Insulin Binding Leads to the Formation of Covalent (-S-S-) Hormone Receptor Complexes*

(Received for publication, September 3, 1981)

Stella Clark‡ and Len C. Harrison

From The Endocrine Laboratory, University of Melbourne Department of Medicine, Royal Melbourne Hospital, Parkville, Victoria, 3050, Australia

The recent finding that the lymphocyte insulin receptor is covalently labeled after 125I-insulin binding (Saviolakis, G., Harrison, L. C., and Roth, J. (1981) J. Biol. Chem. 256, 4924-4928) led us to directly test a previous hypothesis that insulin binds via disulfide bonds. 125I-Insulin was bound to isolated rat adipocytes at 37 °C, the cells were washed extensively at 37 °C to remove nondissociable radioactivity, and then extracted in Triton X-100/Dulbecco’s buffer containing protease inhibitors. The solubilized radioactivity obtained after centrifugation at 100,000 × g was chromatographed on Sephadex G-50 and resolved as a major high molecular weight (HMW) peak and two smaller peaks representing intact and degraded 125I-insulin, respectively. The formation of the HMW component was dependent on the concentration of 125I-insulin, although the total formed did not exceed 10% of the specifically bound radioactivity. Its formation was inhibited in a dose-dependent manner by sulfhydryl-blocking agents in concentrations that did not alter specific binding. The HMW component was also formed in paraformaldehyde-treated cells and in adipocyte membranes. It eluted at the position of the native Triton-solubilized insulin receptor on Sepharose CL-6B chromatography and was immunoprecipitated by antireceptor antibodies. Radioactivity bound to the receptor in the HMW complex was dissociated by the reducing agent dithiothreitol and eluted off Sepharose CL-6B at the position of reduced 125I-insulin. Oxidative sulfhydrylation revealed that the radioactivity in the HMW complex was present in both A and B chains of insulin, in the same proportions as in intact 125I-insulin.

We conclude that a fraction of bound insulin becomes covalently linked to its receptor via a disulfide-sulfhydryl exchange on the cell surface. The significance of this modification in initiating insulin action deserves further study.

A role for sulfhydryl reactions in the action of insulin has been recognized for two decades. Cadenas et al. (1961) showed that N-ethylmaleimide, a sulfhydryl-blocking agent, decreased both insulin uptake and hexose transport in rat heart. More recently Czech (1976a), proposed a sulfhydryl-redox model for hexose transport in which insulin activated the transport system by converting it from a reduced (sulfhydryl) to an oxidized (disulfide) state. Fong et al. (1962) found that some insulin was not dissociated from rat epididymal fat pads and hemidiaphragms by 8 M urea, 0.015 M HCl but could be released with various thiol compounds. They then hypothesized that insulin could bind via disulfide bonds. Edelman et al. (1963) also showed, in purified rat muscle membranes, that bound insulin resistant to 8 M urea could be released by thiol compounds, and that the binding of insulin to membranes was partially inhibited by NEM. However, these early studies measured only the bulk uptake of 125I-insulin whose biological activity was probably quite low.

Direct identification of receptors using biologically active 125I-insulin was accomplished in the early 1970’s (Roth, 1973), but the disulfide-binding hypothesis was not re-examined. Contemporary studies have, nevertheless, confirmed that the interaction of insulin with its receptor is not completely reversible. Thus, after 125I-insulin binding to rat adipocytes there is a time- and temperature-dependent formation of nondissociable radioactivity (Kahn and Baird, 1978). Gel filtration of Triton-acidures extracts of cells reveals that most of the nondissociable radioactivity is either intact or degraded insulin, with a small proportion present as a high molecular weight form. This labeled high molecular weight material has been repeatedly noted and assumed to be aggregated insulin (Marshall and Olefsky, 1979; Hammons and Jarett, 1980; Misbin et al., 1980). Nondissociable 125I-insulin radioactivity has been localized in both plasma membrane and subcellular fractions of adipocytes by Kono et al. (1975), but not further characterized. Morphological studies of the fate of bound 125I- or fluorescent-labeled insulin have also revealed cellular uptake of label (Gordon et al., 1980), but while this process of internalization has been termed receptor-mediated endocytosis (Schlessinger et al., 1978), direct evidence that the labeled hormone is associated with its receptor has been lacking.

The availability of specific receptor antibodies provided a means of identifying the insulin receptor independently of its hormone-binding function (Harrison et al., 1979). With this approach it was recently shown that the radioactive high molecular weight component formed after 125I-insulin binding to cultured human lymphocytes is predominantly covalently labeled insulin receptor (Saviolakis et al., 1981). Although this was the first direct evidence for covalent hormone-receptor complexes, the chemistry of the covalent bond, the nature of the label on the receptor, and the biological significance of this interaction were not investigated. The present study was therefore undertaken to address these questions using a classic insulin target tissue, the adipocyte, and in particular to re-examine the early hypothesis of Fong et al. (1962) that the

† A role for sulfhydryl reactions in the action of insulin has been recognized for two decades. Cadenas et al. (1961) showed that N-ethylmaleimide, a sulfhydryl-blocking agent, decreased both insulin uptake and hexose transport in rat heart. More recently Czech (1976a), proposed a sulfhydryl-redox model for hexose transport in which insulin activated the transport system by converting it from a reduced (sulfhydryl) to an oxidized (disulfide) state. Fong et al. (1962) found that some insulin was not dissociated from rat epididymal fat pads and hemidiaphragms by 8 M urea, 0.015 M HCl but could be released with various thiol compounds. They then hypothesized that insulin could bind via disulfide bonds. Edelman et al. (1963) also showed, in purified rat muscle membranes, that bound insulin resistant to 8 M urea could be released by thiol compounds, and that the binding of insulin to membranes was partially inhibited by NEM. However, these early studies measured only the bulk uptake of 125I-insulin whose biological activity was probably quite low.

Direct identification of receptors using biologically active 125I-insulin was accomplished in the early 1970’s (Roth, 1973), but the disulfide-binding hypothesis was not re-examined. Contemporary studies have, nevertheless, confirmed that the interaction of insulin with its receptor is not completely reversible. Thus, after 125I-insulin binding to rat adipocytes there is a time- and temperature-dependent formation of nondissociable radioactivity (Kahn and Baird, 1978). Gel filtration of Triton-acidures extracts of cells reveals that most of the nondissociable radioactivity is either intact or degraded insulin, with a small proportion present as a high molecular weight form. This labeled high molecular weight material has been repeatedly noted and assumed to be aggregated insulin (Marshall and Olefsky, 1979; Hammons and Jarett, 1980; Misbin et al., 1980). Nondissociable 125I-insulin radioactivity has been localized in both plasma membrane and subcellular fractions of adipocytes by Kono et al. (1975), but not further characterized. Morphological studies of the fate of bound 125I- or fluorescent-labeled insulin have also revealed cellular uptake of label (Gordon et al., 1980), but while this process of internalization has been termed receptor-mediated endocytosis (Schlessinger et al., 1978), direct evidence that the labeled hormone is associated with its receptor has been lacking.

The availability of specific receptor antibodies provided a means of identifying the insulin receptor independently of its hormone-binding function (Harrison et al., 1979). With this approach it was recently shown that the radioactive high molecular weight component formed after 125I-insulin binding to cultured human lymphocytes is predominantly covalently labeled insulin receptor (Saviolakis et al., 1981). Although this was the first direct evidence for covalent hormone-receptor complexes, the chemistry of the covalent bond, the nature of the label on the receptor, and the biological significance of this interaction were not investigated. The present study was therefore undertaken to address these questions using a classic insulin target tissue, the adipocyte, and in particular to re-examine the early hypothesis of Fong et al. (1962) that the

† This work was supported by the National Health and Medical Research Council of Australia and The John Claude Kelion Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Recipient grants from The Sheppard Lowe Foundation (University of Melbourne) and The Victor Hurley Medical Research Foundation of The Royal Melbourne Hospital.

1 The abbreviations used are: NEM, N-ethylmaleimide; SDS, sodium dodecyl sulfate; DTNB, 5,5'-dithiobis(nitrobenzoic acid); HMW, high molecular weight.
covalent interaction of insulin with its receptor occurs through disulfide bonding.

**EXPERIMENTAL PROCEDURES**

**Materials**

Crude collagenase type 1 (EC 3.4.24.3) (125 units/mg) was purchased from Worthington. Porcine monocomponent insulin and A14 (Tyr) 125I-insulin were purchased from the Novo Research Institute, Copenhagen. All isotopes were from The Radiochemical Centre, Amersham. Sephadex G-50 and Sephrose CL-6B were purchased from Pharmacia, South Seas, Inc. Bovine serum albumin (radioimmunoassay grade) was purchased from Sigma. Antireceptor serum, obtained from a patient of severe insulin resistance and acanthosis nigricans (Flier et al., 1975), contained IgG antibodies specific for the binding subunits of the receptor (Kasuga et al., 1981). Guinea pig anti-insulin serum and rabbit anti-guinea pig serum were purchased from Burroughs Wellcome, U.K. All other chemicals were of analytical grade.

**Methods**

**Preparation of Adipocytes and Membranes—**Male Sprague-Dawley rats fed ad libitum and weighing 150-200 g were killed by cervical dislocation. Isolated adipocytes were prepared by incubating small pieces of epididymal fat pads in Dulbecco's buffer, pH 7.4 (Dulbecco and Vogt, 1954) with collagenase (3 mg/ml) and albumin (40 mg/ml). After 60 min cells and partially digested tissue were washed through a plastic strainer, centrifuged at 500 X g for 1 min, and washed twice by centrifugation in a buffer containing albumin (10 mg/ml). Adipocyte membranes were prepared by a slight modification of the method of McKeel and Jarett (1970). Fat pads from two rats were blended and immediately homogenized in 10 ml of extraction medium (10 mM Tris-HCl, 2 mM ethylene glycol bis[β-aminoethyl ether]-N,N,N',N'-tetraacetate acid, 0.24 M sucrose) at 0 °C with a Polytron PT-20 homogenizer (2 X 3 s on position 3). The homogenate was centrifuged at 15,000 X g for 15 min, and the pellet was resuspended in 50 ml of medium and recentrifuged at 100,000 X g for 60 min. The supernatant was then centrifuged at 16,000 X g for 45 min to pellet the crude membrane fraction. The membranes were washed and resuspended in Dulbecco's buffer at a final concentration of about 4 mg/ml of protein.

**125I-Insulin-binding Studies—**Porcine insulin (100-150 μg/ml) was prepared by a modification of the chloramine-T method (Roth, 1975). Adipocytes (2-4 X 10^6 cells/ml) or membranes (approximately 1 mg of protein/ml) were incubated with 125I-insulin (1-25 ng/ml) in Dulbecco's buffer, pH 7.4, containing albumin (10 mg/ml), for 10 min at 20 °C. Insoluble complex was precipitated by the addition of 100 μg/ml of carrier bovine insulin and was dissolved by the addition of 1 M NaOH, and radioactivity was determined. To test the effect of Triton X-100, porcine insulin (1-25 ng/ml) in Dulbecco's buffer, pH 7.4, containing 2% Triton X-100 and the protease inhibitors bacitracin (100 units/ml) and leupeptin (10 μg/ml), and solubilized by stirring for 45 min at 25 °C. The extracted solubilized radioactivity was recovered after centrifugation at 100,000 X g for 90 min, and the pellet was discarded.

**Column Chromatography—**Solubilized radioactivity was chromatographed initially on Sephadex G-50 (1.5 X 30 cm), equilibrated, and eluted with 0.1 M sodium phosphate buffer, pH 7.4, containing 0.1% Triton X-100 at 4 °C. The high molecular weight component from this column, as well as samples of the original extract, was chromatographed on Sepharose CL-6B (1.6 X 70 cm) at 24 °C, equilibrated, and eluted in buffer containing 0.1% Triton X-100 alone, with 100 mM dithiothreitol and 5% mercaptoethanol, with 0.1% SDS, or with a combination of all these. The samples were first boiled in the reducing agents or in 2% SDS before being applied to the respective columns. The elution profiles were expressed in terms of the distribution coefficient, Kd, defined as (Vr - Vo)/(Vt - V0), where Vr = void volume, V0 = total column volume, and Vt = elution of sample.

**Oxidative Sulphhydrolysis—**This procedure was carried out according to the method of Du et al. (1965). Carrier unlabeled insulin (1 mg) was added to aliquots of either the HMW component eluted from Sephadex G-50 or 125I-insulin. The protein was precipitated with 10% trichloroacetic acid and the pellets were redisolved in 1 ml of 0.2 M Tris, pH 9.2. Sodium sulfite (14 mg) and sodium tetrathionate (7 mg) prepared freshly according to the method of Gilman et al. (1946), were added, and the solution was incubated for 1 h at 37 °C. The B chain of insulin by adjusting the pH to 6.4 with 50% acetic acid and centrifuging at 3000 X g for 10 min. The supernatant (A chain) was further adjusted in two steps to pH 5.5 and then to pH 5.0, centrifuging between steps to precipitate any residual B chain. The original pellet (B chain) was resuspended in 1 ml of H2O, dissolved by the addition of 1 M N,N,N',N'-tetraacetic acid, and precipitated by adjustment to pH 6.4. This procedure was repeated twice. Cross-contamination of A and B chains prepared in this way is about 2% (Du et al. 1965). The radioactivity in each chain was then determined by counting in an Auto-Gamma spectrometer.

**Immunoprecipitation—**Radioactivity was precipitated using human antiserum (B-2) specific for the insulin receptor (Harrison et al., 1979) or guinea pig antiserum to porcine insulin. To precipitate insulin receptors an aliquot (100 μl) of the HMW component eluted from the Sephadex G-50 column was incubated with 10 μl of human control serum with or without antireceptor serum (final dilution 1:400) for 16 h at 4 °C. Sheep anti-human IgG (80 μl; titer 2 mg/ml) was then added for a further 4 h at 4 °C to precipitate receptor complexes.

**RESULTS**

After binding of 125I-insulin to adipocytes, and extensive washing at 37 °C, a significant fraction of the radioactivity remained nondissociable (Fig. 1). The formation of the nondissociable radioactivity paralleled the time course of specific binding, being maximal at 10-15 min and thereafter decreasing (Fig. 2). When this radioactivity was solubilized and chromatographed on Sephadex G-50, it was resolved into a major peak at the void volume, and two smaller included peaks representing insulin and probably iodotyrosine (Fig. 3). The major peak, designated the high molecular weight component, was approximately 6% of the specifically bound counts (Fig. 1). This peak was not present when the initial binding of 125I-insulin to the cells was performed in the presence of excess...
Experimental procedures. Results are expressed as a percentage of the nondissociable radioactivity extracted and are the mean ± S.E. (n = 6).

(10⁻⁶ M) unlabeled insulin, but was unaltered when binding was performed in the presence of 10⁻⁶ M thyrotropin, glucagon, or vasopressin, or 10⁻¹⁰ M NaI (not shown). Using dilutions of antireceptor serum known to precipitate more than 90% of the recovered as HMW complex compared with 4.5% at 25 ng/ml, it was possible to precipitate 70–75% of the high molecular weight component, but only 8% of this component could be precipitated by anti-insulin antibodies.

The concentration of ¹²⁵I-insulin used initially to demonstrate the HMW component was supraphysiological (~25 ng/ml). At 5 ng/ml of insulin 1.5% of the specific binding was recovered as HMW complex compared with 4.5% at 25 ng/ml insulin (Fig. 4). Irrespective of the concentration of ¹²⁵I-insulin used, the Sephadex G-50 column profiles of nondissociable radioactivity showed little difference in the ratios of the three peaks (data not shown).

On Sepharose CL-6B the HMW component eluted with a Kav of 0.38. Treatment of the HMW component with the denaturant SDS had no effect on the Kav of the HMW component, whereas treatment with reductants led to the appearance of a diffuse nonsymmetrical peak with a Kav of 0.49. When both SDS and reductants were used the HMW component eluted as a symmetrical peak with a Kav of 0.49, corresponding to the position of reduced insulin (Fig. 5).

NEM, a sulfhydryl-blocking agent, when added to adipocytes at the same time as ¹²⁵I-insulin produced a dose-dependent decrease in the formation of HMW complex, with no effect on specific binding of ¹²⁵I-insulin (Fig. 6). When NEM (5 mM) was added for 10 min before ¹²⁵I-insulin, specific binding was decreased by 50% but HMW complex formation was decreased by 90%; NEM added after ¹²⁵I-insulin had little effect (Table I). DTNB (5 mM), a sulfhydryl-blocking reagent that does not penetrate cells like NEM (Czech, 1976b), and iodoacetate (10 mM), also decreased HMW complex formation (Table I). Furthermore, in preliminary studies we have observed that labeling of the adipocyte by [³H]NEM is reduced by up to 30% in the presence of excess unlabeled insulin (data not shown).

Since the radioactivity from reduced HMW complex coeluted with reduced insulin (Fig. 5), it seemed probable that the radioactivity covalently bound was either intact insulin, A chain, or B chain. After oxidative sulphydrylation followed by a step to separate A and B chains based on their different solubilities, the distribution of radioactivity in the HMW complex was similar to that in our ¹²⁵I-insulin: 77 ± 4% A
Fig. 6. Formation of HMW complex in the presence of increasing concentrations of NEM. Adipocytes (2-4 x 10^6/ml) were incubated with ^251-insulin (25 ng/ml) in the presence of increasing concentrations of NEM at 37°C for 10 min. Specific binding was measured and nondissociable radioactivity was extracted and chromatographed over Sephadex G-50, as described under "Experimental Procedures." The amount of HMW complex formed was expressed as a percentage of that formed in the absence of NEM. Results are the means ± S.E. (n = 9). In control cells not exposed to NEM, specific binding was 18.2% of the total; 7.2% was present as HMW complex.

TABLE I
Effect of sulphhydryl-blocking agents on covalent labeling of the receptor

Adipocytes at 37°C were treated with the agents listed below, either for 10 min prior to a 10-min incubation with ^251-insulin (25 ng/ml), for the 10-min incubation with ^251-insulin, or for 10 min after incubation with ^251-insulin. Specific binding was determined and nondissociable radioactivity was extracted and chromatographed over Sephadex G-50 to estimate the amount of HMW complex formed, as described under "Experimental Procedures." Results are expressed as a percentage of controls not exposed to sulphhydryl-blocking agents. Specific binding in controls was 15.3% of the total radioactivity added. 6.0% of the specific binding was present as HMW complex.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Addition relative to incubation of ^251-insulin</th>
<th>Specific binding</th>
<th>HMW complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEM (5 mM)</td>
<td>Before</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>NEM (5 mM)</td>
<td>At the same time</td>
<td>110</td>
<td>38</td>
</tr>
<tr>
<td>NEM (5 mM)</td>
<td>After</td>
<td>105</td>
<td>73</td>
</tr>
<tr>
<td>DTNB (5 mM)</td>
<td>Before</td>
<td>96</td>
<td>60</td>
</tr>
<tr>
<td>DTNB (5 mM)</td>
<td>At the same time</td>
<td>95</td>
<td>66</td>
</tr>
<tr>
<td>Iodoacetate (10 mM)</td>
<td>Before</td>
<td>97</td>
<td>50</td>
</tr>
</tbody>
</table>

This study shows that the specific binding of ^251-insulin to adipocytes is associated with covalent labeling of the receptor due to a disulfide exchange between a small fraction of specifically bound insulin and its receptor. Our findings confirm and extend previous studies in the cultured lymphocyte (Saviolakis et al., 1981).

Several criteria indicate that the labeled HMW component extracted from adipocytes after ^251-insulin binding is the insulin receptor. First, up to 75% of the HMW component was immunoprecipitated with antireceptor serum whose specificity and lack of cross-reactivity (e.g. with insulin-degrading enzymes and insulin-like growth factor receptors) has been documented previously (Harrison et al., 1979; Harrison and Itin, 1980; Kasuga et al., 1981). We were never able to precipitate all of the HMW radioactivity, and in the earlier study with lymphocytes (Saviolakis et al., 1981) only 6% was immunoprecipitable. However, insulin has been shown to impair the ability of the antireceptor serum to precipitate the receptor (Harrison et al., 1979; Kasuga et al., 1981; Van Obberghen et al., 1981) and when ^125I-insulin was chemically cross-linked to the receptor only 10-20% of the complex was immunoprecipitable (Kasuga et al., 1981). It is therefore likely that occupancy by insulin or conformational changes within the receptor prevent its complete immunoprecipitation. Second, the HMW component was eluted from the Sepharose CL-6B column in a position similar to that reported by several workers for the native insulin receptor (Saviolakis et al., 1981).

Finally, the formation of the HMW component is a function of insulin receptor binding. It was only inhibited by unlabeled insulin and not by unrelated hormones.

As shown previously in the lymphocyte (Saviolakis et al., 1981) the label on the extracted adipocyte receptor is resistant to SDS. However, we have now shown that it can be dissociated from the receptor by the addition of reducing agents (Fig. 5). That the covalent labeling of the receptor involves disulfide bonds is further supported by the fact that the reaction is inhibited by the sulphhydryl-blocking reagents, NEM, DTNB, and iodoacetate, present before and/or during the binding of ^125I-insulin to cells (Table I and Fig. 9). It should be noted that when the sulphhydryl reagents were added together with ^125I-insulin they did not impair the specific binding of ^125I-insulin to its receptor. This suggests that the formation of the disulfide complex is a "postbinding" event. Vasopressin, which possesses an intrachain disulfide bond, did not alter covalent labeling by ^125I-insulin. This is important with regard to specificity because the original studies of Fong...
et al. (1962) had suggested that vasopressin, as well as insulin, could bind to tissues through disulfide bonds.

Initially, we considered that the radioactivity covalently bound to receptor might have been transferred to sulfhydryl groups simply as $^{125}$I. However, this reaction is not likely under the conditions used (Fraenkel-Conrat, 1955) and, furthermore, unlabeled NaI did not prevent covalent labeling. The Sepharose CL-6B column had to be run under reducing conditions in order to dissociate the radioactivity from the labeled receptor, and therefore it was not possible to tell whether the radioactivity was on intact insulin, A chain, or B chain. But the distribution of radioactivity following oxidative siltolysis strongly suggests that it was originally present within intact insulin on the receptor. Thus, the proportion of radioactivity isolated in “A chain” and “B chain” from the labeled receptor was the same as in the $^{125}$I-insulin used in the initial binding step. Unfortunately, anti-insulin antibodies were relatively ineffective in precipitating the native labeled receptor, and were completely ineffective, as might be expected, after its reduction. When $^{125}$I-insulin was chemically cross-linked to its receptor in adipocytes no more than half of the complex could be precipitated by anti-insulin serum (Heinrich et al., 1974). The low precipitability of labeled receptor using anti-insulin antibodies may therefore be due to conformational changes in the bound insulin molecule rendering its antigenic sites less accessible.

Several lines of evidence suggest that the disulfide exchange occurs on the cell surface soon after binding, rather than after receptor “endocytosis.” The reaction is inhibited by DTNB, a sulfhydryl reagent reported not to enter cells (Czech, 1976b), and still occurs in isolated cell membranes and parafomaldehyde-fixed cells. Parafomaldehyde treatment has been used to freeze membrane turnover processes (Willingham et al., 1979). In the previous study on lymphocytes (Savolakis et al., 1981) covalent labeling paralleled specific binding even at $4^\circ$C when endocytosis might be expected to be inhibited, and was not affected by the addition of so-called inhibitors of endocytosis such as bacitracin and methylamine.

The biological significance of disulfide exchange warrants further investigation. It has been known for many years that NEM inhibits insulin-stimulated glucose transport (Cadenas et al., 1961; Fong et al., 1962), and these findings were used by Czech et al. (1974a) and Czech (1976a,b) to support a sulfhydryl-redox model for insulin activation of glucose transport. Czech did not find an effect of NEM on specific insulin binding and concluded that the effect of NEM was on the transport system. The present findings indicate that the receptor is also subject to sulfhydryl modification. Importantly, the concentration range over which NEM was shown to decrease insulin-stimulated glucose transport (Carter and Martin, 1969; Czech et al., 1974a) corresponds exactly to that required to decrease the formation of covalent labeled receptor (Fig. 6). This suggests that disulfide exchange between insulin and its receptor may play a crucial role in the activation of glucose transport, both processes requiring the presence of free sulfhydryl groups. Although only a minority of insulin-receptor complexes appear to be involved in disulfide linkage under steady state conditions, it should be remembered that only a small fraction of the total population of receptors needs to be occupied by specifically bound insulin in order for the hormone to exert its maximum biological effects (Kono and Barham, 1971; Gliemann et al., 1975). It would be interesting to know whether the whole population of the receptors, or only a subgroup in a reduced state, is involved in the disulfide exchange reaction. We might speculate that the coupling of binding to receptor activation requires disulfide exchange and that this accounts for the majority of receptors being “spare.”

Finally, the relevance of these findings to the actions of agents that mimic the effects of insulin deserves comment. Oxidants such as spermine, hydrogen peroxide, vitamin K$_3$ dianime (Czech et al., 1974b; Czech, 1976b), and vanadate (Dubysak and Kleinzeller, 1980; Shechter and Karlish, 1980) have all been shown to stimulate glucose oxidation, and in some cases glucose transport, in adipocytes. These agents oxidize sulfhydryl groups and thus favor disulfide bond formation. Insulin might also be considered to play a similar role in the present context since its disulfide exchange might lead to the formation of an “insulin bridged” disulfide bond. Multivalent lectins such as concanavalin A and wheat germ agglutinin (Cuatrecasas, 1973; Katzen and Sodeman, 1975) and insulin receptor antibodies, either naturally occurring (Flier et al., 1976; Kahn et al., 1977) or experimentally induced (Jacobs et al., 1979), stimulate glucose transport and have other insulin-like effects (Harrison and Kahn, 1980). Receptor antibodies, at least, appear to act via a cross-linking mechanism since their monovalent F(ab) fragments are not “insulinominimetic” unless cross-linked by an anti-F(ab) antibody (Kahn et al., 1978). Disulfide exchange could be a mechanism whereby insulin itself cross-links the receptor subunits, thereby activating glucose transport.

The results of this study validate the original notion of Fong et al. (1962) that nondissociable insulin is bound to its receptor by a process of disulfide exchange. Further studies are required to determine if this covalent modification of the insulin receptor results in conformational changes that are important in initiating insulin action.

Acknowledgment—We are indebted to Linda Stafford for expert secretarial assistance.

REFERENCES

Cuatrecasas, P. (1973) J. Biol. Chem. 248, 3528-3534
Czech, M. P., Lawrence, J. C., Jr., and Lynn, W. S. (1974b) J. Biol. Chem. 249, 5421-5427
Flier, J. S., Kahn, C. R., Roth, J., and Bar, R. S. (1975) Science (Wash. DO 190, 63-65
Covalent (-S-S-) Insulin Binding to Adipocytes

DC 200, 1283–1284