The Relationship between the Insulin-binding Capacity of Fat Cells and the Cellular Response to Insulin

STUDIES WITH INTACT AND TRYPsin-TREATED FAT CELLS*

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SUMMARY

The effects of trypsin on the insulin-binding capacity of fat cells were studied with [125I]iodoinsulin, which was shown to be a valid tracer of native insulin. The binding of insulin to isolated fat cells was approximately 5 microunits/100 mg when the concentration of the hormone in the incubation medium was 100 microunits per ml. The initial step of the insulin receptor interaction followed the law of mass action. When the cells were exposed to trypsin (1 mg per ml) for 15 sec and for 15 min, the binding capacity was reduced by more than 80 and 98%, respectively. Upon subsequent incubation of trypsin-treated cells for 2 hours after inactivation of the enzyme, the binding capacity was partly restored. However, the maximum binding capacity of "recovered" cells was only 9 and 4 microunits/100 mg (depending upon the length of the initial trypsin treatment) while that of untreated cells was 62 microunits/100 mg. The apparent dissociation constant of the insulin receptor system (approximately 1 milliunit per ml or 7 nM) was not significantly altered by the above treatment.

Glucose metabolism in recovered cells was less sensitive to insulin as compared with that of untreated cells; however, the metabolism in all cell preparations responded normally to higher concentrations of insulin when more than 1.5 microunits of insulin were bound to 100 mg of fat cells. This indicates that glucose metabolism in intact fat cells is stimulated maximally when only approximately 2.4% of the total insulin receptors on the cells are occupied by insulin. Since the binding follows the law of mass action, it appears that the presence of "excess" receptors on fat cells renders the cells highly sensitive to insulin.

Upon incubation with fat cells, trypsin initially mimics the effects of insulin on cellular metabolism but subsequently renders the cells unresponsive to both insulin and trypsin (1, 2). Our previous data suggested that these effects of trypsin were induced upon interaction of the enzyme with a certain cellular element,

which was possibly the insulin receptor (1, 2) that is generally considered to be located on the cell surface (3). Accordingly, the present work was initiated to examine whether trypsin modifies the insulin-binding capacity of fat cells.

Previously, the binding of insulin to muscle or adipose tissue cells has been studied with either modified insulin labeled with 125I, 131I, or 131I (4-11), or native unlabeled insulin (12, 13). In the latter case, the uptake of insulin was determined by measuring the changes in the hormone concentration in the incubation medium. Since the changes in hormone concentration were relatively small as compared with the background, the method was not very sensitive. Nevertheless, it had a definite advantage over the "tracer" method since the biological activities of insulins modified by labeling were not known with certainty (cf. Reference 13). Consequently, prior to the work presented in this paper, attempts were made to study the effects of trypsin on the binding of native insulin to fat cells. The results were compatible with the assumption that trypsin lowered the insulin-binding capacity of fat cells (14, 15). In addition, it initially appeared that upon further incubation of trypsin-treated cells for recovery, the cells rapidly regained their insulin-binding capacity (14, 15). However, this observation was not substantiated in subsequent experiments, although the physiological response to the hormone (at 1 milliunit per ml) was restored on all occasions.

In the meantime, separate tests in this laboratory indicated that an iodoinsulin preparation labeled with 125I was a valid tracer of insulin for studying the insulin-binding capacity of isolated fat cells. Accordingly, the effects of trypsin on the insulin-binding capacity were re-examined with the [125I]iodoinsulin preparation as the tracer. Results of this work are presented in this paper along with certain observations that show the validity of the iodoinsulin preparation as the tracer. A preliminary account of the present work has been published (16).

MATERIALS AND METHODS

Iodinated pork insulin labeled with 125I (approximately 100 μCi per μg) was purchased from Cambridge Nuclear Radio-pharmaceutical. According to the manufacturer, most of the iodoinsulin in the preparation was a monoiodo derivative. The biological activity of the preparation in the fat cell system (determined in this laboratory) was approximately 80% of that of native insulin on a weight basis, and approximately 80% of the

1 T. Kono, O. B. Crofford, and C. R. Park, unpublished data.
radioactivity was precipitable with 5% trichloroacetic acid. The preparation was used without any further purification since, as shown later, radioactive impurities in the preparation did not bind to fat cells to any significant extent. The native pork insulin and anti-insulin serum were purchased from Schwartz-Mann; HEPES was obtained from Calbiochem. The specifications of other reagents have been described previously (1, 2).

The isolated fat cell preparations (untreated, trypsin-treated, and recovered) were prepared from rat epididymal adipose tissue as described previously (1, 2). The rate of glucose utilization by fat cells was estimated by measuring the rate of conversion of uniformly labeled n-[14C]glucose into 14CO2 and [14C]lipid (1). The binding of [125I]iodoinsulin to fat cells was determined according to the procedures described below. The medium used for the incubation of cells with iodoinsulin and for the subsequent washing of cells was Krebs-Ringer-HEPES buffer containing albumin (20 mg per ml; bovine serum Fraction V). The composition of the buffer was identical with that of Krebs-Ringer bicarbonate buffer supplemented with albumin (1) except that the bicarbonate was replaced with an equivalent quantity of HEPES. The buffer was adjusted to pH 7.4 (in the air) at the temperature to be used. The cells (50 to 100 mg) were incubated with [125I]iodoinsulin (usually 100 microunits; 200,000 to 300,000 cpm) for 20 min at 25°C in a total volume of 1 ml. The incubation mixture was then cooled down to 0-2°C. The chilled cells were washed by repeated centrifugation (usually three to five times3 with 5-ml portions of buffer) in the cold. The washed cells were separated from the medium by vacuum filtration (pressure: -10 cm of H2O) with a Millipore membrane made of nylon (Code 0.45-μm; 1.0-μm; and 0.45-μm). The exposed cells were filtered with a Millipore membrane and the filter disc (the latter absorbed a part of the radioactivity during the drying process) in a toluene base scintillation fluid.

The filter membrane loaded with fat cells was then dried over a disc of filter paper placed on a planchet; drying was aided by an infrared lamp. The amount of dry cells was determined by gravimetry. The radioactivity was determined by the liquid scintillation method upon immersing both the Millipore membrane and the filter disc (the latter absorbed a part of the radioactivity during the drying process) in a toluene base scintillation fluid (1).

In some experiments (when insulin was used at a level of 200 microunits per ml or more), the [125I]iodoinsulin preparation was mixed with cold native insulin; the data were then evaluated on the assumption that the affinities of fat cells for native and iodinated insulins are equal since, as it is shown later (see "Discussion"), the amounts of the two compounds taken up by fat cells are not greatly different. The apparent maximum insulin-binding capacity of fat cells (Bmax) and the apparent dissociation constant of the insulin receptor system (Kd, "e" for effector) were estimated with one of the Woolf plots (cf. Reference 17) commonly known as the Scatchard plot (18). The quantities of fat cells are shown as dry weights, which in the case of fat cells are almost the same as the wet weights, since more than 90% of the cellular material is fat.

### Table I

| Experiment | Washing conditions | Relative radioactivity bound to 100 mg of fat cells |
|------------|--------------------|--------------------------------------------------|
| A          | Method             | Time (min) | Relative radioactivity bound to 100 mg of fat cells |
|            | I                   | 0-2        | 1-2 | 100 ± 13 (6) |
|            | II                  | 0-2        | 15-25 | 94 ± 2 (6) |
| B          | II                  | 0-2        | 15-25 | 100 ± 3 (3) |
|            | III                 | 0-2        | 45-55 | 104 ± 8 (3) |
|            | III                 | 0-2        | 75-85 | 95 ± 10 (3) |
| C          | II                  | 0-2        | 25-30 | 100 ± 1 (5) |
|            | IV                  | 0          | 25-30 | 70 ± 2 (6) |
|            | IV                  | 25         | 25-30 | 45 ± 3 (3) |
|            | IV                  | 37         | 25-30 | 14 ± 0 (2) |

Method I: The cells exposed to iodoinsulin were filtered and washed as quickly as possible. The results were corrected for the blank value (cf. Footnote 3). Method II: The cells were washed by centrifugation, collected by filtration, and washed again under standard conditions. Method III: The cells were washed by centrifugation as above, kept in a fresh buffer for 30 or 60 min as shown, washed again by centrifugation, filtered, and washed again as above (Method II). The temperature was kept at 0-2°C during the entire operation. Method IV: The cells were washed and filtered as in Method II but at the temperatures shown.

a Mean value ± standard error of the mean (number of observations).

3 The abbreviation used was: HEPES, N-2-hydroxyethylpipera- zine-N’-2-ethanesulfonic acid.

It was important to wash the cells extensively to minimize the amount of free radioactivity (iodoinsulin?) in solution since, under the conditions described above, the nylon membrane retained (bound?) approximately 2% of the total radioactivity in solution in the absence of fat cells. When Millipore filters made of cellulose esters or polyvinylchloride were used, larger amounts of radioactivity were retained under the same conditions.

![Fig. 1. Correlation between bound radioactivity and dry weight of fat cells. Different amounts of fat cells were incubated with [125I]iodoinsulin and processed under standard conditions. The amounts of radioactivity found on Millipore filters (see "Materials and Methods") are plotted against the dry weights of fat cells. Each point in this figure represents the result of a single observation.](http://www.jbc.org/)

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Factors That Affect Binding of Iodoinsulin to Fat Cells

Factors That Affect Binding of Iodoinsulin to Fat Cells--When fat cells were exposed to $^{[125]I}$iodoinsulin, washed, and filtered, a certain amount of radioactivity was found on the filter membrane; the amount was proportional to the dry weight of fat cells (Fig. 1). The amount of radioactivity (per cell) was not significantly lowered when the cells were washed at higher temperatures (Table II).

In Experiments 1 and 2, aliquots of fat cells were exposed to insulin for 15 min; these cells are referred to as "treated cells." Subsequently, portions of the treated cells were washed and incubated in an insulin-free buffer for 2 hours to prepare "recovered cells." In Experiments 3 through 6, the "trypsin-treated cells" and recovered cells were prepared as usual. Different batches of fat cells were used in different experiments shown in this table; hence, two sets of data are presented in Experiments 1 through 6. The initial iodoinsulin concentration in the incubation medium was 100 microunits (as insulin) per ml.

The rate of binding of iodoinsulin to fat cells was temperature dependent (Fig. 2). At 37°C, the reaction proceeded rapidly, but the amount of binding was decreased after 15 min. This was probably caused by a partial destruction of iodoinsulin by either a fat cell enzyme (19) or by a remnant of proteolytic enzymes present in the crude collagenase preparation (cf. Reference 20). At 25°C, the binding was almost complete within 20 min. The inactivation of insulin during a 20-min incubation

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**Table II**

**Effects of anti-insulin serum (AIS) on binding of iodoinsulin to fat cells**

| Preliminary treatment of iodoinsulin preparation with | Binding |
|------------------------------------------------------|---------|
|                                                      | Radioactivity | Iodoinsulin |
|                                                      | % of control | microunits/100 mg |
| Nothing                                              | 100 ± 2.8    | 4.99 ± 0.2  |
| AIS (10 eq)                                           | 7.5 ± 0.4    | 0.15 ± 0.08 |
| AIS (100 eq)                                          | 2.0 ± 0.2    | 0.45 ± 0.04 |

* Microunits (as insulin) per 100 mg of fat cells.

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**Table III**

**Effects of insulin and trypsin on iodoinsulin-binding capacity of fat cells**

| Experiment | Cells treated with | Iodoinsulin binding |
|------------|--------------------|---------------------|
|            | Untreated cells    | Treated cells       | Recovered cells    |
| 1: Insulin, 100 million units per ml (15 min) | 4.30 ± 0.05 (100) | 0.094 ± 0.005 (2.1) | 4.53 ± 0.20 (101) |
| 2: Trypsin (15 sec) | 4.88 ± 0.08 (100) | 0.081 ± 0.016 (1.7) | 4.22 ± 0.08 (86) |
| 3: Trypsin (15 min) | 4.36 ± 0.06 (100) | 0.063 ± 0.012 (15.6) | 0.795 ± 0.010 (18.7) |
| 4: Trypsin (15 min) | 4.86 ± 0.17 (100) | 0.344 ± 0.018 (7.1) | 0.377 ± 0.022 (11.9) |
| 5: Trypsin (15 min) | 4.88 ± 0.04 (100) | 0.064 ± 0.005 (6.2) | 0.321 ± 0.011 (6.6) |
| 6: Trypsin (15 min) | 4.31 ± 0.06 (100) | 0.062 ± 0.004 (1.4) | 0.265 ± 0.007 (6.2) |
| 7: T-S Complex | 4.88 ± 0.09 (100) | 5.00 ± 0.006 (102) | Not determined |

* Mean value (microunits per 100 mg of cells) ± standard error of the mean (four observations).

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Fig. 2. The binding of $^{[125]I}$iodoinsulin to fat cells at different temperatures. The binding of $^{[125]I}$iodoinsulin to fat cells was determined under standard conditions, but the temperature and the time for the incubation were changed as shown. Each point in this figure represents the mean value of two observations.
Fig. 3. Estimation of the maximum insulin-binding capacity ($B_{\text{max}}$) and the dissociation constant of the insulin receptor system ($K_a$) of untreated fat cells. Aliquots of fat cells were incubated with a fixed amount of $[125I]$iodoinsulin (3.83 microunits per ml) supplemented with different amounts of cold native insulin to make the total insulin concentrations as shown. The binding of radioactivity to the cells was determined as usual, and the results were plotted in the figure. The amount of total (native plus iodinated) insulin bound to fat cells ($IR$) was estimated on the assumption that the affinities of fat cells for native and iodinated insulins are equal (see “Discussion”). The $B_{\text{max}}$ and $K_a$ values were estimated by the Scatchard plot (see “Materials and Methods”) as shown. In the plotting, however, a few data obtained at extremely high insulin concentrations were rejected since it was observed that a small amount of radioactivity would always bind to fat cells even in the presence of a large dose of cold insulin (see also Figs. 4 and 5). The amount of this nonspecific binding was 0.05 to 0.07% of the total radioactivity added to the incubation mixture. Each point in this figure represents the mean value of two observations. IR, free insulin (microunits per 100 mg in 1 ml); I, free insulin (milliunits per ml).

at 25° (or under standard conditions for the incubation; see “Materials and Methods”) was less than 10% of the total (the data are not shown), as it might be expected from previous data (12, 13). The rate of reaction was very slow at 0°.

Effects of Insulin and Trypsin on Binding—The binding of iodoinsulin to fat cells was greatly inhibited by the addition of cold native insulin (Table III, Experiments 1 and 2), or by preliminary exposure of cells to trypsin for either 15 sec or 15 min (Table III, Experiments 3 through 6). The degree of inhibition was greater when the cells were exposed to the enzyme for a longer period (i.e., 15 min). In contrast, no inhibition was observed when the cells were mixed with trypsin and soybean trypsin inhibitor in the reverse order (Table III, Experiment 7). These data were consistent with an interpretation that both iodoinsulin and trypsin interact with the insulin-binding sites of fat cells. As it has been reported previously, trypsin induces insulin-like effects in fat cells during a 15-sec treatment (2) and abolishes the responsiveness of the cells to the hormone during a 15-min treatment (1).

When the cells exposed to insulin were washed and incubated for 2 hours in an insulin-free medium, the capacity to bind iodoinsulin was restored almost in full (Table III, Experiments 1 and 2). This observation was consistent with the data of our physiological experiments (2). In contrast, when the cells treated with trypsin for either 15 sec or 15 min were incubated for recovery, only a fraction (a few per cent) of the original binding capacity was restored in 2 hours (Table III, Experiments 3 through 6). These results were unexpected since, according to our physiological experiments, fat cells were able to regain near normal metabolic activity within a few hours of trypsin treatment (1, 2), although the “recovered” cells were not as sensitive to insulin as untreated fat cells (2).

The results presented in Figs. 3 through 5 indicated that the apparent maximal insulin-binding capacity ($B_{\text{max}}$) of untreated fat cells was approximately 62 microunits/100 mg of fat cells (Fig. 3), but those of cells recovered from a 15-sec treatment and a 15-min treatment were only approximately 9 and 4 microunits, respectively, per 100 mg (Figs. 4 and 5). In contrast, the apparent dissociation constant ($K_a$) of the insulin receptor...
Correlation between $B_{\text{max}}$ values and insulin-binding characteristics of fat cell preparations

With the equations shown below, the following values were calculated: (a) the amounts of insulin to be bound to the indicated cell preparations when the hormone concentration is 100 microunits per ml, and (b) the concentration of insulin in the incubation medium when a fixed amount of the hormone (1.5 microunits/100 mg) is bound to the indicated cell preparations. The calculated values are compared with the corresponding data observed in the experiments shown in Table III and Figs. 6 and 7. The equations used were:

$$B_{\text{max}} = IR + R$$

and

$$\left[ I \right] \cdot \left[ R \right] = K_s \cdot \left[ IR \right]$$

where $B_{\text{max}} =$ the apparent maximum insulin-binding capacity; $IR =$ insulin-bound receptor (or insulin binding); $R =$ free receptor; $I =$ free insulin; and $K_s =$ the apparent dissociation constant. The amount of fat cells was 100 mg in 1 ml.

**Table IV**

| Cell preparation | Project A | Project B |
|------------------|----------|-----------|
|                  | $B_{\text{max}}^{a}$ |        |
|                  | microunits/100 mg | microunits/100 mg |
| Untreated        | 62       | 5.6       |
| Recovered from 15 sec. | 9       | 0.82      |
| Recovered from 15 min. | 4       | 0.36      |

*Estimated in the experiments shown in Figs. 3 to 5.

*Calculated from the $B_{\text{max}}$ values shown, on an assumption that $K_s = 1$ milliunit per ml.

*Observed in the experiments shown in Table III; mean values are listed.

*Observed in the experiments shown in Figs. 6 and 7.

**Fig. 7.** A correlation between the physiological effect and the binding of insulin to fat cells. The untreated fat cells ($I$), cells recovered from a 15-sec trypsin treatment ($II$), and those recovered from a 15 min treatment ($III$) were prepared as usual (recovery period was 2 hours). In Panel $A$, the rate of glucose oxidation (in 30 min) was determined as usual but in the presence of different amounts of insulin as shown at the bottom of the figure. In Panel $B$, the binding of total insulin (native and iodinated) was estimated as described in Fig. 3 on an assumption that the affinities of native and iodinated insulins for fat cells are equal. Each point in Panel $A$ represents the mean value of three observations; the standard errors, which were reasonably small, are not shown for the sake of clarification. Each point in Panel $B$ represents the mean value of two observations.

**Fig. 8.** Relative effects of insulin concentration on the binding of iodoinsulin to fat cells and on the rate of glucose oxidation. The binding of iodoinsulin to fat cells (•) and the rate of glucose metabolism by the cells (○) were determined in the presence of the indicated doses of iodoinsulin (as insulin) and native insulin, respectively. Each point in this figure represents the mean value of two observations.

The $K_s$ values estimated above. As it can be seen in Table IV (Project A), the calculated values were not greatly different from the corresponding data that were obtained in separate experiments.

The mean value of the $K_s$ values estimated above was approximately 1 milliunit per ml. However, the apparent $K_s$ value for the glucose metabolism was only approximately 7 microunits per ml. As a measure of the validity of these estimated figures, amounts of insulin that would be bound to fat cells at a given hormone concentration were calculated with the $B_{\text{max}}$ and $K_s$ values estimated above. As it can be seen in Table IV (Project A), these values were comparable to those that were calculated from the $B_{\text{max}}$ and $K_s$ values estimated earlier.
TABLE V
Partial protection by insulin of iodoinsulin-binding capacity of fat cells from effect of trypsin

The untreated, treated, and "recovered" cells were prepared as usual, except (a) that the indicated amounts of insulin were added to the reaction mixture 10 min before trypsin treatment and (b) that all the cell preparations were extensively washed to remove insulin from the "plus-insulin" preparations prior to the recovery period. The time for recovery was 2 hours. The binding of iodoinsulin was determined under standard conditions.

| Cell preparations | Insulin added | Binding of iodoinsulin to fat cells |
|-------------------|---------------|-----------------------------------|
|                   | 12-sec treatment | 12-min treatment |
|                   | milliunits/ml | % of control | milliunits/ml | % of control |
| Untreated . . . . . | 0              | 100 ± 2a | 100 ± 3 |
| Treated . . . . .  | 0              | 30 ± 0   | 2.8 ± 0.2 |
| "Recovered": . .  | 0              | 31 ± 1   | 5.7 ± 0.3 |
| "Recovered": . .  | 100            | 76 ± 3   | 6.2 ± 0.4 |

* Mean value ± standard error of the mean (four observations).

Partial Protection of Hormone-binding Site with Insulin from Effect of Trypsin—When trypsin treatment for 15 sec was carried out in the presence of insulin (100 milliunits per ml), the decrease in the binding capacity (as observed in recovered cells) was significantly smaller than the control (Table V). In contrast, no such protective effect of the hormone was observed when the cells (and insulin) were exposed to the enzyme for 15 min (Table V), presumably because insulin was digested by the enzyme under these conditions (2).

DISCUSSION

Although the exact physiological activities of iodoinsulin have not been established, it has been suggested by Izso et al. (21), by Garratt (22), and by Freychet, Roth, and Neville (11) that an iodoinsulin preparation is a valid tracer of native insulin provided that the compound is not too heavily iodinated. In agreement with this suggestion, it was observed in the present work that a monoiodoinsulin preparation was a valid tracer of insulin in studying the binding of the hormone to fat cells. Thus, the binding of the compound was almost entirely blocked upon preliminary treatment of the iodoinsulin preparation with antinsulin serum, or upon addition of native insulin to the incubation mixture. Furthermore, it was noted that the binding could be reversed (as are the physiological effects of native insulin) upon washing the loaded cells at room temperature. These results differ from some of the earlier observations which indicated that the binding of iodoinsulin to fat cells or adipose tissue was only partially blocked by either anti-insulin serum (9) or cold native insulin (11).

The amount of iodoinsulin bound to fat cells was estimated to be approximately 5 microunits (as insulin) per 100 mg when its concentration was 100 microunits per ml (Tables II and III). Previously, it was reported by Crofford (13) that the amount of native insulin taken up by fat cells was approximately 10 microunits/100 mg when the insulin concentration was 100 microunits per ml. However, a smaller value, 4.2 ± 0.5 microunits/100 mg (n = 9), was obtained when the uptake experiment was repeated prior to the work reported in this paper (unpublished, but a part of the data is in Reference 15). Therefore, as a first approximation, it was postulated in the present work that the activities of fat cells for native and iodinated insulins are equal (Figs. 3, 4, 5, and 7). Although certain errors may have been introduced by this postulate, the effects of the approximation are probably insignificant to the present discussion.

The binding of insulin to fat cells was saturable (Fig. 3). Nevertheless, the physiological effect of the hormone on glucose oxidation was saturated when only approximately one-fortieth (1.5 microunits/100 mg) of the apparent total binding sites (62 microunits/100 mg) were occupied by the hormone (Figs. 6 and 7). The difference in the apparent Kp values for the binding (1 milliunit per ml; Figs. 3 through 5) and for the glucose oxidation (approximately 7 microunits per ml; Fig. 6) was more than 100-fold, which was too great to be accounted for by a possible difference in the affinities of fat cells for native and iodinated insulins. In addition, similar results have previously been obtained in studies on the uptake of iodoinsulin by frog muscle (8), rat diaphragm (6, 7), and rat adipose tissue (10), and of native insulin by isolated fat cells (13).

Because of these observations mentioned above, it was suggested in the past that either (a) there is little correlation between the observed binding and the physiological effects of insulin (6, 9) or (b) only a fraction of the observed binding is "specific" or "active," while the rest is not (7, 8, 10). However, it was noted in the present work that both the binding and metabolic effects of insulin at low physiological (see below) concentrations were greatly affected by the apparent maximal insulin-binding capacity (Bmax) of the cell preparation (Fig. 7 and Table IV), and that glucose oxidation in fat cells was stimulated in full when a fixed amount of insulin was bound to the cell preparation (Figs. 6 and 7). It appears that these and other observations described in this paper are consistent with the interpretation (a) that normal fat cells can generate, or accumulate, a certain intracellular hormonal signal strong enough to stimulate the cellular metabolism when only a fraction of the total insulin receptors are occupied by hormone, and (b) that the cells equipped with a large number of receptors are highly sensitive to insulin since, according to the law of mass action, these cells are able to bind the necessary amount of insulin even when the hormone concentration is low.

With this interpretation, one can easily explain how the metabolism in recovered cells, which are equipped with a small number of receptors, can be stimulated to a near normal level when the hormone concentration is increased (cf. Fig. 7).

The above argument does not imply, however, that the individual insulin receptor is inefficient. The estimated Kp value for the binding was approximately 7 nM (or 1 milliunit per ml), which is very good when compared with the Kp values of many enzymes and is roughly comparable to the Kp value of (a) the receptor systems for estradiol (0.7 nM) and estriol (2 nM) of uterus (23, 24) and (b) the glucagon receptor system (approximately 4 nM) of liver (25). Nevertheless, the normal insulin concentration in the blood of rats is probably less than 1 nM (150 microunits per ml; e.g. see Reference 26) and the estimated Kp value for glucose utilization in fat cells was only approximately 50 pm (or 7 microunits per ml; Fig. 6). Besides, it has been known that in both fat cells and adipose tissue insulin regulates glucose utilization (13, 27; Fig. 6), lipolysis (2, 27, 28), and protein synthesis (29, 30) at approximately the same concentration range (roughly 7 to 300 pm or 1 to 50 microunits per ml), although it is also known that isolated cells are somewhat more...
sensitive to the hormone as compared with intact adipose tissue (e.g. Reference 27). Incidentally, it was reported previously that protein synthesis in fat cells was greatly stimulated when the insulin concentration was increased from 50 to 800 microunits per ml (29), but the physiological significance of the observation is questionable.

If the total insulin-binding capacity of fat cells is assumed to be 62 microunits/100 mg as estimated in Fig. 3, one can calculate that this value is roughly equivalent to (a) 4 pmoles of insulin per g of fat cells, or (b) 100,000 receptor sites in a single cell when the mean cell diameter is 50 μm, or (c) 21 receptors per μm² of the cell surface if the receptors are evenly distributed on the surface. Likewise, one can calculate from the present data that glucose metabolism in fat cells is stimulated to one-half of the maximum and to the maximum when approximately 1,200 and 4,000 insulin molecules, respectively, are bound to each cell. The latter value is not greatly different from that of Crofford and Minemura, who estimated that both glucose and amino acid metabolism in fat cells are stimulated maximally when approximately 3,000 insulin molecules are taken up by each cell (15).

Finally, the present data are consistent with our previous hypothesis that trypsin induces insulin-like effects upon interaction (presumably binding) with the insulin receptor site, which is modified by the enzyme upon prolonged incubation (2). The data are also compatible with our theory that fat cells have a capacity to restore, or regenerate, the insulin receptors after trypsin treatment (1, 14, 15); however, it is now suggested that the rate of restoration is only a few percent per hour (cf. Table III).

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REFERENCES
1. Kono, T., J. Biol. Chem., 244, 5777 (1969).
2. Kono, T., and Barham, F. W., J. Biol. Chem., 246, 6204 (1971).
3. Cuatrecasas, P., Proc. Nat. Acad. Sci. U. S. A., 63, 450 (1969).
4. Stadler, W. C., Haugeard, N., and Vaughan, M., J. Biol. Chem., 199, 729 (1952); 200, 745 (1953).
5. Cadenas, E., Kazi, H., Park, C. R., and Rasmussen, H., J. Biol. Chem., 235, PC03 (1961).
6. Malaisse, W., and Franksson, J. R. M., Arch. Int. Pharmacodynam. Ther., 155, 184 (1965).
7. Garratt, C. J., Cameron, J. S., and Menzinger, G., Biochim. Biophys. Acta, 115, 179 (1966).
8. Wohltmann, H. J., and Naramura, H. T., J. Biol. Chem., 241, 4931 (1966).
9. Behnke, R. S., Hilleman, C. C., and Ashmore, M., Mol. Pharmacol., 2, 227 (1966).
10. Crofford, C. J., Jarrett, R. J., and Keun, U., Biochem. Biophys. Acta, 121, 143 (1966).
11. Freychet, P., Roth, J., and Neville, D. M., Jr., Biochem. Biophys. Res. Commun., 43, 400 (1971).
12. Antoniades, H. N., and Gershoff, S. N., Diabetes, 15, 655 (1966).
13. Crofford, O. B., J. Biol. Chem., 243, 362 (1968).
14. Kono, T., Crofford, O. B., and Park, C. R., Diabetes, 18, 335 (1969).
15. Crofford, O. B., Minemura, T., and Kono, T., Advan. Enzyme Regul., 8, 219 (1970).
16. Kono, T., and Barham, F. W., Fed. Proc., 30, 104 (1971).
17. Haldane, J. B. S., Nature, 179, 832 (1957).
18. Satchard, G., Ann. N. Y. Acad. Sci., 91, 600 (1969).
19. Di Grollam, M., Barham, D., Makin, M. F., and Garcia, L. A., Diabetes, 14, 87 (1965).
20. Kono, T., Biochemistry, 7, 1106 (1968).
21. Izoz, J. L., Koncone, A., Izoo, M. J., and Bales, W. F., J. Biol. Chem., 239, 3749 (1964).
22. Garratt, C. J., Nature, 201, 1324 (1964).
23. Toft, D., Shyama, G., Gorski, J., Proc. Nat. Acad. Sci. U. S. A., 67, 1740 (1967).
24. Gorski, J., Shyama, G., and Toft, D., Curr. Top. Develop. Biol., 4, 149 (1969).
25. Rodbell, M., Krans, H. M. J., Pohi, S. L., and Bierer, L., J. Biol. Chem., 246, 1861 (1971).
26. Morgan, C. R., and Lazarow, A., Diabetes, 12, 115 (1963).
27. Challoner, D. R., and Yu, P., Diabetes, 19, 259 (1970).
28. Heff, D., Poppem, P. L., Essnack, J. W., and Williams, R. H., Metabolism, 15, 393 (1967).
29. Miller, L. V., and Beigelman, P. M., Endocrinology, 81, 386 (1967).
30. Minemura, T., Lacy, W. W., and Crofford, O. B., J. Biol. Chem., 245, 3872 (1970).
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