fractions of rat liver is reported. A method for the isolation of liver plasma membranes, which permits one to follow quantitatively the distribution of all the parameters of interest, was modified and applied to the study of the cellular topography of insulin binding. The insulin-binding capacity did not follow closely the enzyme marker (5′-nucleotidase) for plasma membranes when differential centrifugation schemes were used, and the divergence from this marker was more prominent when separations were performed on discontinuous sucrose gradients. A significant amount of insulin binding capacity was always present in fractions with higher density than those containing the majority of 5′-nucleotidase. Results of studies on linear sucrose gradients have disclosed in some of the purified membrane fractions small but consistent differences in density of the insulin binding, and plasma membrane particles. It is suggested that there may be several types of intracellular membranes to which insulin can bind besides the plasma membranes.

Kahn, C. Ronald (Diabetes Branch, NIAMDD, NIH, Bethesda, Maryland): MEMBRANE RECEPTORS FOR HORMONES AND NEUROTRANSMITTERS. J. Cell Biol. *70*:261, 1976.

Over the past 20 yr. evidence has come from a variety of sources to suggest that the primary event in the action of peptide hormones and some neurotransmitters is binding to a specific site on the plasma membrane of the cell known as a receptor site. Most early studies attempted to define these hormone receptors by studying the steps of hormone action in reverse order, i.e. by investigation of effects of various factors on hormonally induced biological responsiveness in intact animals or intact tissues. More recently, with development of methods for preparation of high specific activity, biologically active labeled hormones, and hormonally responsive cells or cell membranes, direct study of hormone-receptor interactions has become possible. Such studies have now been employed for a wide variety of tissues and a large number of peptide hormones and neurotransmitters. These studies have resulted in a significant increase in our understanding of the chemical nature of receptors and how they interact with their hormonal ligands. Many aspects of the hormone-receptor interaction have been discussed in previous reviews. The purpose of this review is to bring together some concepts about the hormone receptor as a component of the cell membrane, the chemical nature of hormone receptors, and cellular regulation of hormone receptors as they relate to the biology of the cell. Only the receptors for peptide hormones and for the cholinergic and the β-adrenergic neurotransmitters will be considered. Receptors for viruses, immunoglobulins, and drugs demonstrate many features similar to those of the hormone receptors; however, a review of these is outside the scope of this article.

Kolb, Helmut J.; Renner, Rolf; Hepp, K. Dieter; Weiss, Ludwig; and Wieland, Otto H. (Institut für Diabetes, Forschung, 8000 Munchen 40, Kolner Platz 1, Germany): RE-EVALUATION OF SEPHAROSE-INSULIN AS A TOOL FOR THE STUDY OF INSULIN ACTION. Proc. Natl. Acad. Sci. *72*:248, January, 1975.

The biological activity of Sepharose-insulin in different assays *in vitro*, e.g., stimulation of glucose oxidation, lipogenesis, and antilipolysis and activation of pyruvate dehydrogenase (EC 1.2.4.1) activity, has been investigated. According to amino acid analysis, between 270 and 330 μg (6.9–8.2 U) of insulin were coupled per ml of packed beads. Related to the total insulin content, 0.2–0.7% of the insulin was biologically active. Comparable biological activity was observed with isolated fat cells and fat pad pieces. After incubation with tissue or cells, Sepharose-insulin particles were separated by centrifugation from the medium. The clear supernatant was assayed for biologically and immunologically reactive insulin and contained soluble insulin activity. A quantitative evaluation of the soluble biological and immunological insulin activity in the supernatant accounted for the total insulin activity of Sepharose-insulin.

Lee, Norman D.; and Williams, Robert H. (Dept. of Med., Univ. of Washington Sch. of Med., Seattle, Wash.): THE INTRACELLULAR LOCALIZATION OF LABELED THYRONINE AND LABELED INSULIN IN MAMMALIAN LIVER. Endocrinology *54*:5, 1954.

Using I^{131} as a label, it was shown that the intravenous administration of either thyroxine or insulin to the rat is followed by concentration of these hormones in the liver. This concentration process involves passage through the cell wall and fixation to the various cytoplasmic structures and to the nucleus. The distribution of thyroxine-I^{131} among the cytostructural elements is different from that for insulin-I^{131} and such differences are maintained with respect to the time studies.

Insulin, localized in the rat liver, appears to be completely resistant to removal by perfusion whereas this technique removes a relatively large quantity of the localized thyroxine.

The nature of the binding of thyroxine intracellularly changes

with respect to time and is a characteristic of the cytostructural element under consideration.

Nordquist, Robert E.; and Palmieri, Genaro M. A. (VA Hosp., Oklahoma Med. Rsch. Foundation, and Depts. of Path. and Med., Univ. of Oklahoma Health Sci. Centr., Oklahoma City, Oklahoma): INTRACELLULAR LOCALIZATION OF PARATHYROID HORMONE IN THE KIDNEY. Endocrinology 95:229, 1974.

Partially purified bovine parathyroid hormone (PTH) and highly purified ^{125}I-PTH were infused simultaneously into dogs. The kidneys were studied by fluorescent microscopy and light and electron autoradiography. By immunofluorescence PTH was found to be present only in the cytoplasm of renal tubules. Light microscopic examination of autoradiographs revealed a selective localization of labeled PTH in cells of renal proximal tubules. Electron autoradiographic studies showed most of the labeled hormone to be in the mitochondria of proximal tubular cells. No localization of labeled PTH in renal cortex was observed when ^{125}I-PTH was administered after a load of unlabeled PTH. Biologically active ^{3}H-PTH demonstrated identical localization at the light microscopic level in renal proximal tubules in the rat.

These studies demonstrate that exogenous PTH is selectively localized in the cytoplasm of renal proximal tubular cells conserving at least partially its immunoreactivity and that labeled PTH is found predominantly in mitochondria. Whether the intracellular localization of PTH is related to a biological effect of the hormone or to its destruction or both cannot be answered by the present studies. It is however interesting that a hormone thought to exert its physiological effect by stimulating adenyl cyclase at the plasma membrane level of target organs may be found *in vivo* in the intracellular space and in the mitochondria.

Ryser, J. J.-P. (Dept. of Pharmacol., Harvard Med. Sch., Boston, Mass.): UPTAKE OF PROTEIN BY MAMMALIAN CELLS: AN UNDERDEVELOPED AREA. Science *159*:390, 1968.

Although it is accepted on the basis of biological and morphological evidence that mammalian cells will take up macromolecules, little is known about the kinetics, the specificity, and the functions of this uptake. With labeled proteins used as models, it is found that the transport proceeds at very low rates, requires little energy, and is markedly enhanced by polybasic compounds. Molecular charge and size are important factors: cells clearly favor cationic macromolecules of large molecular weights. Neither factor, however, can fully account for the selectivity detected in the uptake of different proteins. Ingested albumin undergoes rapid and extensive degradation. This fact suggests that macromolecules have only a limited chance to express their biological activity in target cells, a finding that is relevant also to the role of foreign nucleic acids and the possibility of achieving genetic transformation in animal cells. There are concrete indications, however, that in spite of their short half-life, proteins can act as carriers, as precursors of active agents, and as regulators of metabolic functions in host cells. They may also be important in the control of growth and differentiation. These functions of exogenous proteins are still largely unexplored.

Stein, Olga; and Gross, Jack (Dept. of Exp. Med. and Cancer Rsch., The Hebrew Univ., Hadassah Med. Sch., Jerusalem, Israel): THE LOCALIZATION AND METABOLISM OF I^{131} INSULIN IN THE MUSCLE AND SOME OTHER TISSUES OF THE RAT. Endocrinology *65*:707, 1959.

The localization of I^{131} labelled-insulin in muscle and other organs of the rat was investigated. The kidneys and muscles were found to be the main regulators of the fate of injected insulin. The dose of insulin approaching the tracer dose was found to lie in the range of 0.025-0.05 μg./100 gm. b. w.

10 minutes after intravenous injection, insulin was shown to be distributed in a volume amounting to 19% of the volume of muscle. After subcellular fractionation of muscle cells a concentration in the soluble protein fraction was revealed. Autoradiographically this localization of I^{131} was shown to be evenly distributed throughout the muscle fiber, without any evidence of sarcolemmal concentration. An intracellular distribution was also found in heart muscle, liver and lungs.

In the diaphragm, following in vitro incubation with I^{131} insulin, the hormone was shown to localize mainly on the fascia covering the muscle. The radioactivity in all the experiments with muscle was shown to be 85% insulin and 15% iodinated amino acids, monoiodotyrosine or diiodotyrosine. In the liver the iodinated amino acids were shown to contribute about 45% of the total TCA precipitable radioactivity.

Terris, Susan; and Steiner, Donald F. (Dept. of Biochem., Univ. of Chicago, Chicago, Ill.): RETENTION AND DEGRADATION OF ^{125}I-INSULIN BY PERFUSED LIVERS FROM DIABETIC RATS. J. Clin. Invest. *57*:885, April, 1976.

The retention and degradation of insulin by isolated perfused liver have been examined. Noncyclically perfused livers from streptozotocin-diabetic rats retained 25% and degraded 10% of ^{125}I-insulin administered as a 1-min pulse. On gel filtration (Sephadex G50F), the degradation products released into the vascular effluent eluted in the salt peak. During the 45-min interval after the end of the ^{125}I-insulin infusion, 0.19% of the total dose was excreted in the bile. 60–90% of this material consisted of iodinated, low-molecular-weight degradation products.

Inclusion of native insulin with the ^{125}I-insulin in the pulse depressed both the retention and degradation of iodinated material; however, this reflected increased retention and degradation of the total insulin dose (^{125}I-insulin plus native hormone). The log of the total amounts of insulin retained and degraded were linearly related to the log of the total amount of insulin infused at concentrations between 12.7 nM and 2.84 μM. Increasing the amount of native insulin in the infused pulse also depressed the total amount of iodinated material found in the bile and led to the appearance in the bile of intermediate-sized degradation products that did not simultaneously appear in the vascular effluent.

Addition of high concentrations of glucagon to the infused ^{125}I-insulin had no effect on the retention or degradation of the labeled hormone, or on the apparent size and amount of iodinated degradation products found in the vascular effluent or in the bile. Preinfusion of concanavalin A inhibited both ^{125}I-insulin retention and degradation. A greater depression by concanavalin A of degradation than binding was also observed with isolated hepatocytes.

In contrast to ^{125}I-insulin, the retention and degradation of two

iodinated insulin analogues of relative low biological potency, proinsulin and desalanyl-desasparaginyl insulin, were small. The amount of radioactivity appearing in the bile after infusion of these analogues was almost negligible. However, degradation products of these analogues that appeared in the bile and in the vascular effluent were qualitatively similar to those found after the infusion of ^{125}I-insulin.

Our findings suggest that the rapid initial uptake of ^{125}I-insulin after its infusion into noncyclically perfused liver, as well as its subsequent degradation, behaves in a qualitatively similar fashion to the binding of ^{125}I-insulin and its degradation by isolated rat hepatocytes. This uptake and the subsequent phase of degradation may be attributable to binding of insulin at specific recognition sites, preliminary to its transfer to a degradative site(s) presumed to be located inside the cell.

Unanue, Emil R.; Perkins, William D.; and Karnovsky, Morris J. (Dept. of Path., Harvard Med. Sch., Boston, Mass.): LIGAND-INDUCED MOVEMENT OF LYMPHOCYTE MEMBRANE MACROMOLECULES. J. Exp. Med. *136*:885, 1972.

The fate of different complexes on the membrane of thymocytes and spleen lymphocytes was studied with the use of both immunofluorescence and ultrastructural radioautography. The complexes of anti-immunoglobulin (Ig) with the surface Ig of B lymphocytes were present all around the membrane at 4° C; an increase in temperature produced a rapid aggregation of the complex into a cap which was readily interiorized in vesicles. Ultrastructural details of this process were given. The movement of the complexes depended upon the amount of anti-Ig and the temperature.

The complexes of anti-lymphocyte antibody with surface antigen(s) did not result in formation of a single large aggregate (or cap) unless an anti-antibody was brought into the reaction. The caps formed by this trilayered complex were not interiorized. Concanavalin A (Con A) bound to cell surface carbohydrate moieties and the complexes of Con A readily formed a cap and were interiorized. Finally, antibodies to *H-2* determinants did not form in most instances a single cap aggregate even when anti-antibodies were used. With time the *H-2* complexes tended to form several large aggregates with some endocytosis.
