MECHANISM OF ACTION OF INSULIN

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INTRODUCTION

The literature on the mechanism of action of insulin on cells lacks a unifying concept despite the welter of experimental data. References to the classic experiments on insulin action and to recent reviews on various aspects of insulin action are found at the end of this article (1–10). Though insulin action has not been previously reviewed in this series, we restrict ourselves to work done over the last four to five years. This review reports in biochemical terms the effects of insulin on carbohydrate, fat, protein, and nucleic acid metabolism in the principal insulin sensitive tissues. After noting the individual effects of the hormone, we attempt to delineate possible mechanisms of action that account for these separate effects. This review emphasizes the effects of insulin action on carbohydrate metabolism, protein, and nucleic acid synthesis, and on the level of cyclic adenylate in cells.

EVIDENCE THAT THE ACTION OF INSULIN RESULTS FROM INITIAL INTERACTION WITH THE PLASMA MEMBRANE

Narahara (260) has reviewed past work on binding of insulin to tissues in relation to biological activity. The binding of insulin to tissue was first reported by Stadie et al (25) twenty years ago. This work was carried out using the isolated rat diaphragm exposed to iodoinsulin in vitro. The results were difficult to interpret, however, because of uncertainties as to whether the modified insulin had full biological potency (250, 251) and whether the hormone was actually bound or simply trapped in the interstices of the tissue. Antoniades & Gershoff (261) and later Crofford (22) showed that isolated fat cells could take up native insulin in a reaction that was so rapid that the uptake could be ascribed with reasonable certainty to binding to the plasma membrane. Since the insulin-membrane complex is stable in the cold (23, 251), these cells could be washed extensively at 0° to remove all

hormone not actually bound. Crofford (22), and subsequently Kono in detailed studies (43, 45), and others (23, 40, 54, 270, 271) have shown that the binding constant (K_e) for the hormone is much higher (3–7 nmol) than the concentration that gives half maximal activation of glucose utilization (K_e = 50 pmol). Cuatrecasas (27), and others (271, 272) have reported the existence of binding proteins of much higher affinity than the above. Kono (43, 45) and others (22, 23, 251) have proposed that only a small fraction of insulin receptors needs to interact with the hormone to generate a sufficient signal to induce metabolic changes. Kono (53) emphasizes that some processes (e.g. protein synthesis or lipolytic activation by insulin; see later), require much stronger signals than others (e.g. antilipolysis or glucose transport). The presence of many receptors allows strong signals to be developed, if necessary, and establishes sensitivity of the cell to low concentrations of hormone at all times. Sensitivity is promoted because the presence of receptors in large numbers, by mass action, increases the probability of hormone-receptor collision. Others (23, 27, 262) have suggested that cells have a small number of high affinity "specific" receptor sites and a large number of nonspecific sites. Kono & Colowick (263), Wohltmann & Narahara, (23) and Crofford (22) found that adipocytes washed at room temperature quickly lose insulin. Crofford (22) reported that insulin could not be found in the wash medium in an immunologically or biologically active form. This observation, along with his subsequent study (252, 253) with adipocyte plasma membranes, suggests that binding to the receptor may be followed by inactivation of the hormone. This is in line with the thought that any rapidly acting regulatory system must have very effective mechanisms to turn signals off as well as on. Others (27, 40, 264) using higher concentrations of insulin or unphysiological methods of dissociating the insulin receptor complex have found some of the hormone to be released in an active form. Recent work (264) suggests that the insulin receptor and an insulin degrading system in liver cell membranes are different.

Freychet et al (26) showed that lightly iodinated, fully biologically active insulin is bound to purified plasma membranes of rat liver. A number of investigators have extended this work and shown that plasma membranes from liver and fat cells (27–31), fibroblasts (32), lymphocytes (32), and the central nervous system (33) contain insulin-binding protein. The insulin-binding protein has been successfully dispersed from plasma membranes from fat cells and liver membranes (34, 35) using detergents. The binding protein of liver cells and adipocytes appears to be an asymmetrical protein, molecular weight about 300,000 with a frictional ratio of 1.7 and a Stokes radius of 71 Å (34). Cuatrecasas (35) has used affinity chromatography to isolate and purify an insulin-binding protein. It is not known, however, whether this is the physiological receptor, because no biological tests have been applied to it. Furthermore, it has been observed that membrane fractions exposed to phospholipase (C or A) show a great increase in insulin binding (27), whose significance is unknown.

It has been postulated that the binding of insulin to the membranes involves formation of disulfide bonds between the hormone and receptor (24, 36–38). Wohltmann & Narahara (23), however, were unable to relate the quantity of radioactive

insulin that is covalently bound through disulfide bridges with the effect of the hormone on transport. Recently, Cuatrecasas (27, 39) and Pilkis et al (40) showed that insulin binding to fat cell membranes or purified liver plasma membranes is not affected by sulfhydryl-blocking agents.

Exposure of muscle (254) or fat cells (41–43) to proteases in low concentrations can induce insulin-like effects. Kono first noted that treatment of fat cells with trypsin or other proteases in very high concentrations results in a loss of insulin binding (42–43) and of biological effects. Cuatrecasas (44) reported that the affinity of the binding protein for insulin is diminished by such treatment, whereas Kono & Barham (45) found a decreased number of binding sites with no change in affinity. The insulin "receptors" regenerate upon further incubation of the cells in the presence of soybean trypsin inhibitor at a rate of about 3% of the total number per hour (42, 44, 45). Prior addition of insulin protects the "receptor" from proteolytic attack (30). Cuatrecasas found that exposure of fat cells to neuraminidase reduces the basal rate of glucose uptake and prevents the action of insulin (49). Neuraminidase digestion, however, does not affect insulin binding to fat cells (49) or to purified liver plasma membranes (50).

Reduced binding of insulin has been found in liver membranes of obese-hyperglycemic mice (51) suggesting that a deficiency of receptor may be an important component of some insulin-resistant states. No alteration in the binding of insulin to fat cells of rats treated with steroid or streptozotocin has been found (52).

Cuatrecasas (11) has reported that insulin bound covalently to sepharose beads will increase glucose oxidation and inhibit lipolysis in isolated fat cells. He claimed that the insulin sepharose complex is stable during incubation with tissue and that the sepharose bead is not taken up by cells. Cuatrecasas concludes, therefore, that insulin does not need to enter the cell to exert an effect. Others have shown that insulin-Sepharose or insulin-Dextran has insulin-like effects in various cell types and in the intact animal in these ways: it stimulates incorporation of precursors into RNA in mammary cells (12), accumulation of amino isobutyric acid in isolated mammary cells (13), glycogen synthesis in tadpole liver (14), glucose oxidation and glycogen synthesis in diaphragm muscle (15), and causes hypoglycemia in the rat (265).

These data must still be accepted only with reservations. Davidson (16) & Fritz (17) have presented evidence that insulin begins to leak off the Sepharose bead, commencing shortly after washing and continuing progressively. Davidson et al (16, 21) have concluded that insulin-Sepharose may be useful for isolating detergent-dispersed insulin-binding protein but it is not a suitable model for studying the biological effects of bound insulin. Katzen & Vlahakes (18) have recalculated the data of Cuatrecasas (11) on insulin-Sepharose and question the validity of his results. For example, only a few beads, and in some cases, only a fraction of a bead per milliliter would have been present in an incubation flask in his experiments, although the biological effects obtained were purportedly equal to those given by the same amount of unbound insulin (19, 20). It is important to clarify these Sepharose-insulin experiments because they constitute potentially an ingenious and useful tool for determining that a hormone need not enter a cell to exert a biological effect.

Much data already suggest that this is true for insulin, in particular the studies (42, 43, 46–48) with trypsin in which the evidence seems good that the enzyme attacks only structures on the surface of the plasma membrane.

The insulin-receptor complex formation can be postulated to induce a conformational change in the receptor that leads to generation of a signal. Light exposure to trypsin (41–43) or to *p*-chloromercuribenzoate (273) apparently also triggers effectively the generation of this or a similar signal which activates glucose utilization, inhibits lipolysis, and lowers the level of cAMP. Heavy exposure to trypsin or other proteases destroys the capacity of the receptor to bind hormone or to generate a signal. A very light exposure to N-ethylmaleimide blocks the insulin stimulation of transport without affecting the basal rate of transport or other metabolic parameters (266, 268). Binding of insulin is not blocked under these conditions but is apparently blocked by substantially heavier exposure to maleimide (22). These results suggest that the receptor is in part, at least, a peptide or protein. Kono & Barham (43, 45) estimate that a fat cell possesses about 160,000 receptors and that glucose utilization is maximally stimulated when 4000 of these sites are occupied. Crofford (22) reported that binding of 3000 molecules of insulin would stimulate glucose utilization maximally and that complexing of about 100 insulin molecules can generate a detectable metabolic response.

What is the nature of the signal generated by insulin-receptor interaction? This is the central, unanswered question with regard to the mechanism of insulin action. Some speculations are considered later.

EFFECTS OF INSULIN ON MUSCLE

Protein Metabolism

The molecular mechanism whereby insulin controls protein metabolism has been most extensively studied in muscle (55). So far as presently known, insulin appears to stimulate synthesis of all proteins in muscle, whereas in some other tissues, synthesis of specific enzymes is involved. Wool & Krahl (56) first demonstrated that the effect of insulin on incorporation of amino acids into diaphragm muscle protein was independent of the action of the hormone on glucose transport. This is also true of the perfused rat heart (55).

A number of observations regarding insulin effects on protein synthesis have been made using ribosome preparations. It has been shown that ribosomes from heart and skeletal muscle of diabetic animals do not synthesize protein as well as do preparations from normal animals (55, 57). Furthermore, insulin treatment of the diabetic animal increases the assembly of polysomes and the synthesis of protein by ribosomes by a mechanism that does not require RNA synthesis (58). That is, the effect of insulin is on translation of mRNA, not its synthesis. Martin & Wool (59) showed a defect in the 60S ribosomal subunit of diabetic muscle as regards its ability to carry on poly(U)-stimulated protein synthesis and to bind phenylalanine tRNA. This defect probably derives from the fact that ribosomes from normal liver retain greater amounts of bound peptidyl-tRNA when dissociated than do ribosomes isolated from

the diabetic tissue (60, 61). Apparently peptidyl-tRNA bound to the ribosome acts as an initiator of peptide bond formation and aminoacyl-tRNA binding. Since some peptidyl-tRNA remains bound to the 60S ribosomal subunit when ribosomes are dissociated (60), the differing amounts of peptidyl-tRNA bound to muscle ribosomes from normal and diabetic animals would appear to be a plausible explanation for the difference in activity between the two preparations.

The reduction in peptidyl-tRNA bound to ribosomes from diabetic animals is probably explained by decreased initiation of endogenous protein synthesis. Rannels et al (62) have shown that levels of ribosomal subunits are increased in skeletal muscle but not in hearts from diabetic animals. Moreover, when protein synthesis of normal hearts perfused with glucose and amino acid is stimulated by addition of insulin, levels of subunits decrease and levels of polysomes rise, indicating that insulin has stimulated the initiation of peptide chains (63). Jefferson et al (64), employing an isolated perfused preparation of rat hemicorpus, have also shown that insulin decreases the levels of ribosomal subunits in this tissue.

In an attempt to study the initiation process itself, Wool et al (61) have studied the translation of polyuridylic acid and a viral mRNA by ribosomes from normal and diabetic animals. Ribosomes from diabetic animals were less efficient than ribosomes from normal animals. However, the difference tended to disappear after ribosomes were dissociated to subunits and then reconstituted (61). Wool et al (61) have also reported that ribosomal subunits isolated from muscle of diabetic animals reassociate less readily than normal in the presence of an initiation factor from rat liver.

Chain & Sender (65) report that protein synthesis by the perfused heart as well as the polysome profiles and amino acid incorporation by ribosomal preparations of these hearts are unaltered by streptozotocin-induced diabetes. These findings agree with those of Rannels et al (62) but are at variance with those of Wool et al (55).

It has been suggested that in heart, but not in skeletal muscle (65), elevated plasma concentrations of free fatty acids may be important in maintaining normal rates of protein synthesis (64).

Sender & Garlick (66) report that insulin stimulates labeling of all proteins separated by disc gel electrophoresis from extracts of the heart perfused with radioactive amino acid. They could detect no selective effect of the hormone on synthesis of specific proteins. Wool et al (55) earlier presented evidence that insulin stimulates the synthesis of a specific protein in diaphragm muscle, but this observation has not been confirmed.

The question of whether insulin may affect protein metabolism, and ultimately even ribosomal activity, by an effect on transport of amino acids across the cell membrane is still unanswered. It has been shown, in this connection, that raising the concentration of amino acids perfusing a rat heart (63) or liver preparation (274) will promote ribosome aggregation and increase incorporation into protein, thus duplicating effects of the hormone. Insulin in vitro stimulates the transport of some amino acids and not of others (67) whereas it stimulates incorporation of all naturally occurring amino acids, including those formed intracellularly into protein (68).

This would suggest that increased incorporation is not due entirely to increased uptake. This conclusion remains uncertain, however, because it is unclear whether amino acids entering the cell are incorporated preferentially into protein or mix with the pool of free amino acids within the tissue (67, 69). Manchester (69) has reviewed the effects of insulin on uptake of amino acids into muscle and the relation of this accumulation to the stimulation of amino acid incorporation into protein. In general, enhancement of amino acid transport in muscle by insulin does not appear to be a sufficient explanation for the hormone effect on protein synthesis (69).

Pozefsky et al (70) have shown recently that insulin reduces the plasma content of amino acids, in part at least, through suppression of release from msucle. All amino acids are affected, though quantitatively to different degrees.

Carbohydrate Metabolism in Muscle

The effects of insulin on carbohydrate metabolism have been well reviewed (5, 17, 88).

Glucose transport is the principal rate-limiting step for use of glucose in muscle under physiological conditions. The conclusion that insulin accelerates glucose transport has been confirmed by many investigators. Effects of insulin on kinetic parameters of transport have been investigated in perfused heart where the hormone increases the maximal rate of transport and appears to decrease the affinity somewhat (71–74). An increase in the maximal rate is also observed in skeletal muscle (75). The biochemical mechanism accounting for these changes is unknown (78), except that the movement of the carrier across the membrane is presumably increased. The rapidity of insulin action suggests that synthesis of new carrier is not involved. Weis & Narahara (254), using the frog sartorius preparation, have shown that trypsin stimulates glucose transport in a manner similar to insulin. This has also been observed in adipose cells (see later).

Gould & Chaudry (76) have studied the effect of cations on the action of insulin on glucose uptake by isolated rat soleus muscle. They found that stimulation of glucose uptake by insulin does not depend on monovalent cations in the incubation medium. Muscle depleted of Ca^{2+} and Mg^{2+} by EDTA treatment has a depressed glucose uptake which is not stimulated by insulin. Replacement of Mg^{2+} and Ca^{2+} restores the basal uptake to normal. However, Mg^{2+}, and not Ca^{2+}, is required for the stimulation by insulin. It is postulated that Mg^{2+} participates in the mechanism whereby insulin promotes the binding of glucose to the transport carrier. In our opinion, however, these studies do not distinguish between effects on transport and intracellular events. Gould & Chaudry also report (77) that anoxia does not stimulate glucose uptake in EDTA-treated muscle but does so in the presence of a low concentration of insulin, which by itself has no effect.

Tarui et al (15) have shown that insulin bound to dextran (molwt 40,000) stimulates glycogen formation in parallel with the facilitation of glucose transport in a manner similar to that of native insulin. This suggests that insulin exerts its effects in muscle on glycogen synthesis as well as glucose transport without entering the cell. Under certain conditions, an effect of insulin to control transport is seen without an effect on glycogen synthesis, while insulin can, under other conditions, affect

have studied the effect of insulin on the pattern of glucose metabolism in the isolated

perfused rat heart. In agreement with many previous studies, insulin stimulates glucose oxidation to CO_2, glycogen synthesis, and formation of phosphorylated sugar and lactate. Increasing the concentration of glucose in the perfusion medium leads to changes in the pattern of glucose metabolism that are quite different from those brought about by insulin. The effect of a work load on the pattern of glucose metabolism is also different from that of insulin. Beitner & Kalant (91), using rat hemidiaphragms, and Ozand & Narahara (92), using frog sartorius muscle, have shown that insulin stimulates the rate of glycolysis in muscle independently of its effect on glucose transport. Both groups conclude that insulin stimulates the phosphofructokinase reaction.

The Adenylate Cyclase System and Insulin Effects in Muscle

Wool et al (55) have reported that cAMP and caffeine inhibit amino acid incorporation in the isolated rat diaphragm. However, protein synthesis by ribosomes isolated from normal and diabetic rats is not influenced by addition of cAMP and caffeine to the cell-free systems (55).

Walaas et al (79) and Craig et al (80) have found no effect of insulin on cAMP content in the rat diaphragm. Insulin can increase the level of glycogen synthetase I without influencing that of cAMP (81), although, under certain conditions, a decreased cAMP concentration may be seen. Insulin injection into rats causes a slight increase in skeletal muscle cAMP content (82). Insulin does not alter the level of cAMP in perfused rat heart under conditions in which glucose transport is strongly stimulated (275). Goldberg et al (82) have reported that insulin in the presence of epinephrine elevates cAMP slightly in the isolated diaphragm. Although incubation of diaphragm with insulin augmented the rise in cAMP induced by epinephrine, glycogen phosphorylase activation was unaffected. Keely et al (83) have found that insulin in combination with epinephrine elevates cAMP in heart as in diaphragm.

Drummond et al (86) have reported that insulin in vitro does not affect the adenylate cyclase of purified plasma membranes of skeletal muscle in the presence or absence of epinephrine. Das & Chain (87) have reported a stimulating effect on cAMP phosphodiesterase in extracts of the isolated rat heart perfused with insulin. At present, it seems unlikely that cAMP is directly involved as a mediator of insulin action in muscle.

Larner (84) has postulated that insulin leads to formation of a nucleotide intermediate (in analogy to Murad et al, 85) that is in chemical equilibrium with cAMP. This "second messenger" would mediate the effects of insulin. The idea of a second messenger for insulin is an old one (269) but continues to be the leading theory of insulin action in many laboratories. There is, however, no experimental evidence whatsoever for the existence of such a compound.

EFFECTS OF INSULIN ON MAMMARY GLAND

Study of the effect of insulin and other hormones on cell proliferation in organ culture of mammary gland has yielded much information concerning the control of DNA, RNA, and protein synthesis. It is clear that this regulation in many types of cells is dependent upon exposure to several hormones in a well-defined sequence.

In explants of mammary glands cultured on chemically defined medium, 70% or more of the epithelial cells can be induced by insulin to proliferate (93, 94). Insulin stimulates incorporation of precursors into RNA and protein during the first 8–12 hr of culture, and shortly thereafter an increase in DNA synthesis is observed (94). Turkington and co-workers (95, 96) have also observed increased precursor incorporation into ribosomal RNA and rapidly labeled RNA after addition of insulin to intact cells. Addition of insulin to cells also results in an increased RNA polymerase activity measured in isolated nuclei (95, 96). Associated with these changes are increased rates of phosphorylation of histones and nonhistone proteins (97). Insulin induces initiation of DNA replication in the epithelial cells (93, 98) and, subsequent to this, cell division occurs. Both growth hormone and epithelial growth factor have similar effects on these cells (93, 99, 100).

Alevolar cell differentiation is dependent on the interaction of many hormones (94). Insulin, hydrocortisone, and prolactin increase the number of functionally differentiated cells (101, 102). The addition of all three hormones is necessary for a maximal response, but insulin or epithelial growth factor alone is adequate to stimulate cell division and differentiation in the proliferative stage (103). However, the effects of prolactin seen in the postmitotic period require insulin (103).

Stimulation of precursor incorporation into rapidly labeled RNA of isolated mammary cells has been reported using insulin covalently bound to Sepharose (12). Insulin did not change the specific activity of the intracellular precursor pool (12). These observations suggest action of the hormone at the cell membrane to stimulate an intracellular process. The reliability of insulin-sepharose as a tool is questionable, however, in view of the reservations already discussed.

Majumder & Turkington (104) have provided evidence that the level of cAMP is not rate limiting in the developing mammary gland. In organ cultures of mouse mammary tissue and in isolated epithelial cells, prolactin induces the synthesis of protein kinases in cells formed in vitro by insulin-mediated cell division (104). Insulin acts synergistically with prolactin for induction of the catalytic subunit of protein kinase, but induction of the cAMP-binding protein requires prolactin only. Neither hormone affects the activity of mammary cell plasma membrane adenylate cyclase. Addition of cAMP, dibutyryl cAMP, or theophylline to the incubation medium does not substitute for insulin, hydrocortisone, or prolactin. The regulatory polypeptide hormones in this system interact initially with the cell membrane (12, 104), but they do not affect adenylate cyclase activity. These data on the lack of role of cAMP in regulation of metabolism in developing mammary gland have not yet been confirmed by other workers.

INSULIN EFFECTS ON LIVER METABOLISM

Effects

As in muscle, insulin appears to have a general anabolic (or anticatabolic) effect in liver, but, in addition, insulin influences the levels of a number of specific proteins. Miller and co-workers have emphasized the essential nature of insulin in promoting positive nitrogen balance in the perfused liver (105). Mortimore & Mondon (106)

have reported that insulin diminishes the release of 1-C^{14}-valine from prelabeled proteins in the perfused liver. They have evidence that this is an effect of the hormone on lysosomal-induced proteolysis. It has also been postulated that insulin may stabilize hepatic lysosomes (107). Vavrinkova & Mosinger (177) reported that insulin administration decreases rat hepatic acid phosphatase, while glucagon elevates this activity. These authors conclude that the release of lysosomal enzymes in liver is hormonally sensitive and suggest that this process may well be under control of cAMP.

Microsomal and purified ribosomal preparations from livers of diabetic animals incorporate amino acids less efficiently than do such preparations from normal animals (108–111). Pilkis & Korner (110) find that this defect is most prominent in the free polysome fraction which has a lower than normal proportion of large polysomal aggregates. Insulin administration for 4 hr in vivo completely restores the activity and profile of the free polysome fraction to normal. As in diabetic muscle, ribosomes from diabetic liver have less bound peptidyl-tRNA, which may account for their reduced ability to incorporate amino acids into protein. Wittman et al have reported detectable effects of insulin injection within 5 min on rat liver polysome profiles (112).

The effect of insulin on the biosynthesis of hepatic enzymes has been well reviewed by Steiner (116). Insulin administration to diabetic rats over a period of days decreases the levels of gluconeogenic enzymes (pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase, and glucose-6-phosphatase), and increases the levels of the glycolytic enzymes (glucokinase, phosphofructokinase, and pyruvate kinase) (113). Protein and RNA synthesis are required for these hormone effects, but there is no evidence that insulin acts directly as an inducer or repressor of enzyme synthesis (116). In general, it seems likely that these slow changes in enzyme levels in liver of diabetic rats are a result of altered levels of substrates which protect the enzymes against degradation or promote their induction.

Surprisingly, there have been few detailed studies on insulin's effect on liver RNA synthesis despite the above data on hepatic enzyme synthesis (116). Steiner and associates have reported that insulin administration to diabetic rats stimulates incorporation of precursors into rapidly labeled total RNA and increases total RNA content in the liver (117). It has subsequently been shown that insulin administered to diabetic animals stimulates incorporation of labeled precursors into total nuclear RNA (118). Both nucleolar and extranucleolar labeling is stimulated, although the effect is first seen in the extranucleolar fraction (119). Insulin administration stimulates both the nucleolar and extranucleolar RNA polymerase activities. It seems likely that the effect of the hormone on incorporation of precursors into RNA is on the activity or quantity of the synthetic enzymes involved rather than on the pool size of precursor (118).

Relation between Insulin Action in the Liver and Hepatic cAMP Level

Exton and associates (reviewed by Park, 120) have presented evidence that many of the hepatic actions of insulin can be accounted for by a fall in intracellular levels

of cAMP (121, 122). It should be pointed out, however, that these investigators do not believe that this phenomenon can account for all the effects of insulin in this or other tissues (120). Hepatic cAMP is elevated by both glucagon and catecholamines in the isolated perfused rat liver (122, 123). Many if not all the effects of these hormones on metabolic parameters can be mimicked by the addition of exogenous cAMP. If insulin action were due, even in part, to the lowering of cAMP levels, the hormone should counteract these effects. Park et al (120) have summarized the effects of cAMP (or glucagon or catecholamines) on the isolated rat liver and the antagonistic action of insulin. Insulin opposed the effects of glucagon (and epinephrine and exogenous cAMP where tested) on glycogenolysis (122, 124), gluconeogenesis (122), ureogenesis (122, 124), K^+ loss (122, 125), and proteolysis (107). Mackrell & Sokal (124) have also found that insulin generally antagonizes the metabolic effects of glucagon in the perfused liver. However, these authors showed that low doses of insulin also block urea production from endogenous substrate in the absence of glucagon. Williams et al (126) have shown that insulin antagonizes the glucagon stimulation of K^+ loss from the liver, and that the hormone also suppresses basal K^+ loss under conditions where there is no detectable alteration in cAMP levels.

In general, an effect of insulin on cAMP-mediated processes is most clearly seen when the level of cAMP is elevated submaximally by another hormone (123). A doubling of the basal level of cAMP is sufficient to stimulate maximally the rate of glucose output (glycogenolysis plus gluconeogenesis) in the perfused liver. A stimulation of this high degree can be understood if one postulates that most of the basal cAMP is bound or inactive. Thus, under basal condtions, the "free" cAMP level is near to zero as also suggested by the extremely low leakage (presumably by a transport system) of the nucleotide from the liver (122, 123). Upon addition of glucagon, relatively large amounts of cAMP appear in the perfusate, suggesting formation of another pool of cAMP that has better access to the plasma membrane (122, 123). In line with this idea, insulin is without detectable effect on the basal level of cAMP but does suppress the hormone-induced rise in tissue as well as movement of the nucleotide into the perfusate (122, 123). This effect is seen clearly with epinephrine or with low, physiological doses of glucagon but becomes undetectable at a high concentration of the latter hormone, for reasons that may be technical in nature (122, 123). Insulin also lowers glucagon-stimulated cAMP levels in isolated liver cells (127) and in organ culture of mature rat liver (128).

Mackrell & Sokal (124) reported that insulin inhibits urea formation but not phosphorylase activation in perfused livers in the presence of glucagon. An explanation for this selectivity of insulin action might be the presence of multiple adenylate cyclase systems controlling the concentrations of different intracellular cAMP pools. Some evidence has been presented for separate adenylate cyclase systems for glucagon and epinephrine (129), but this point is not firmly established. Another explanation for the finding of Mackrell & Sokal is based on the much greater sensitivity of phosphorylase to activation by cAMP than urea formation. Thus a reduction in cAMP sufficient to block stimulation of ureogenesis might be insufficient to inactivate phosphorylase (123).

Epinephrine is a much weaker stimulus than glucagon to cAMP formation in the perfused liver (123) and isolated liver cells (132). For example, epinephrine does not stimulate urea formation, although it does activate glycogenolysis and gluconeogenesis fully. In line with this relatively weak effect of the catecholamine on cAMP, its effect is very readily suppressed by insulin (123).

The mechanism by which insulin lowers cAMP in liver is controversial. Hepp & Renner (130) and Illiano & Cuatrecasas (131) have reported that insulin in vitro inhibits glucagon-stimulated adenylate cyclase in particulate preparations of mouse and rat liver. Neither of these preparations can be designated plasma membranes but rather are microsomal membranes contaminated with small amounts of plasma membranes. Both preparations have adenylate cyclase specific activities that are 1/8 to 1/10 that of purified plasma membrane preparations (132). The inhibitory effect of insulin on liver adenylate cyclase activity has not been seen in other laboratories (Thompson et al 133, Pilkis et al 127, and Pohl et al 144) in studies of similar crude or purified plasma membrane preparations. Failure to show in vitro effects of insulin on adenylate cyclase have been attributed to excessively high insulin concentrations (134–139). Illiano & Cuatrecasas (131) reported maximal inhibition of the cyclase at a concentration of 50 μU/ml ($3 \times 10^{-11} M$) whereas higher concentrations were stimulatory. However, the insulin concentration in portal venous blood of fasting humans is 40–50 μU/ml and levels as high as 1000 μU/ml have been observed in fed individuals (140). Very high concentrations of insulin suppress cAMP levels in the perfused rat liver (123).

It is possible that insulin could affect cyclic AMP production by an effect on membrane ATPase. Hepp & Renner (130) employed low ATP concentrations and an ATP-regenerating system in their assay. A direct action of insulin on plasma membrane ATPase activity in human lymphocytes has been reported (141), whereas the hormone did not affect the activity of adenylate cyclase. Krahl (142) has also suggested that insulin may alter membrane ATPase in cultured rat uteri. Luly et al (143) report that epinephrine, glucagon, cAMP, and insulin affect rat liver plasma membrane ($Na^+ + K^+$)-ATPase. In both the reports of Illiano & Cuatrecasas and Hepp & Renner (130, 131), a radioactive assay of adenylate cyclase employing ^{32}P-ATP was used. The specificity of this assay for cAMP is uncertain when used with crude enzyme preparations. Pohl et al (144), using this radioactive assay, have not detected an effect of insulin on adenylate cyclase in partially purified rat liver plasma membranes. Rosselin et al (145), using a radioimmunoassay to assay adenylate cyclase, were also unable to observe an insulin effect on the enzyme. In considering all the above reports, it would appear that an effect of insulin has not been demonstrated in a way that can be generally reproduced. This is reminiscent of many claims of insulin action on broken cell preparations in the past, virtually all of which have proved not to be reproducible.

Senft et al (146) first reported that insulin treatment increases the activity of phosphodiesterase in liver and adipose tissue, but others could not confirm their findings (147–151) until recently (153, 154). House & Weidemann (152) reported that insulin in vitro activates a liver plasma membrane-bound phosphodiesterase, but this has not been confirmed (153, 154). Thompson et al (153) and Pilkis et al

(154) have found that the rat liver plasma membrane, low K_m phosphodiesterase activity is decreased in liver of diabetic rats and increased after insulin treatment of the animal. These effects on phosphodiesterase in liver of diabetic animals are probably secondary to effects on protein synthesis (146).

Carbohydrate Metabolism

Mondon & Burton (155) have shown that when livers from fed animals are perfused with a glucose concentration of 240 mg% in 90% rat blood, glucose uptake is equal to glucose release, and insulin does not affect the net carbohydrate balance. When glucose release is increased by perfusion with 45% blood, which limits O_2 utilization in the liver, insulin decreases glucose output. Glycogen synthesis in the liver in vitro is slower than in vivo and is increased by raising the perfusate glucose concentration but not by addition of insulin or acetylcholine. However, insulin plus acetylcholine markedly increase glucose uptake and glycogen synthesis. The authors conclude that insulin affects hepatic carbohydrate metabolism by inhibiting catabolic processes and enhances glycogen synthesis when combined with cholinergic stimulation. Kotoulas et al (156) have presented morphological evidence to suggest that insulin and glucose may delay formation of lysosomes and thereby delay mobilization of glycogen in the postnatal period. Seglen (157) and Johnson et al (158) have shown that insulin stimulates glycogen synthesis in isolated liver cells, but only if the cell donors are starved.

Blatt et al (161) have reported that activation of hepatic glycogen synthetase and glycogen deposition in tadpoles are dependent on insulin. They have also shown that insulin in vitro promotes conversion of glycogen synthetase to the I form in minced tadpole liver preparations (162). Addition of ouabain prevents this effect of insulin (163), suggesting involvement of $Na^+ + K^+$ ATPase. Nichols & Goldberg (160) report that the activity of glycogen synthetase D-phosphatase activity is decreased in the alloxan diabetic rat. Insulin administration has been reported to raise this activity to normal (164, 165), and similar observations have been made using leukocytes of diabetic humans (166). Gold (164) reports a stimulation by insulin of glycogen synthetase activity within 15 min but no alteration in phosphatase activity until 1 hr. The control of liver glycogen synthetase D-phosphatase has recently been linked to changes in levels of active phosphorylase (167).

Bishop (179) has reported a rapid increase in liver glycogen synthetase D-phosphatase after insulin infusion into dogs. Activity is reduced by subsequent infusion of glucagon. This rapid activation-inactivation suggests that there are interconvertible forms of the phosphatase. In diabetic animals, however, insulin is unable to elevate phosphatase activity (179). Hornbrook (159) and Nichols & Goldberg (160) have shown that cortisol administration to alloxan diabetic animals fails to promote the activation of glycogen synthetase.

It would seem highly probable that one mechanism by which insulin activates hepatic glycogen synthetase involves a decrease in cellular cAMP. Jefferson et al (121) and Nichols & Goldberg (160) have shown that the elevated levels of hepatic cAMP found in the liver of alloxan diabetic animals are returned to normal within 75 min by injection of insulin into the animal. Insulin also decreases hepatic cAMP

in perfused liver within 20 min when the level has been raised by continuous infusion of a low concentration of glucagon (123). An effect of insulin on glycogen synthetase or phosphorylase in perfused liver was not shown by Glinsmann et al (168) but has recently been obtained by Miller & Larner (180). Nichols & Goldberg (160), Miller & Larner (180), and Curnow et al (181) have reported a stimulatory effect of insulin on glycogen synthetase I formation without any significant change in cAMP levels under appropriate conditions. As mentioned earlier, insulin may lower cAMP in a small free (active) pool of cAMP, a change which may be undetectable in the presence of a large pool of bound cAMP. It is possible, as suggested for muscle by Villar-Palasi & Wenger (169) and others in this group (170–172), that the sensitivity of glycogen synthetase kinase (i.e. protein kinase) to cAMP is altered by insulin. There are, of course, other possible mechanisms not involving cAMP.

An insulin effect to suppress gluconeogenesis in the perfused rat liver has been obtained by Jefferson et al (21). The findings of Friedmann et al (173) and others (174–176) also support the view that insulin can suppress gluconeogenesis and enhance glycogenesis by moderating the level of cAMP. This effect of insulin is rapid and is not likely to be due to enzyme synthesis.

Inhibitory effects of cAMP on insulin-stimulated enzyme activities in liver have been reported. Pilkis (114) and Ureta et al (115) have shown that in vivo administration of cAMP can prevent the insulin mediated induction of rat liver glucokinase. Lakshmanan et al (178) have shown that the in vivo synthesis of rat liver fatty acid synthetase was stimulated by insulin administration and inhibited by glucagon and cAMP.

Wieland et al (182) have reported that administration of insulin to normal rats causes an increase in the active form of pyruvate dehydrogenase in rat liver (182). They (182) suggest that plasma free fatty acids play an important role in control of pyruvate dehydrogenase interconversion. They find no effect of insulin in vitro on this process in the perfused liver. The control of pyruvate dehydrogenase will be more fully discussed in the next section.

EFFECTS OF INSULIN ON ADIPOSE TISSUE

Carbohydrate, Protein, and Lipid Metabolism in Relation to the Adenylate Cyclase System

Insulin effects on isolated adipose tissue have been extensively reviewed (183–187). Some of the important actions of the hormone in vitro on adipose tissues are as follows: (*a*) enhancement of the transport of sugars that employ the glucose carrier system (glucose, galactose, and 3-O-methyl glucose; 188); (*b*) increased conversion of glucose intracellularly to CO_2, glyceride glycerol, fatty acids, and glycogen (186, 187); (*c*) stimulation of transport of amino acids, such as α-amino isobutyric acid and methionine, independent of the presence of glucose in the medium (189); (*d*) increased incorporation of amino acids into protein (5); (*e*) inhibition of lipolysis induced by epinephrine or other agents which enhance lipolysis, independent of the presence of glucose in the medium (187).

In intact fat cells, Sneyd et al (267) have shown that insulin stimulates sugar transport apparently independently of the level of cAMP, and Rodbell (186) has shown that various lipolytic hormones, which elevate cyclic AMP in fat cell ghosts, do not block insulin-stimulated glucose utilization (glucose transport). The possibility cannot be excluded, although it seems highly unlikely, that insulin affects transport through changes in cAMP level in a small compartment of the cell.

Avruch et al (190) have reported that a sonicated plasma membrane preparation from insulin-treated adipocytes of the rat epididymal fat pad shows an accelerated uptake and/or release of D-glucose. Addition of insulin directly to the plasma membranes was without effect (190). The hormone effect is presumably on glucose transport in vesicles formed in processing the preparation. No changes in the physical properties and protein composition of the plasma membrane were observed (190).

Soifer et al (192) report that insulin promotes microtubule assembly in fat cells. Colchicine inhibits this effect and also the stimulation of lipid and glycogen synthesis but does not affect acceleration of glucose oxidation (presumably glucose transport). Colchicine did not block the insulin-like, metabolic effects of high cocentrations of glucose (192). Murthy & Steiner (193) have shown that insulin increases lipogenesis from acetate in brown adipose tissue through an effect that is independent of any action on glucose transport or metabolism. Jungas (194) and Halperin & Robinson (195) reported that insulin augments the rate of fatty acid synthesis in white adipose tissue by a mechanism distinct from the stimulation of glucose transport. The insulin effect on fatty acid synthesis does not appear to involve changes in the level of cAMP.

Jungas and others have shown that insulin treatment of adipose tissue stimulates pyruvate dehydrogenase activity. This regulation may explain, in part, how insulin promotes fatty acid synthesis from lactate, pyruvate, or endogenous sources (see 196–198 for review). This effect of insulin appears also to be independent of any effect on glucose transport (197). Coore et al (199) have shown that the effect of insulin on pyruvate dehydrogenase activity is inhibited by adrenaline, ACTH, or dibutyryl cAMP. The adipose tissue enzyme is similar to the pyruvate dehydrogenase in heart, kidney, and liver where evidence has been given that activation and inactivation were catalyzed by an ATP-dependent kinase and Mg^{2+}-dependent phosphatase (200). Insulin has been postulated to act by increasing the proportion of active (dephosphorylated) pyruvate dehydrogenase (199, 201). It seems unlikely that insulin activation of the enzyme is mediated by cAMP directly. Taylor et al (202) found no effect of cAMP on the protein kinase or phosphatase for pyruvate dehydrogenase (198). Furthermore, they were unable to demonstrate any effect of cAMP or cGMP on the pyruvate dehydrogenase complex in adipose tissue. Coore et al (199) have obtained similar negative results. Martin et al (259) have very interesting evidence to suggest that the insulin effect on this enzyme system is mediated by a change in the cellular distribution of Ca^{2+}. This ion is an activator of the pyruvate dehydrogenase phosphatase.

Sica & Cuatrecasas (203) report that insulin stimulates the total activity of adipose tissue pyruvate dehydrogenase by a process that is inhibited by high concentrations of puromycin and cycloheximide but not by actinomycin.

Addition of insulin in vitro to adipose tissue stimulates the incorporation of labeled amino acids into protein (5). It does so in isolated fat cells, in the absence or presence of glucose in the medium. Minemura et al (204) have reported that insulin affects peptide bond synthesis rather than amino acid transport. These workers also find that insulin counteracts the inhibition of protein synthesis caused by ACTH, suggesting involvement under these conditions of cAMP. However, insulin added by itself to adipose tissue stimulates protein synthesis with no detectable alteration in cAMP levels (205). Under conditions in which protein synthesis is inhibited by cycloheximide, insulin has an inhibitory effect on proteolysis. This hormone effect is too small, however, to account for the increased net incorporation of amino acids into protein noted above. Little or no work on subcellular systems has been attempted in adipose tissue, probably because of technical difficulties. Insulin stimulates uridine incorporation into adipose tissue RNA (206).

Insulin inhibits lipolysis in isolated fat cells (207, 208) in the absence of glucose. This effect can be accounted for under certain conditions by a lowering of cellular cAMP. Under physiological conditions, however, where glucose is always present, the antilipolytic effect of insulin may for the most part be due to the stimulation of fatty acid reesterification consequent to increased glucose uptake (187). The effect of insulin to lower cAMP is seen most readily if the nucleotide level has been elevated by low (215–217) or moderate (210, 211) concentrations of epinephrine, caffeine, or both. A small lowering of the basal level has been reported recently (210). Insulin counteracts the lipolytic effect of exogenous cAMP (210, 211, 218), which must be added in very high concentrations because of poor penetration, and counteracts low but not high concentrations of exogenous dibutyryl cAMP (148, 150, 218, 255–257). Insulin is strongly antilipolytic in fat cells stimulated by ACTH. The level of cAMP may be reduced only slightly under these conditions and to an insufficient degree to account for the antilipolysis unless compartmentation of cAMP is invoked (210). Kuo et al (212) have presented evidence for separate pools of cAMP in adipose tissue. Jarett et al (205) and Khoo et al (220) suggest that insulin does not lower cAMP levels sufficiently to account for antilipolysis in adipose tissue exposed to catecholamine under certain conditions. There have been reports that insulin inhibits adipose tissue adenylate cyclase (130, 131) and other reports that it has no effect (134–139). Loten & Sneyd (214) and Vaughan (213) have found a small, activating effect of insulin on phosphodiesterase activity in homogenates of adipose tissue pretreated briefly with insulin. The effect is greatest on the membrane-bound enzyme (213). It has been reported (146) and denied (147) that phosphodiesterase is reduced in adipose tissue of diabetic rats. It is not stimulated by injection of insulin into diabetic or normal animals (148–151). Crofford and co-workers (219) have postulated that the ability of lipolytic agents to inhibit the stimulation by insulin of glucose oxidation and protein synthesis is a consequence of their ability to elevate cAMP levels. Fain (216) has suggested that this inhibition of insulin action by lipolytic agents is due to an accumulation of intracellular fatty acids which, in turn, could be the consequence of lipolytic activation by cAMP.

Khoo et al (220) have studied the effects of epinephrine and insulin on the control of lipase, phosphorylase kinase, phosphorylase, and glycogen synthetase in adipose cells. They suggest that the antagonistic effects of insulin and epinephrine on the

activation of these enzymes cannot be fully accounted for by changes in the level of cAMP.

Lavis et al (257) and Kono (210, 211) showed that insulin in a concentration of 150 μU/ml or higher enhances the lipolytic activity of epinephrine or ACTH but not of caffeine. Increased lipolysis is associated with a rise in the level of cAMP. Insulin by itself does not have this effect. Hepp & Renner (130) and Illiano & Cuatrecasas (131) report that low but not high concentrations of insulin inhibit hormone-stimulated adipose tissue adenylate cyclase.

Effects of Insulin on Adipose Tissue; Relationship to Ions

Taubai & Jeanrenaud (221) have reported that insulin counteracts concomitantly the lipolysis and decreased K^+ uptake induced by lipolytic hormones in fat tissue. These authors suggest that insulin prevents a fall in phosphate bond energy level by reducing reesterification. In this connection, Bihler & Jeanrenaud (222) have shown that insulin partly prevents the depression of ATP levels induced by lipolytic agents in the absence of glucose.

Rodbell (223) reported a potassium requirement for the insulin stimulation of glucose transport in isolated fat cells of the rat. Letarte & Renold (224) and Letarte et al (225), however, found no consistent effect of potassium deficiency on the hormone effect in mouse adipocytes. In contrast to muscle, adipose tissue does not require magnesium for an insulin effect on transport (224).

A number of investigators have found that insulin effects on glucose oxidation and lipogenesis are inhibited in a medium low in sodium. Clausen (226) concluded that sodium was not required for transport stimulation but that it may effect oxidation and lipogenesis. However, Letarte & Renold (224) concluded that insulin-enhanced glucose transport was sodium dependent. In their experiments sodium lack did not completely prevent the effect of insulin, and there is no conclusive evidence that insulin action on transport in adipose tissue has an absolute requirement for this ion.

Krishna et al (227) have shown that norepinephrine causes a depolarization of the cell membrane of brown fat. Insulin counteracts the depolarization caused by norepinephrine but does not by itself alter the membrane potential. Propranolol also blocks the depolarization. These authors suggested that norepinephrine and insulin modify membrane potential by modifying the entry of K^+ into the cell. Dibutyryl cAMP did not alter the membrane potential of the fat cell (225). The resting membrane potential of adipose tissue cells of young rats is increased by insulin in the absence of glucose (228).

As already noted, Martin et al (259) suggest that activation of pyruvate dehydrogenase in adipose tissue by insulin is associated with an intracellular flux of Ca^{2+}.

EFFECTS OF INSULIN ON CELLS IN CULTURE

Addition of insulin to serum starved 3T3 cells results in what has been referred to as a positive pleiotypic response (229). This is characterized by increased uridine uptake, RNA synthesis, polysome aggregation, protein synthesis, increased glucose utilization, and decreased protein degradation.

A number of investigators have studied the effect of insulin on tyrosine transaminase activity in hepatoma cell cultures. In general, insulin increases the activity of this enzyme (230–233) and also of pyruvate kinase in a rat liver cell line. Insulin completely supresses the stimulatory effects of dexamethasone and dibutyryl cAMP on phosphoenolpyruvate carboxykinase in Reuber H35 cells. Immunochemical-isotope data indicate that insulin specifically stimulates the synthesis of tyrosine transaminase at the post-transcriptional or -translational level (232, 233). The effect of insulin on pyruvate kinase requires de novo RNA synthesis (235). Insulin increases the uptake of α-aminoisobutyrate in hepatoma cell cultures (234).

In BHK-21 cells insulin stimulates glucose uptake and protein synthesis (236). Jimenez de Asua et al (237) have reported that insulin has a dramatic stimulatory effect on the growth of fibroblasts in culture. Insulin inhibits adenylate cyclase in crude homogenates of these cells (237) and also in homogenates of neurospora (276).

Gerschenson et al (238) have shown that insulin stimulates growth and formation of polyribosomes in cultured rat liver cells. Insulin causes morphologic changes in explanted chicken embryos (239).

EFFECTS OF INSULIN ON OTHER TISSUES

Goldfine and associates (240, 241) have shown that insulin stimulation of α-aminoisobutyric acid transport in rat thymocytes correlates with ^{125}I-insulin binding and that the change in α-aminoisobutyric acid influx is blocked by inhibitors of protein synthesis. cAMP does not appear to be involved (241).

Peck and co-workers (242, 243) have reported that insulin enhances the incorporation of labeled uridine into the free uridine pool and into RNA in isolated bone cells. These workers suggest that insulin acts by stimulating uridine uptake and phosphorylation. Dithiothreitol has an effect similar to insulin. Hahn et al (244) have shown that insulin stimulates amino acid transport in fetal rat calvaria by a process that is blocked by inhibitors of protein synthesis and by ouabain.

The human red blood cell is generally thought to be an insulin insensitive tissue. In line with this is the fact that insulin does not bind appreciably to red blood cells. However, Zipper & Mawe (245) report that insulin elevates the maximal net flux of glucose across the cell membrane.

RELATION OF INSULIN STRUCTURE TO FUNCTION

The three-dimensional structure of the insulin molecule has been determined by single crystal X-ray analysis by Hodgkin and associates (246). They have shown that the B chain has a rigid central helical region extending between residues B9 and B19. This central structure holds the cysteines B7 and B19 a definite distance apart. The terminal residues on both sides of the helix extend as arms to form a pocket for the A chain. The A chain conformation is compact and is confined by the interchain disulfide bonds connecting to A7 and A20 and by the interchain bond connecting A6 and A11.

In the crystals, the monomers of insulin align in pairs in such a way as to bury the hydrophobic residues that make up the helices of the B chains. In the presence of zinc, there is aggregation of these dimers to form hexamers. This aggregation occurs not only in the crystals used for X-ray analysis but also in the pancreas (258). Hydrophobic residues are very evident in intermolecular contacts, but only two hydrophobic groups, A10 isoleucine and B25 phenylalanine, are exposed to the surface. On the other hand, certain hydrophilic residues of the A chain, A1 glycine, A5 glutamine, A19 tyrosine, and A21 asparagine are on the surface of the molecule and are not involved in aggregation. Their deletion affects activity and function. Arquilla and associates (247), using immunological data, have been able to construct a three dimensional model of insulin that is amazingly similar to the model constructed by X-ray analysis.

The question of whether the physiologically active form of insulin is monomeric or dimeric is as yet unanswered. Evidence for an active monomer is the retention of biological activity of insulin after covalent linkage to sepharosebeads where dimer interaction is unlikely.

Frazier et al (248) have summarized a large amount of data that points out that nervegrowth factor and insulin (or proinsulin) are similar in structure, function, and origin. Both effectors stimulate many anabolic processes (248).

Somatomedin competes with insulin for binding sites on liver plasma membranes (249) suggesting that insulin and somatomedin also are related structurally and functionally.

CONCLUSIONS

The plasma membrane of insulin sensitive cells contains specific receptors for the hormone. These receptors have peptide elements that face the external surface of the cell and are presumably the sites of insulin complex formation. It seems likely that there is a single class of specific receptors, on adipocytes at least, with an insulin-receptor dissociation constant of about 5×10^{-9}mol. An insulin binding protein, which may be the specific receptor, has been extensively purified. It has a molecular weight of about 300,000.

It seems likely that only a small fraction of the total number of receptors in a cell needs to complex with insulin in order to generate a signal of sufficient strength to modify cell function. It is speculated that the signal results from a conformational change in the receptor. Complexing with the receptor may be coupled to inactivation of the hormone.

As regards the nature of the signal, it is frequently theorized that an intracellular mediator substance, *X*, may be generated. The compound is postulated to affect transport systems in the membrane and intracellular enzymes to produce the changes characteristic of insulin action. Certain cyclic nucleotides (277), Ca^{2+}, and other ions havebeen suggested to fulfill the role of *X*, in part, at least. Another theory is that the signal consists of a modification of cell structure. As a consequence, transport systems and enzyme reactions would be affected. This theory would also account for the persistent problem of demonstrating a reproducible effect of insulin

preparations. Neither theory has any firm experimental support. It is possible that more than one type of signal is generated.

Some of the consequences of signal generation are rapid. These include effects on transport of sugars, amino acids, some inorganic ions, and probably many other compounds. Other consequences are relatively slow and include acceleration of growth and the synthesis of specific proteins. The signal affects protein turnover in several ways. It may modify the levels of substrates that protect or "induce" various enzymes; it may affect the level of cAMP or act by other as yet unknown mechanisms. The signal, probably through many steps, stimulates RNA polymerase activity, the initiation of peptide chains, and ribosomal aggregation, It also probably reduces protein catabolism by stabilization of lysosomes.

The signal can lead to a reduction in the level of cAMP in adipocytes, hepatocytes, and probably certain other cells, but not detectably in skeletal muscle or cardiac cells. The reduction is prominent if the level of cAMP has first been elevated slightly or moderately by other hormones, notably catecholamines (β activity) and glucagon. In this way, insulin action is antagonistic to these hormones in all of their cAMP-mediated effects. From a physiological point of view, the effect on the level of cAMP would seem particularly important in hepatic metabolism (glycogenolysis, gluconeogenesis, ion fluxes, protein turnover), and it probably contributes to antilipolysis in the adipocyte.

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